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Investigating the trigger for human parturition using metabolomic and phosphoproteomic techniques within case-control and cohort studies

INVESTIGATING THE TRIGGER FOR HUMAN PARTURITION USING METABOLOMIC AND PHOSPHOPROTEOMIC TECHNIQUES WITHIN CASE-CONTROL AND COHORT STUDIES

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ABSTRACT

The mechanism for human labour remains poorly understood, restricted by difficulties in human pregnancy experimentation, and this limits our ability to manage complications of parturition including spontaneous preterm birth and induction of labour. The studies presented in this thesis utilise metabolomic and phosphoproteomic techniques to improve our understanding of the pathways involved in the trigger for labour in humans.

The first study in the thesis compared mass spectrometry metabolomics analysis of maternal and cord plasma at the time of delivery, and the results indicate that the trigger for spontaneous labour in humans may involve endocannabinoid, sphingolipid and ceramide pathways. The second study presented investigates this further, comparing the metabolic profiles of maternal, cord artery, and cord vein blood sampled from women during low-risk pregnancies, including comparisons of metabolite concentrations between different labour onsets and delivery. This study indicates that there are inherent differences in fetal and maternal signals between women who spontaneously labour and women who have an induction of labour. The third study assessed potential associations between the maternal metabolic profile measured in the second trimester with gestational age at delivery within the Born in Bradford cohort study (analysis sample n = 7440). This study indicates a metabolic profile of dyslipidaemia may be associated with shorter gestational age at delivery, and that non-pathological human labour may involve pathways related to inflammation and vascular function. The final study used phosphoproteomics analysis of fresh myometrium *in vitro*, and the findings indicate that there may be a myometrial phenotype associated with failed induction of labour and failure to progress.

Taken together, these results provide novel leads to follow in order to progress our quest for a full understanding of the trigger for spontaneous labour in humans.

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And to my family - I could not have done this without you.

DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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DECLARATION OF PUBLICATION ARISING FROM PhD

The results from Chapter 2 of this thesis have been published in a peer-reviewed journal, and Chapter 2 itself is adapted from the published article (Table 2A1 in the Appendices):

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We declare that the above article was written by the author of this thesis, Katherine Alice Birchenall, with support from the PhD supervisors Professor Andrés López Bernal and Dr Gavin Welsh.

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Professor Andrés López Bernal

SIGNED: DATE: 11.03.2020

Dr Gavin Welsh

ABBREVIATIONS USED IN TEXT

17βHSD	17β hydroxysteroid dehydrogenase
3β-HSD	3beta-hydroxysteroid dehydrogenase
95%CI	95 per cent confidence interval
ACTH	Adrenocorticotropic hormone
ADP-ribose	Adenosine 5'-diphosphoribose
AEA	Anandamide
AHNAK	Neuroblast differentiation-associated protein AHNAK
ALSPAC	Avon Longitudinal Study of Parents and Children
AMP	Adenosine 5’-monophosphate
ANP	Atrial natriuretic peptide
ASAH1	N-acylsphingosine amidohydrolase 1
ASIC	Acid-sensing ion channel
BASIC	Bile acid-sensitive ion channel
BiB	Born in Bradford cohort study
BLINaC	Brain, liver, intestinal Na^+ channel
BMI	Body mass index
CA	Cord artery
Ca^{2+}	Calcium ion
Ca-CaM-dependent enzyme	Calcium ion-calmodulin-dependent enzyme
$CaCl_2$	Calcium chloride
cAMP	Adenosine 3',5'-cyclic monophosphate
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CDS	Central delivery suite
cGMP	Cyclic guanosine monophosphate
COX-2	Cyclooxygenase-2
CPMG	Car-purcell-meiboom-gill
CRH	Corticotrophin-releasing hormone
CRMP	Collapsin response mediator protein
CS	Caesarean section
CV	Cord vein
CYP450scc	Cytochrome P450
DAG	Directed acyclic graph
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone-sulphate
DPYSL3 protein	Dihydropyrimidinase-related protein 3

DYRK	Dual tyroxine-regulated kinase
DZ	Definitive zone
EC	The metabolite quantity identified in cord plasma from women who did not labour and delivered via elective caesarean section
ECM	Extracellular matrix
ECS	Elective caesarean section
EDD	Estimated date of delivery
ELISA	Enzyme-linked immunosorbent assays
EM	The metabolite quantity contained in intervillous plasma from women who did not labour and delivered via elective caesarean section
eNOS	Endothelial nitric oxide synthase
ER	Endoplasmic reticulum
ER	Oestrogen receptor
Erα	Oestrogen receptor alpha
ERα	Oestrogen receptor alpha
ERβ	Oestrogen receptor beta
ERβ1	Oestrogen receptor beta 1
ERΔ7	Oestrogen receptor delta 7
FAAH	Fatty acid amide hydrolase
FC	Fold change
FDR	False discovery rate
FISH	Fluorescent in situ Hybridisation
FMD	Flow mediated dilatation
FZ	Fetal zone
GC	Gas chromatography
GC-MS	Gas chromatography coupled with mass spectrometry
GDM	Gestational diabetes mellitus
GGCX	Gamma-glutamyl carboxylase
GSK3	Glycogen synthase kinase 3
GWAS	Genome-wide association study
HBMD	Human metabolome database
HCD	Higher-energy collisional dissociation
HDL	High density lipoprotein
HEK293 cell line	Human Embryonic Kidney 293 cells
HIF1A	Hypoxia inducible factor 1 subunit alpha
hINaC	Human intestinal Na^+ channel
HPA axis	Hypothalamic-pituitary-adrenal axis

HPLC/UPLC	High or ultrahigh performance Liquid Chromatography coupled to ultraviolet or fluorescence detection
HSP	Heat shock protein
IDL	Intermediate density lipoprotein
IGF1	Insulin-like growth factor 1
IL-1	Interleukin-1
IL-1β	Interleukin-1 beta
IL-4	Interleukin-4
IL-6	Interleukin 6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12	Interleukin-12
INF-γ	Interferon-gamma
IOL	Induction of labour
IV	Intervillous
KCL	Potassium chloride
KEGG	Kyoto Encyclopedia of Genes and Genomes
KO	Knock-out
LC	Liquid chromatography
LCA	Latent class analysis
LC-MS	Liquid chromatography coupled with single-stage mass spectrometry
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
LDL	Low-density lipoprotein
LDL	Low density lipoprotein
LIF	Leukaemia inhibitory factor
LMP	Last menstrual period
LMW	Low-molecular weight
LMWM	Low molecular weight molecules
LOX	Lysyl oxidase
LOXL2	Lysyl oxidase homolog 2
Lp(a)	Lipoprotein a
LTQ	Lysine tyrosylquinone
m/z	Mass-to-charge ratio
MAM	Mitochondria-associated membrane
MBRRACE-UK	Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries
MEF2C	Myocyte enhancer factor 2C
MGP	Matrix gla protein

$MgS0_4$	Magnesium sulphate
MHC	Myosin heavy chain
miR	MicroRNA
ml	Millilitre
MLC	Myosin light chain
mM	Millimoles
MME	Membrane metallo-endopeptidase
MR	Mendeilian randomisation
MS	Mass spectrometry
MYLK	Myosin light chain kinase
MYLP	Myosin phosphatase
MYOCD	Myocardin
Na^+	Sodium ion
NaH_2PO_4	Monosodium phosphate
$NaHCO_3$	Sodium bicarbonate
Nano-LC MS	Nanoscale-Liquid Chromatography coupled to tandem Mass Spectrometry
NF-kB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NHS	National health service
NICE	National Institute for Clinical and Health Excellence
nM	Nano-moles
NMR	Nuclear magnetic resonance
NO	Nitric oxide
N-PS	N-palmitoylserine
OEA	Oleoyl-ethanolamide
OGTT	Oral glucose tolerance test
OPPTIMUM	Does progesterone prophylaxis to prevent preterm labour improve outcome? Trial
OR	Odds ratio
Otr	Oxytocin receptor
OXT	Oxytocin
OXT-CON	Oxytocin-stimulated contraction snap frozen at peak phasic contraction
OXT-REL	Oxytocin-stimulated phasic contraction snap frozen 20 seconds following the end of a phasic contraction
PACS-2	Phosphofurin acidic cluster sorting protein 2
PAH	Pulmonary arterial hypertension
PDGFR-alpha	Platelet-Derived Growth Factor Receptor alpha
PEA	Palmitoyl-ethanolamide
PET	Pre-eclampsia

PFOA	Perfluorooctanoate
PFOS	Perfluorooctanesulfonate
PGRMC1	Membrane-associated progesterone receptor component 1
PHLDA5	Pleckstrin homology-like domain, family A, member 5
PKA	Protein kinase A
PLD	Phospholipase D
PPAR- α	Peroxisome proliferator-activated receptor-alpha
PPAR- α interacting complex 285	Peroxisomal proliferator-activated receptor A interacting complex 285
PPI	Protein-protein interaction
PR	Progesterone receptors
PRA	Progesterone Receptor type A
PRB	Progesterone Receptor type B
PRC	Progesterone receptor c
PRE	No tension applied and considered pre-contraction
PRISM trial	Progesterone in spontaneous miscarriage trial
PROMISE trial	Progesterone in Recurrent Miscarriages trial
PSMs	Peptide spectral matches
PTB	Preterm birth
PTL	Preterm labour
PUFA	Polyunsaturated fatty acid
QC	Quality control
RCT	Randomised controlled trial
REL/PRE	Ratio between (spontaneous contraction, snap frozen 20 seconds following the end of a phasic contraction) and (no tension applied and considered pre-contraction)
RIPA	Radioimmunoprecipitation assay
ROS	Reactive oxygen species
RR	Risk ratio
RSD	Relative standard deviation
RU486	Mifepristone
SCOPE	Screening for Obstetric and Pregnancy Endpoints
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SEP	Socioeconomic position
SGTA	Small glutamine-rich tetratricopeptide repeat-containing protein alpha
SL	Spontaneous labour
SMC	Smooth muscle cell

SP-CON	Spontaneous contraction snap frozen at peak phasic contraction
SP-REL	Spontaneous contraction, snap frozen 20 seconds following the end of a phasic contraction
SRF	Serum response factor
TGF	Transforming Growth Factor-β
TGFBI	Transforming Growth Factor-Beta-induced Protein ig-h3
$Ti0_2$	Titanium dioxide
TIMP-1	Tissue inhibitor of metalloproteinase-1
TMAP	N,N,N-trimethyl-alanylproline betaine
Tmod	Tropomodulins
TMT	Tandem mass tag
TNFα	Tumour necrosis factor α
TPR	Tetratricopeptide repeat
TRPV1	Transient receptor potential vanilloid type 1
TZ	Transitional zone
UHPLC/MS	Ultrahigh performance liquid chromatography-tandem mass spectrometry
UK	United kingdom
v/v	Vol/vol
VC	Metabolite quantity identified in cord plasma from women who laboured and delivered vaginally
VC/EC	Represents the difference in the means between VC, the metabolite quantity identified in cord plasma from women who laboured and delivered vaginally, and EC, the metabolite quantity identified in cord plasma from women who did not labour and delivered via elective caesarean section
VC/EM	VM/EM represents the difference in the means between VM, the metabolite quantity identified in intervillous plasma from women who laboured and delivered vaginally, and EM, the metabolite quantity contained in intervillous plasma from women who did not labour and delivered via elective caesarean section
VD	Vaginal delivery
VLDL	Very low density lipoprotein
VM	The metabolite quantity identified in intervillous plasma from women who laboured and delivered vaginally
WHO	World health organisation
βhCG	Beta human Chorionic Gonadotropin
βig-H3	Transforming Growth Factor-Beta-induced Protein ig-h3

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CHAPTER 1. INTRODUCTION

1.1 Rationale for research

Parturition is essential for mammalian existence, however despite decades of research the mechanism for labour in humans remains poorly understood[1-3], with negligible advances in safe and effective methods for the inhibition or stimulation of uterine contractility in recent years[4, 5]. This has many implications, including limiting our effectiveness at managing preterm birth (PTB) and induction of labour (IOL)[1, 6, 7]. While infection and inflammatory processes have been implicated in some episodes of PTB, in many cases no cause is found, and it is possible that there is an early triggering of the events of parturition which would normally occur at term[8, 9]. A better understanding of human labour would therefore allow development of methods for improved prevention of PTB, as well as more effective strategies for IOL.

There is strong interest in using epidemiological, clinical and molecular markers to identify women at risk of complications of pregnancy[5, 10, 11], with advances in prediction models for complications of pregnancy. These include clinical tests to evaluate cervicovaginal fluid, such as: quantitative measurement of fetal fibronectin for prediction of preterm labour[12]; determination of the presence of human placental-alpha-microglobulin-1 with the PartoSure test for prediction of PTB within 7-14 days[13] ; and measurement of phosphorylated insulin-like growth

factor binding protein-1 with the Actim Partus bedside test which is also used to predict PTB within the next 7 days[14]. Further potential markers for prediction of PTB include 4-hydroxyglutamate (identified from mass spectrometry assessment of approximately 900 gestation serum metabolomic markers) as a predictor for preeclampsia[15], and a predictor model based on four maternal serum metabolites (from the same mass spectrometry platform as that for 4-hydroxyglutamate) for prediction of fetal growth restriction at term[16]. The latter two of these studies involved the use of metabolomics, which is one of a number of recent technologies that are enabling new insight into these important clinical research questions. Further, a nested case-control study (n=81) within the prospective Proteomic Assessment of Preterm Risk (PAPR) study was conducted in the United States between 2011 and 2013. Proteomic assessment of maternal serum was performed between 17 and 28+6 weeks' gestation in order to develop a mass spectrometry-based serum test for prediction of spontaneous preterm labour in asymptomatic pregnant women[17]. The log ratio of insulin-like growth factor-binding protein 4 (IBP4)/Sex Hormone-Binding Protein 4 (SHBG) was found to have an odds ratio of 5.04 for spontaneous preterm delivery before 37 weeks' gestation, with a sensitivity of 75% and specificity of 74%[17]. This group have since conducted a prospective case-control study (9 cases; 838 non-cases) within an independent cohort to show that this ratio is predictive of preterm

birth <32 weeks' gestation, as well as significantly associated with length of stay on the neonatal intensive care unit[18]. The authors of this study also suggest that as IBP4 is produced by the placenta and present in increased concentrations in women with small for gestational age fetuses, that is may also be a marker for placental insufficiency. This is interesting as it supports a potential link with placental function and PTB, and therefore raises the possibility that timing of gestational age at spontaneous delivery may be related to changing placental function towards term. This is a theme which is returned to throughout this thesis.

While such prediction of pathologies of pregnancy are valuable so that women who are at risk may be identified early and monitored, these biomarkers do not necessarily reflect the causal pathway for the conditions they predict and do not always provide answers as to the best way to treat and prevent[19-22]. It is therefore useful to continue research into the mechanistic pathways underlying these processes, in parallel with developing prediction models, in order to further direct potential development for treatment and prevention of these conditions. The overall ethos for the research included in this thesis was to improve our understanding of the molecular triggers for spontaneous labour, including PTB, by integrating evidence from clinical, laboratory and epidemiological studies.

1.2 Background

1.2.1 Human gestation

The average gestational length for human pregnancy is 40 weeks (280 days) following the first day of the last menstrual period (LMP)[23, 24]. In many settings, including in the United Kingdom (UK), an initial estimated date of delivery is calculated by adding 280 days to the LMP. This may be adjusted following fetal measurements taken at the dating scan, routinely conducted between 11- and 14-weeks' gestation, which measures the developing fetus and gives an estimated gestational age according to the calculated fetal size[25]. However, the reality is that human gestational length can vary considerably between individuals, and the estimated date of delivery (EDD) is rarely precise[24, 25]. Only around 4% of pregnancies deliver at 280 days, and approximately 70% deliver within ten days of their predicted EDD[24]. Gestational length has been studied since ancient times, and Aristotle remarked that, unlike other animals which have a uniform gestation, humans may have pregnancies of seven to eleven months' gestation[26]. The reason for this variation is unclear, and while it may be due to inherent errors in the EDD calculation, as it is not possible to detect precisely when fertilisation occurs, there is also likely variation in the rate of fetal maturation and individual differences in the timing of natural delivery. Importantly, it is unclear whether there is a single healthy gestational length for

humans[24]. Interestingly, cell-free RNA in maternal blood has been shown to have a distinct phenotype at different stages of pregnancy[27]. A pilot study (n=31) indicated that measurement of nine cell-free RNA transcripts within maternal blood estimated current gestational age with a similar accuracy to the gold-standard ultrasound. The same authors also described another pilot study of 38 women with risk-factors for PTB which showed that seven cell-free RNA transcripts could predict preterm birth up to two months in advance. Larger studies are required to investigate this further[28].

1.2.2 Gestational length definitions

A pregnancy is considered to deliver at "term" if the baby is born between 259 days and 294 days (37 and 42 completed weeks' gestation)[29, 30], "preterm" if delivered before 259 days (37 completed weeks) and "post-term" if delivered after 294 days (42 completed weeks)[29, 31]. However, recent discussions among experts suggest that the five weeks described as term, i.e., 37 completed weeks to 42 completed weeks, may be too long; and that neonatal outcomes for babies born within this time frame should not be considered the same, particularly with regards to neonatal respiratory morbidity. A further sub-categorisation has been suggested, creating the following categories: "early term" (37 completed weeks to <39 completed weeks), "full term" (39 completed weeks to <41 completed weeks), "late term" (41 completed weeks to <42 completed weeks) and "post-

term" (42 completed weeks and above)[23]. However, this suggestion has not currently been taken up in the UK. In general, babies who are born outside of the "term" boundaries are considered to be at increased risk for perinatal complications[29, 30].

1.2.3 Mechanisms of parturition

Parturition describes the process of birth in mammals. In humans this is a series of events, colloquially referred to as labour, which involve rupture of the amniotic membranes (which may not occur until immediately before the baby is born), cervical softening and dilatation, and activation of the smooth muscle of the myometrium to produce contractions that result in delivery of the baby[32]. Although some mechanisms are shared among mammalian species[33], the triggers for these events in humans remain unclear[3, 34].

Progesterone and oestrogen are key hormones for human reproduction and pregnancy, with antagonistic actions on myometrial contraction. During pregnancy, progesterone appears essential for general quiescence of the uterine myometrium, and *in vitro* a decrease in progesterone results in increased contractile activity[35]. In contrast, oestrogen promotes transcription of genes which encode proteins that increase myometrial excitability and contractility, such as cyclooxygenase-2 (COX-2) and oxytocin receptor (OTr); and an increase in oestrogen concentrations can thereby stimulate the myometrium

to contract and labour[32]. In many mammals, parturition seems to be triggered by a change in the balance between progesterone and oestrogen so that at the time of labour there is a relative decrease in progesterone concentrations, or increased sensitivity of tissues to oestrogen compared with progesterone. Much research in this area has been conducted in rodents and sheep, although this has not been easy to translate to humans, and this will be discussed in the coming chapters[35].

1.2.4 The epidemiology of preterm birth

As described above, PTB is defined by the World Health Organisation (WHO) as birth which occurs before 37 completed weeks' gestation[31, 33]. PTB is a global problem, affecting women within both developed and developing countries, and 15 million babies are born prior to 37 weeks' gestation each year[36]. In 2015, more than a million babies died because of complications of PTB, which comprised 16% of global deaths in children under five years of age [37]. Many more of these children have life-long disabilities including cerebral palsy, and visual, learning and hearing impairments[36, 38]. Within the UK, PTB is the largest cause of neonatal morbidity and mortality. According to the "Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries – UK" (MBRRACE-UK) report, 7% (57441 out of 780 114) of UK live births were delivered prior to 37 weeks gestation in 2015[39], a statistic which has not substantially changed for

over a decade[31, 40]. The risk for poor neonatal outcome secondary to PTB is inversely proportional to the gestational age at delivery, so that those babies that are born moderately preterm (between 34 and 37 completed weeks' gestation) have better outcomes than those that are born extremely preterm (before 28 weeks' gestation[31]). For example, approximately 35% of babies born preterm are diagnosed with cerebral palsy, and the risk increases the shorter the gestational age at delivery. The incidence of cerebral palsy in those born before 28 completed weeks is 111.8 per 1000 neonatal survivors, or 82.25 per 1000 live births[41], compared with an overall prevalence 2-3.5 per 1000 live births in the UK[42].

1.2.5 Induction of labour

Induction or augmentation of labour may be desirable if there are concerns about the mother or baby, or because the pregnancy has become very overdue (likely to deliver beyond 42 completed weeks' gestation)[43, 44]. Current methods for IOL can be inefficient, with potentially severe complications for the mother and neonate including fetal distress, emergency caesarean section, instrumental delivery and postpartum haemorrhage[4,43, 44]. These complications can result in lengthy hospital stays which potentially have a negative impact on the birth experience and psychological wellbeing of the women and their families, and with financial cost to the National Health Service (NHS)[4,43, 44]. According to the

National Institute for Clinical and Health Excellence (NICE), in 2004 and 2005 one in every five UK deliveries was induced; and over a third of these women required interventions to deliver their babies, including instrumental delivery or caesarean section[44]. Further, the proportion of pregnancies with IOL in England has increased from 20% in 2006-7 to 29% in 2016-17 [45], thought to be in part due to changing guidelines about which women should be offered IOL at term[46-48].

1.3 Macro- and micro-structure of the human uterus

1.3.1 The uterus

The uterus is the major maternal organ involved in parturition and will be described here. The non-pregnant uterus can structurally be considered to comprise of three main layers: an inner endometrium, a thick muscular myometrium and an outer serosal layer[49] (Figure 1.1). The tissues of the uterus undergo changes throughout a woman's life that are largely under the influence of oestrogen and progesterone, the main steroid hormones of reproduction and pregnancy[50-52]. The uterus is an essential organ for pregnancy, facilitating implantation, placentation and development of the fetus[52].

The endometrium is composed of a basal and a functional layer, the stratum basalis and the stratum functionalis, respectively. The basal layer is adjacent to the myometrium and is where regeneration of the endometrium occurs. The

functional layer is receptive to hormonal influence and changes throughout the menstrual cycle, sloughing off with menstruation[52]. For the majority of pregnancy, the myometrium is in a state of relative quiescence and it transitions to a contracting state at the time of parturition[32].

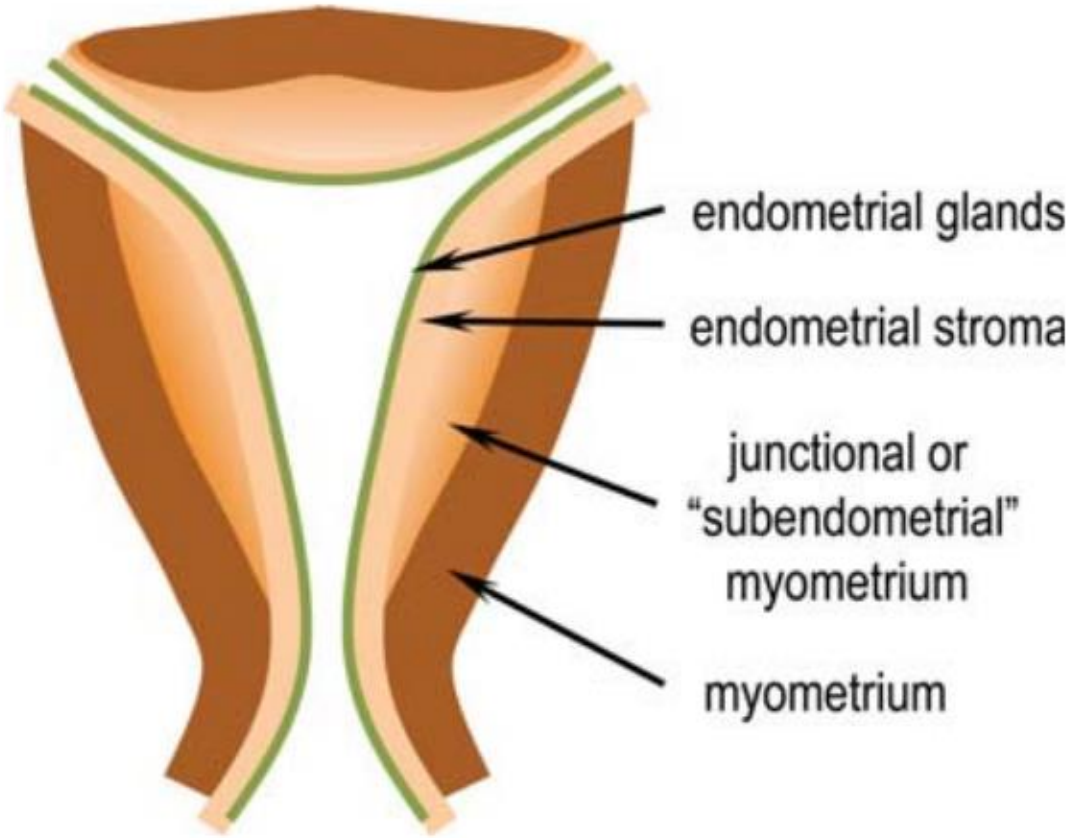

Figure 1.1. Illustration of non-pregnant uterine structure (adapted from Aguilar & Mitchell (2010)[49]).

1.3.2 Myometrial structure and electrophysiology

Given that the myometrium is the organ which undergoes coordinated contractions to expel the fetus at the time of labour, it is essential to understand its role and response to the changing environment during pregnancy,

particularly at the time of parturition, in order to answer the overarching question of this thesis[3]. The myometrium is composed of smooth muscle which contracts both spontaneously and in response to agonists, the rhythmicity of which is related to the generation of slow waves and superimposed action potentials [53]. Small contractions occur throughout the menstrual cycle as well as in pregnancy, and the amplitude, frequency, duration and direction of propagation change according to the physiological or pathophysiological environment. In non-primates the myometrium is made up of a distinct inner circular layer and an outer longitudinal layer, however it is less distinct in humans in whom there are intertwined muscular bundles that often surround vascular channels[49]. The contractions which occur in the non-pregnant state appear to involve only the junctional or sub-endometrial layer of the myometrium (Figure 1.1), producing endometrial waves that are distinct to the stronger propulsive contractions of the outer layer during miscarriage and parturition. This inner junctional layer has been shown to have an abundance of receptors for oestrogen and progesterone which are regulated throughout the menstrual cycle[49].

1.3.3 Myocytes and contractility

Approximately 80-90% of the volume of myocytes is made up of contractile machinery including myofilaments and the dense bodies associated with them.

The main proteins expressed in myocytes are actin and myosin and, unlike skeletal muscle, myocytes contain more actin than myosin. Actin forms thin myofilaments of diameter 6-8nm, while myosin forms thick myofilaments of diameter 15-18nm. These myofilaments tend to run in parallel along the longitudinal axis of the myocytes. Myosin in myocytes is primarily composed of myosin II, a hexamer molecule made up of two pairs of myosin light chains (MLC) and two heavy chains (MHC). There are three domains to the molecule: a head comprised of the globular N-terminal end of MHCs, constituting the actin-binding region, and an ATP hydrolysis site, termed the "motor domain" as it provides the energy for the force of contraction; a tail of thick filaments of the C-terminal ends of the MHCs; and a neck domain which creates an angle between the head and tail where each of the MLCs are attached via a non-covalent bond to each of the MHCs[49, 54, 55]. The head and neck domains make cross bridges which interact with the actin filaments during contractile activity. There are two main isoforms of the MLCs: one has a molecular mass of 17 (MLC_{17}), termed the essential light chain, and the other 20 (MLC_{20}), termed the regulatory light chain. MLC_{20} has an important role in regulating muscle contraction in uterine smooth muscle. To enable relaxation following contraction, the cross-bridge attachment needs to be released, and this involves dephosphorylation of MLC_{20}. The dominant MHC in smooth muscle is coded for

by a single gene, with splice variants resulting in four distinct isoforms. Different genes code for non-muscle MHC which may also be contained within smooth muscle. Two isoforms of MLC_{17} exist due to alternate splicing. In contrast to this, two different genes encode the two different MLC_{20} isoforms, one of which associates with smooth muscle MHC, and the other associates with non-muscle MHC[49, 55, 56].

The resting membrane potential of the myometrium is determined largely by the inward ionic flux of sodium and calcium ions, and the outward ionic flux of potassium and chloride ions. As a pregnancy reaches term, the resting membrane potential of the myometrial cells becomes gradually more depolarised, and *in vitro* this gradual depolarisation results in an increased number of contractions[53]. The myometrium is autonomous and contracts without nervous or hormonal input, and it is unclear whether there are specific pacemaker cells or if all cells have inherent pacemaker activity[57].

1.3.4 The cervix

During normal pregnancy, the cervix remains firm and closed and prevents passage of the developing fetus until term[50]. The uterus is separated from the cervix by the squamocolumnar junction, and this is where the squamous mucosal epithelium, which covers the outer (ectocervix) and inner (endocervix) part of the cervix, meets the columnar epithelium of the uterus. During normal

labour, the cervix softens and dilates to facilitate delivery of the baby[50]. Monitoring the rate of cervical dilatation allows clinicians to assess labour progress.

While the cervix is functionally distinct from the uterus, cervical remodelling during labour is commonly coordinated with uterine contractions[58]. The cervix is predominately made up of the extracellular matrix (ECM) proteins elastin, collagen, and glycosaminoglycans. In addition, 10-15% of cervical tissue is comprised of smooth muscle cells. The primary cause of cervical softening is believed to be realignment and rearrangement due to mechanical forces of elastin, collagen and smooth muscle cells; and the additional rearrangement of collagen which occurs as glycosaminoglycans content within the cervix changes with time[58]. Dilatation of the cervix is believed to be in part due to the orientation of elastin and a ratcheting effect[58]. During labour, the cells of the cervix are metabolically active, and cervical smooth muscle cells have been shown to undergo apoptosis during cervical softening. This has prompted suggestions that, as apoptosis is an event that is genetically timed, this could explain the differences in gestational length between species [58]. Mouse models have been used in many studies of the function of the cervix during labour and in PTB[50].

1.3.5 Placenta

The placenta is the organ via which the fetus and the maternal circulations communicate, and it has multiple functions including metabolism, biosynthesis, and transport of molecules such as steroids and cholesterol, eliminating waste, gas exchange, and prevention of rejection of the pregnancy as a fetal allograft[59-62]. The placenta is also a major site of hormone synthesis during pregnancy, adapting throughout pregnancy in order to best support the fetus[60], and is probably involved in the initiation of labour[61]. Inadequate placentation and placental insufficiency can affect fetal growth, development and survival, as well as contribute to maternal pathologies such as pre-eclampsia[62]. Prior to implantation, at the blastocyst stage, the outer cells of the embryo are separated from the inner cell mass and become the trophoblast, while the inner cell mass develops to become the fetus. Trophoblast cells differentiate into the cytotrophoblast and the syncytiotrophoblast, and these are the cells which invade the maternal endometrium to form the fetal part of the placenta. Lacunae form within the synctiotrophoblast, creating the intervillous spaces that allows circulation of maternal blood into the placental bed[61]. The attachment of the placenta to the uterine wall is essential for successful pregnancy, and also serves to hold the pregnancy within the uterus[61]. The cells of the placental chorionic villi act as a filter between the fetal and maternal circulations, with no

direct contact between the fetal and maternal blood (Figure 1.2). Simple diffusion occurs for some low-molecular weight (LMW) and/or non-polar molecules such as sodium, gases, urea, water, fatty acids and unconjugated steroids. The amount of simple diffusion changes throughout pregnancy as diffusion distances decrease with increasing gestation, and diffusion of many of these molecules increase as the pregnancy advances. Other molecules depend upon active transport[60-62].

The chorion forms a membrane which is continuous with the placental trophoblast and which is immediately adjacent to the maternal decidua. The amnion is a membrane that surrounds the amniotic cavity, which ultimately contains the amniotic fluid and fetus, and lies between the trophoblastic chorion and the inner cell mass. Neither the chorion nor the amnion have lymphatics, blood vessels or nerves, and together they form the fetal membranes which rupture either prior to or during labour[61].

Blood flows between the placenta and the fetus via the umbilical cord which originates from the chorion (Figure 1.2 and 1.3)[62, 63]. The cord typically contains two cord arteries and a cord vein, although some have just one cord artery. The umbilical vein carries blood from the placenta to the fetus, via the ductus venosus, and into the fetal inferior vena cava and right atrium. The blood in the umbilical vein contains nutrients and is typically 80% oxygen saturated. The

umbilical arteries carry blood away from the fetal descending aorta back to the placenta, and the oxygen saturation of the umbilical arteries is around 50% (Figure 1.3)[63]. This indicates that oxygen and nutrients are taken up and metabolised by the fetus, while the products of this metabolism are transported back to the placenta via the umbilical arteries. In addition, the placenta produces some precursor molecules which, as the fetal organs mature, the fetus uses to convert to other essential molecules[62, 63]. In the majority of previous studies identified, cord blood samples are not defined as either the cord vein or cord artery, and instead are likely mixed or cord vein[5, 64-70]. However, it is possible to sample blood separately from the cord arteries and cord vein at delivery, and thereby measure the differing concentrations of different metabolites in the blood derived from the fetus (umbilical cord arteries) and the blood derived from the placenta (umbilical cord vein), as well as blood from the maternal circulation[5]. Importantly, such measurements could identify pathways and interactions between the fetus, placenta and maternal metabolites at the time point of delivery that may be important for labour. This triangulation is utilised in the studies of this thesis to identify potential pathways that are activated between the fetus and the placenta with labour and delivery.

The placenta produces many of the hormones of human pregnancy, many of which are secreted into the maternal circulation, aided by the close proximity of

the placental cytotrophoblast and syncytiotrophoblast cells to maternal blood[71]. In contrast, most maternal hormones are prevented from entering the fetal compartment by the syncytiotrophoblast cells. This means that the endocrine system of the fetal-placental system develops and functions largely in independence from the mother[71]. The syncytiotrophoblast layer is the major source of these hormones as it expresses the enzymes required for several[72]. Hormones produced by the placenta include progesterone, oestriol, pregnenolone, and beta human chorionic gonadotropin (βhCG). Some of the substrates for these hormones are produced by the fetus, and the exchange of molecules between the fetus and the placenta as a feto-placental unit is discussed further below[71, 72].

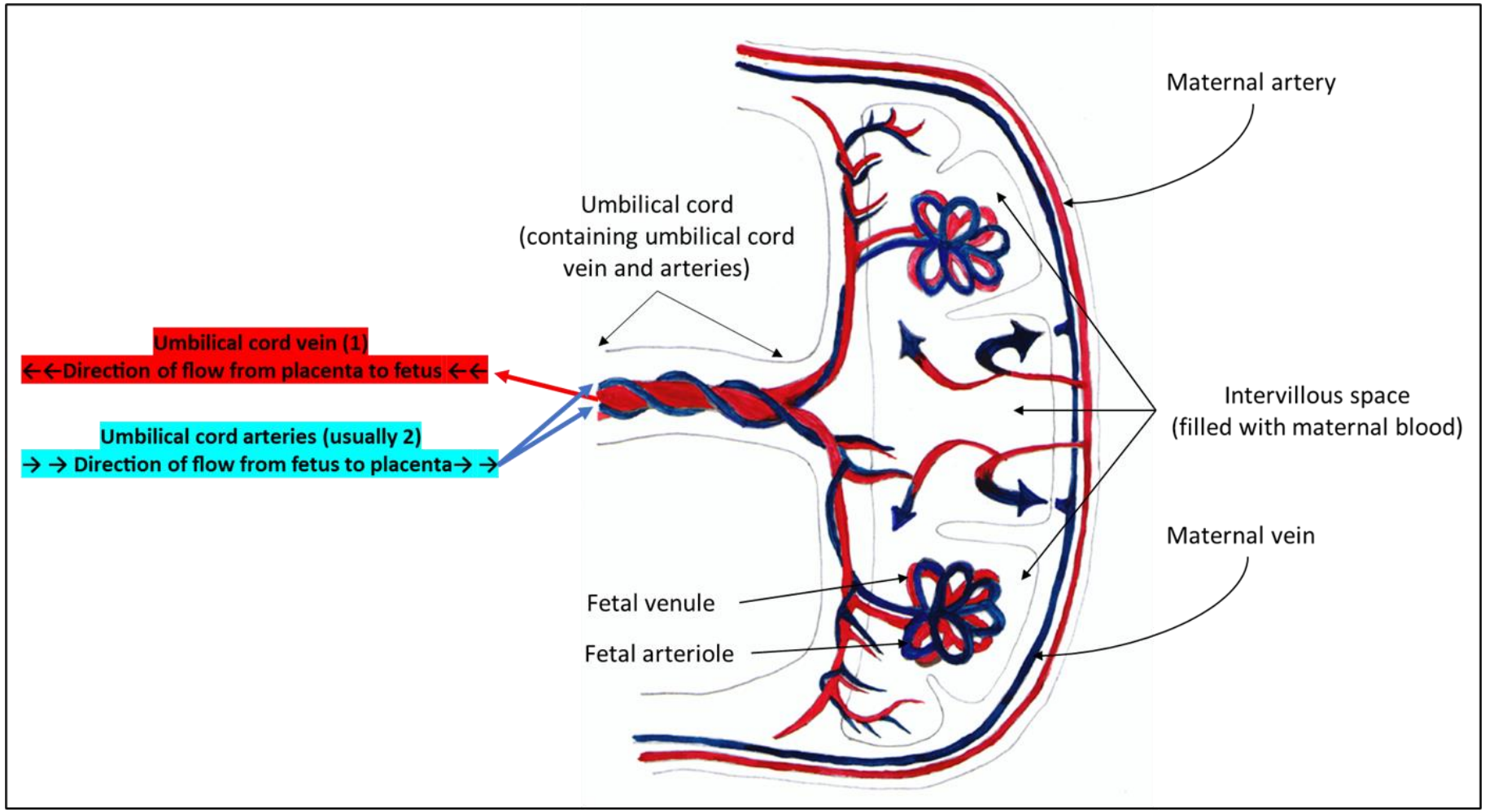

Figure 1.2: Diagram of blood flow within the human placenta, with separated maternal and fetal blood circulations. Maternal blood flows into the placenta via maternal arteries, pools in the intervillous space, and flows out of the placenta via maternal veins. Fetal blood circulation through the placenta occurs via fetal venules and arterioles contained within chorionic villi that protrude into the intervillous space, allowing selective exchange of molecules between the maternal and fetal circulations. Fetal blood flows from the placenta to the fetus via the umbilical cord vein and flows from the fetus to the placenta via the umbilical cord arteries (usually two), contained within the umbilical cord. Red colour = oxygen-rich blood; blue colour = oxygen-depleted blood.

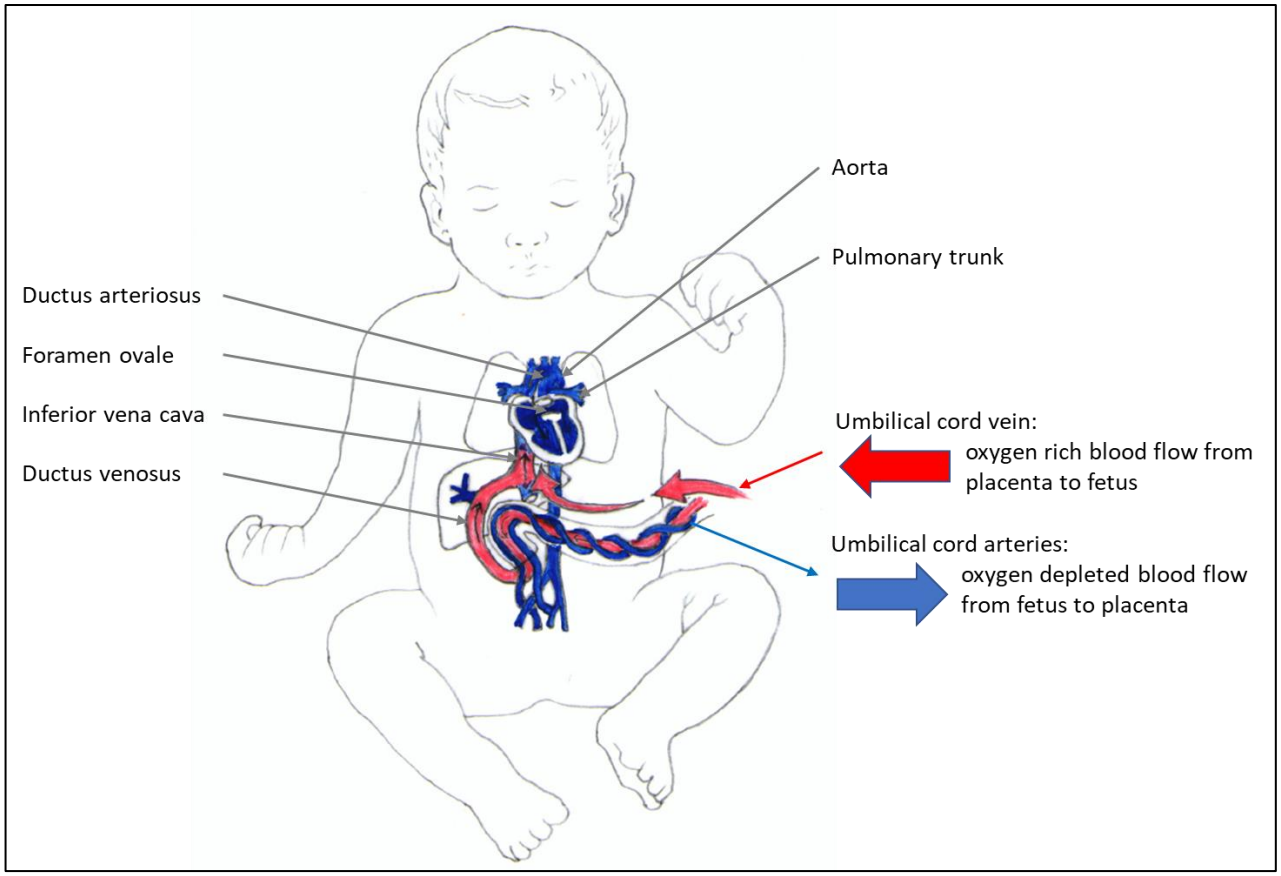

Figure 1.3: Diagram of fetal and umbilical cord blood circulation. The umbilical cord vein carries blood rich in oxygen (80% saturation) and other molecules from the placenta to the fetus. While some blood flows to the fetal liver, the majority is shunted via the ductus venosus to reach the inferior vena cava directly and then flows into the right atrium of the fetal heart. Most of this oxygen-rich blood flows through the foramen ovale, an opening between the two atria which usually closes after birth, into the left atrium. From here the blood flows into the fetal left ventricle before being pumped out into the ascending aorta and from there to the vessels supplying the heart, brain, and other parts of the body. Returning oxygen-depleted blood (approximately 58% saturation)returns to heart via the superior vena cava, via the right atrium into the right ventricle and then into the pulmonary trunk. As the pulmonary vessels have high resistance in the fetus, the majority of the blood entering the pulmonary trunk flows through the fetal ductus arteriosus into the descending aorta, and this blood then returns to the placenta via the umbilical cord arteries[63]. Red colour = oxygen-rich blood; blue colour = oxygen-depleted blood.

1.4 Control of parturition

1.4.1 Control of parturition in animals

One of the challenges of studying human pregnancy is that, in contrast to many other conditions that can be studied in animal models, some aspects of reproduction are species-specific, and while many mammals share similar DNA sequences for many systems, there is far less cross-over for reproductive functions[3, 7]. Such differences are described below and include differences in placental structure and function[62], the role of the corpus luteum[6, 73], functional or measurable progesterone withdrawal[32, 35], and differences in duration and variation in gestational length[24, 25]. Because this has taken some time to realise, and because invasive experimentation on human fetuses is ethically difficult, during the last century animal studies were conducted to better understand parturition[33]. While findings from animal models cannot be directly translated to elucidate mechanisms of labour in humans, these studies can be used to generate hypotheses on the potential function of different hormones involved in the human reproductive process and will be discussed here.

1.4.2 Rabbit, goat, and rodent models

Experiments in rabbits, goats and rodents have shown that the trigger for labour in these mammals is progesterone withdrawal following luteolysis of the corpus luteum, which otherwise maintains the pregnancy through progesterone

synthesis. Luteolysis is triggered by activation of prostanoid FP receptors in the corpus luteum by prostaglandin F2α which is released by the endometrium, causing a fall in maternal progesterone which rapidly leads to uterine activation and labour[73]. This mechanism is not directly relevant to human pregnancy and labour as the corpus luteum is not required for ongoing pregnancy in humans after the first trimester when the placenta takes over progesterone production, and therefore luteolysis is not the trigger for labour in humans[6].

1.4.3 Sheep models

Three important experiments in sheep were conducted in New Zealand by Liggins *et al.* (1968 and 1969)[74, 75] which highlighted the potential role of the fetus in the trigger for human labour. Although later work has shown that these findings are not directly related to humans, as will be discussed below, these experiments are worth discussion as they may give some clues as to potential interactions in humans. The first experiment involved forming lesions in the pituitary gland of lamb fetuses using electrocoagulation. In the first part of this experiment, 11 pregnancies had a pituitary lesion of a single fetus pregnancy and two had a pituitary lesion in both twins of two twin pregnancies. Of these, nine of the pregnancies were abnormally prolonged, and the authors subsequently noted that these fetuses had more than 70% of the pituitary gland destroyed, whereas the four which delivered at a normal gestation had 60% or

less destroyed. A second group included one singleton fetus with the pituitary stalk dissected and one singleton fetus with hypothalamic destruction, and both pregnancies continued beyond term and had to be delivered via caesarean section without signs of approaching labour. In a third group, three twin pregnancies had one unoperated twin and one given a pituitary lesion, and one triplet pregnancy had on unoperated fetus and two with pituitary lesions. All of these pregnancies delivered at term. And in the final group, four ewes had sham operations with no pituitary or hypothalamus destruction, and all four delivered at term. The authors concluded that the fetus was essential for timely parturition in sheep, and that the mechanism for this involved the pituitary gland and probably the hypothalamus[76]. The second study involved continuous administration of adrenocorticotropic hormone (ACTH) directly into the peritoneal cavity of singleton fetal lambs, and the finding that this resulted in premature spontaneous parturition within four days of this administration. Similar results were observed with infusion of cortisol, however infusion of oestradiol-17beta did not cause early parturition. Moreover, neither the infusion of ACTH nor cortisol directly into pregnant ewes resulted in preterm labour. The authors concluded that the pituitary-adrenal system of the fetus was vital for parturition in the ewe[74]. The third study again involved infusion of different substances directly into the peritoneal cavities of lamb fetuses. This

included infusing six single fetuses with normal saline for 14 days, following which pregnancy continued normally; infusing 11 singleton fetuses with different concentrations of dexamethasone (a glucocorticoid), and the nine fetuses with the higher concentration of dexamethasone delivered prematurely; two ewes were infused with dexamethasone while the fetuses were infused with normal saline, and neither of these fetuses delivered early; and corticosterone (mineralocorticoid) was dissolved in normal saline and infused continuously into two further fetuses, again neither of which delivered early; and finally, one fetus had deoxycorticosterone (mineralocorticoid) infused and did not deliver early. The authors concluded that glucocorticoids (dexamethasone) caused premature delivery when infused directly into the lamb fetuses, but premature labour did not occur with direct infusion of the mineralocorticoids into the fetus, nor with infusion of glucocorticoid (dexamethasone) into the ewe rather than the fetus. The researchers also investigated the effect of administering progesterone into the fetus or ewe at the same time as infusing dexamethasone into the fetus, and found that premature labour was not prevented, which indicated that withdrawal of the inhibitory effects of progesterone on the myometrium occurs in these situations despite elevated progesterone in the plasma[75].

Another group conducted an experiment investigating the length of gestation in two breeds of sheep with different mean gestational lengths. The researchers conducted inter-breed and intra-breed and mixed-breed embryo transfers and showed that the breed of the lamb fetus had the greatest impact on gestational length rather than the breed of ewe. Finnish Landrace sheep have a mean gestation of 144.9 days, and Targhee sheep have a mean gestation of 150.4 days, and Finnish Landrace embryos transferred to Targhee sheep had shorter gestational lengths, while Targhee embryos transferred to Finnish Landrace sheep had longer gestations. Mixed breed pregnancies (one of each breed of embryo) had a gestational length between the two. The authors suggested this shows an interaction between fetuses in the timing of parturition[77].

Further experiments in sheep have shown that activation of the fetal pituitary-adrenal axis results in increased fetal cortisol and induction of $P450_{C17}$ enzymes in the placenta, which in turn promote conversion of C21 steroids to C18 steroids, resulting in a decrease in maternal progesterone levels, a rise in maternal oestradiol levels and increased intrauterine prostaglandin production that effects softening of the cervix and myometrial contractility[6, 33, 74, 75]. As with the other mammals described above, the trigger for labour in sheep involves a measurable change in the balance between oestrogen and progesterone, with

increased oestrogen and decreased progesterone resulting in myometrial contractions and labour.

While these are very elegant experiments, they clearly could not be repeated in humans, and therefore to test whether these effects are also present in human pregnancies requires other methodologies. Given increased pituitary and adrenal function in the sheep results in premature labour, and absent or impaired function results in prolonged labour, if this were the same in humans then it would be expected that human fetuses without a pituitary gland would always deliver post-term. However, a study of 147 singleton human pregnancies with anencephaly (where the fetal brain does not develop and therefore the fetus does not have a pituitary-adrenal axis) showed this was not the case. While those fetuses with anencephaly which developed polyhydramnios tended to deliver earlier, likely secondary to increased stretch on the myometrium, those without polyhydramnios (n=29) were found to spontaneously labour at a similar mean gestation to control singleton pregnancies where the fetus had a normally developed brain (Figure 1.2)[78]. However, the authors did observe increased variability in gestational age at delivery in the pregnancies with fetal anencephaly and suggested that increased activity of the fetal pituitary-adrenal axis towards the end of pregnancy in humans may influence rather than trigger the precise timing of spontaneous labour[78]. While the numbers are relatively

low in this study, which is not surprising given anencephaly is rare and that when diagnosed antenatally these pregnancies may be terminated, they do show that an absent pituitary and hypothalamus in human fetuses do not always prolong gestational length. Therefore it is unlikely that maturity of the fetal pituitary-adrenal axis is essential for the timing of parturition in humans; however, it may be that signals from the fetus are required for precise timing of parturition[6, 78] and these may be involved in the trigger for spontaneous human labour.

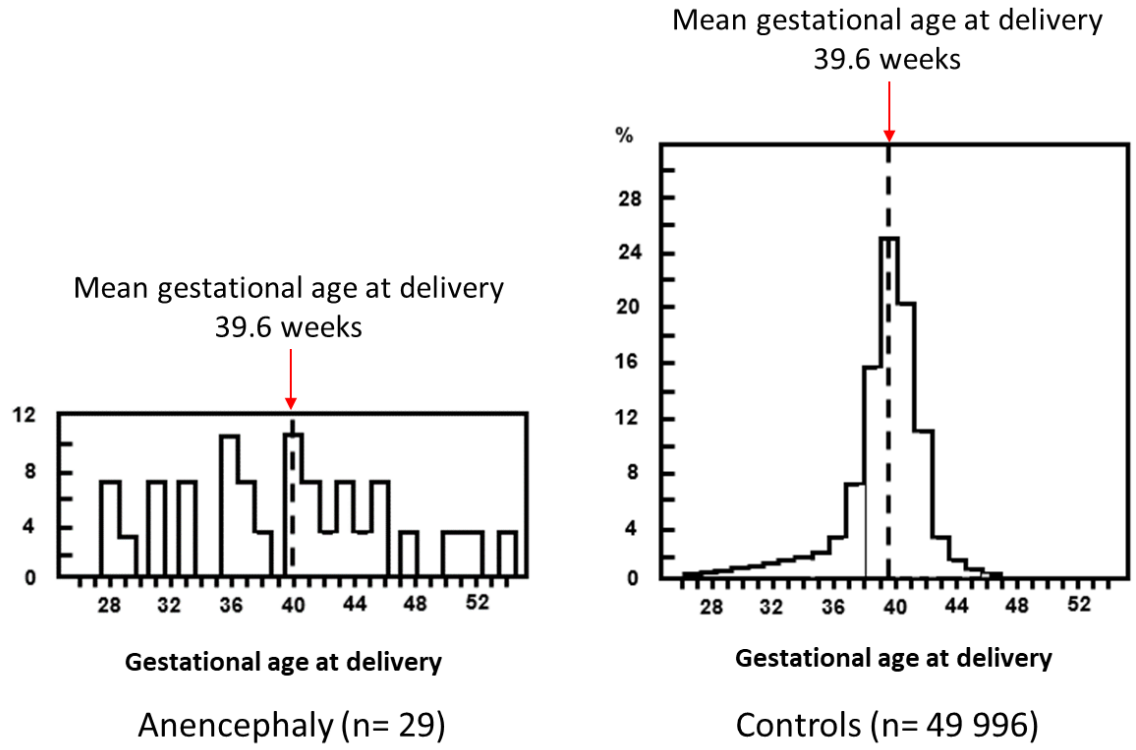

Figure 1.4: Comparison of mean gestational age at delivery between human fetuses with anencephaly and no polyhydramnios (n=29) and human fetuses with normally developed brain (n=49 996). Mean gestational age at delivery for both groups 39.6 weeks. Adapted from Honnebier & Swaab (1973)[78].

While many of the measurable endocrine changes associated with parturition in these smaller mammals are not found in measurement of maternal blood in humans, it remains possible that such changes may occur more locally, for example within the intrauterine tissues or the fetus. Such paracrine or autocrine systemic changes may not be measureable in the maternal blood, but could have important effects on the local tissues that could trigger labour in humans[8]. This thesis aims to investigate whether such local changes are.

1.4.4 Non-human primate models

Humans are closer in evolutionary terms to non-human primates than to mice and sheep. Chimpanzees and humans are both within the primate family Hominidae, and the chimpanzee genome shares 98.7% of its sequences with humans. As with humans, non-human primates do not have a measurable progesterone withdrawal at the time of parturition, and have similar myometrial electromyographic activity[8, 79]. However, despite this, there remain some key differences, for example, the average gestational length in the chimpanzee is 227 days compared with 280 days in humans (although as there are differences in the way in which this is measured between humans (first day of last period) and chimpanzees (last day of maximal sex skin tumescence), the actual difference has been suggested to be around 40 days)[80]. While studies in non-human primates have enabled researchers to better understand human

parturition, the ethical and logistical demands of non-primate experimentation mean that only certain groups have the funding and space to be able to conduct such research[81, 82]. Some findings from non-human primate studies related to the aims of this thesis will now be discussed.

One study investigated gestational length and preterm labour within a chimpanzee community at the private Primate Foundation of Arizona, observing this community for 30 years. Of 97 observed pregnancies, the researchers could confidently determine gestational length for 93 pregnancies by retrospectively reviewing written records. All but one of the chimpanzees born before 208 days gestation was either stillborn or died within a couple of days, and the one survivor was reported to have a possible incorrectly calculated gestation as conception could have occurred one month earlier[80]. Preterm labour in chimpanzees has been defined as births which occur at gestations 2SD or less below the average gestational length, and this was found to occur in 16% of pregnancies in this study population, which is similar to the rate in humans[80, 83].

As with humans, it is not clear why non-primates go into preterm labour[83], and intrauterine infection has been investigated as a potential major cause among non-primates[81, 82]. For example, intra-amniotic infusion of Group B streptococci in rhesus monkeys results in preterm labour following an increase in uterine contractility preceded by an increase in tumour necrosis factor α (TNFα) and

interleukin (IL)-1β, TNF-α, IL-6, prostaglandin E2 and prostaglandin F2α[84]. One group sought to determine whether prostaglandin synthesis is blocked by indomethacin and thereby prevents stimulation of uterine contractions by IL-1β production. The group performed this experiment in 11 pregnant rhesus monkeys. Oral indomethacin was administered for five days to six of the rhesus monkeys. Human recombinant IL-1β was also infused directly into the amniotic cavity on day three of the experiment, and the intrauterine pressures measured following this. Five days after the final indomethacin dose, the same study protocol was repeated but without administration of indomethacin. A further five monkeys received only IL-1β (n=5). In all, the amniotic fluid was periodically sampled and prostaglandin E_2 and $F_{2\alpha,}$ IL-1β, IL-6, IL-8, TNFα, and IL-1 receptor antagonist (all inflammatory cytokines) were measured. The researchers determined that indomethacin prevented the increase in uterine activity that was seen when IL-1β was infused alone. There were no differences in the levels of the inflammatory cytokines between the indomethacin and non-indomethacin groups, however there was an increase in the two prostaglandins in the groups not administered indomethacin. This suggested that, in rhesus monkeys at least, indomethacin blocks the development of uterine activity in response to IL-1β, and that this seems to be through decreased prostaglandin production [81]

Another study by the same group investigated whether the immunomodulators dexamethasone and IL-10 could be used to prevent the uterine contractions stimulated by infusion with IL-1β[82]. Thirteen rhesus monkeys at 135 days gestation (where term is 167 days) were administered either: maternal dexamethasone (1mg/maternal Kg) every six hours for three days, along with intraamniotic infusion of 10μg of human recombinant IL-1β on first day via a catheter over two hours (n=4). A second group (n=5) received simultaneous maternal intravenous human recombinant IL-10 (25μg/kg) and 200μg human recombinant IL-10 into the amniotic fluid every eight hours for three days. Thirty minutes after the first infusion of IL-10, human recombinant IL-1β was infused into the amniotic fluid at a rate of 5 μg per hour over two hours. A third group (n=5) received an intra-amniotic infusion of IL-1β only. The group found that IL-10 and dexamethasone infusion reduced the uterine activity stimulated by IL-1β infusion, as well as concentrations of prostaglandins found in the amniotic fluid. Maternal and fetal cortisol, estradiol and dehydroepiandrosterone sulphate (DHEA-S) were also reduced in the dexamethasone group, and TNF-α was reduced in the IL-10 group. The researchers concluded that dexamethasone and IL-10 may be useful in treatment of preterm labour where it is associated with infection or inflammation[82]. However, in the majority of spontaneous preterm labour in humans no infective cause is found, and where there may be infection

it could be dangerous to prolong a pregnancy where there is infection, both to the survival of the mother and the fetus.

Studies involving non-primates have also informed generation of hypotheses regarding evolutionary influences on gestational length, such as the suggestion that preterm labour has been evolutionarily preserved as a mechanism to protect the mother or fetus from the potential dangers of poor intrauterine or maternal conditions[80]. Such study of the evolution of parturition, with a potential survival benefit for earlier parturition, may shed light onto why some human pregnancies undergo premature parturition[8]. One theory is that there is a metabolic "crossover" effect towards the end of gestation, where the placental provision of nutrients to the fetus becomes inadequate and fails to match the growth of the fetus. The fetus then becomes stressed, and this metabolic stress increases cortisol levels and arachidonic acid production in the placenta, which eventually leads to prostaglandin production and labour. It has been suggested that this crossover period may be on average reached at 240 days gestation in humans, and 228 days in chimpanzees[80, 85].

The apparent similarities between humans and non-human primates do imply that they better represent human parturition than mice and sheep. However, as mentioned above, there are ethical dilemmas as well as practical considerations for such research, requiring large housing areas and professional care for the

animals, with the resulting high costs and ethical concerns often prohibitive for funding[8]. The studies in this thesis utilise techniques to investigate metabolic changes of pregnancy directly in humans, removing the quandary related to experimenting on non-human primates.

1.4.5 Progesterone and human pregnancy

Until approximately seven weeks' gestation, human pregnancy is dependent upon progesterone produced by the corpus luteum in response to hCG stimulation. After this point the placenta takes over as primary producer of progesterone, termed the "luteo-placental shift"[35,72]. Progesterone is mainly derived from maternal cholesterol[71, 72], largely derived from low-density lipoproteins (LDL)[71], the conversion of which occurs in the synctiotrophoblast mitochondria. The enzyme cholesterol side-chain cleavage cytochrome P450 (CYP450scc) is present in the inner membrane of these mitochondria and converts maternal cholesterol to pregnenolone, and this is the rate-determining step for this process. Pregnenolone is then metabolised into progesterone by the enzyme 3beta-hydroxysteroid dehydrogenase (3β-HSD)[71, 72]. The human placenta cannot convert progesterone to 17α-hydroxyprogesterone and P450c17 is not expressed in the human placenta, therefore the placenta cannot convert progesterone to C19 androgens, and instead placental oestrogens are

synthesised from precursors of C19 androgens from the maternal and fetal adrenals, as described below[71].

As with other mammals, progesterone is essential for human pregnancy continuation. Administration of anti-progestins such as the progesterone receptor antagonist mifepristone (RU486) are used to induce abortion of early or mid-trimester pregnancies, medical management of delayed miscarriage and, previously, promotion of cervical effacement for induction of labour[32, 35, 86]. In humans there is no measurable fall in progesterone at the time of parturition, and plasma progesterone and oestrogen levels remain high until the end of third stage when the placenta is delivered[72, 87]. Given that the other mammals described above have a measurable progesterone withdrawal, the potential that human labour may be associated with a functional withdrawal of progesterone is a widely held theory, and researchers have investigated potential forms that this may take, some of which will be explored below[32, 35].

1.4.6 Feto-placental unit

As described above, the findings in sheep indicate a pivotal role for the fetus in the timing of labour in sheep, and this has been investigated in humans. Egon Diczfalusy (1920-2016) was one of the first to describe the feto-placental unit in humans. He observed that the human placenta is deficient in enzymes required for production of essential pregnancy steroids, and that these enzymes are

instead present in the fetal adrenal glands and liver. The feto-placental unit represents interactions between the fetus and the placenta via which precursors to steroids are shuttled between the two for generation of essential steroids. Specifically, the fetal adrenal provides androgen precursors such as DHEA-S, used by the placenta for biosynthesis of oestrogens[88-90] (Figure 1.3). Much of the investigation into the activity of the human feto-placental unit has been performed *in vitro*, particularly isolated primary human cytotrophoblasts or human placental explants, or immortilised trophoblast cell lines, and therefore the exact effects *in vivo* are still unclear[72, 91, 92], although there have been further advances in recent years with new techniques in gene technology and the omics.

During fetal development, the human fetal adrenal develops three distinct zones: the fetal zone (FZ) and an outer definitive zone (DZ), with a transitional zone (TZ) that links the two. During pregnancy, most of the activity of the fetal adrenal appears to originate in the FZ, which is unique to humans and higher primates, and not present in sheep and rodents[93]. After birth the FZ rapidly disappears and androgen secretion decreases, and it is likely that this is via apoptosis[94]. Because this happens immediately after birth, it is thought that the continuation of the FZ during pregnancy is dependent upon placental factors[93]. One study showed that cortisol concentrations in umbilical cord blood sampled from pregnancies delivered via elective caesarean section at various gestations

were found to decrease in the mid-trimester, followed by a steep rise in the third trimester[66].

In pregnancy, the main oestrogen is oestriol rather than the ovarian-cycle steroid 17-oestradiol, and oestriol makes up over 90% of urinary oestrogen during pregnancy[71,91]. Importantly, synthesis of oestriol and other oestrogens occurs in the placenta, however the placenta does not produce oestrogens directly from acetate or cholesterol as it does not have the cytochrome P450 enzyme CYP17 that is required[91]. Placental production of oestradiol and oestrone (see Figure 1.5) requires dehydroepiandrosterone (DHEA) as a precursor, and this is derived from both the maternal adrenal glands[95, 96] and the fetal adrenal cortex. Sulphatase in the placenta rapidly converts dehydroepiandrosterone-sulphate (DHEA-S) to DHEA, which is then converted by 3βHSD to androstenedione. The aromatase enzyme is highly expressed by the placenta, and this converts androstenedione to oestrone, following which the 17β hydroxysteroid dehydrogenase (17βHSD) enzymes then interconvert oestrone and oestradiol[61, 71, 72].

However, as stated, oestriol is the main oestrogen of human pregnancy, and only a small proportion of this is produced from conversion of oestrone and oestradiol by the placenta. The majority results from DHEA-S produced by the fetal adrenals undergoing 16α-hydroxylation to produce 16α-hydroxy-DHEA-S,

taking place primarily in the fetal liver as well as the fetal adrenals. 16α-hydroxy-DHEA-S is then transported to the placenta where a placental sulphatase enzyme converts 16α-hydroxy-DHEA-S to 16α-hydroxy-DHEA, which is subsequently aromatised to produce oestriol. The transportation of 16-hydroxy DHEA as a sulphate conjugate is believed to protect the fetus from the biological effects of the steroid, as the sulphinated form is water soluble and inactive. Placental oestriol synthesis via this pathway is therefore dependent upon the placental sulphatase which is abundant in the placenta, and the high levels of oestriol during human pregnancy are dependent upon the activity of the fetal pituitary-adrenal axis[71, 72]. Figure 1.5 summarises these pathways pictorially.

Given this level of interaction between the fetal/placental/maternal system, and given the findings of the importance of the pituitary-adrenal axis in the timing of parturition in sheep[74, 75], it would not be surprising if part of the trigger for human labour originated in the fetus. However, given the findings of no change in the median gestational age at delivery in pregnancies affected with anencephaly, such a signal may not be essential for eventual delivery but required for appropriate timing of labour, and if present too early could result in PTB. It is also possible that the fetal signal is not dependent upon the pituitary gland, and instead reliant upon another signal from the fetus. There are still many unknowns which the studies included in this thesis aim to investigate.

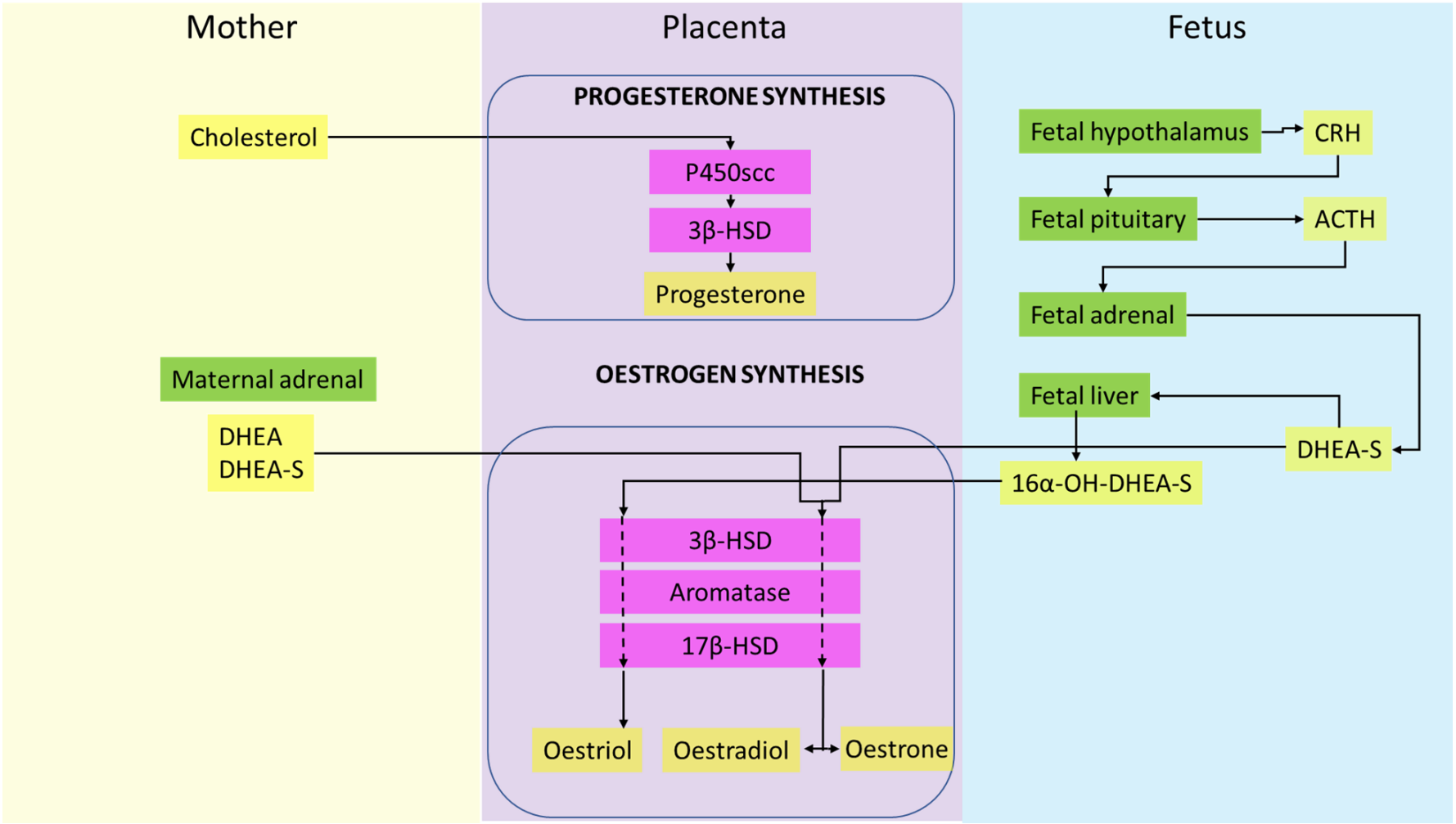

Figure 1.5: Diagram of maternal-feto-placental unit with biosynthesis of progesterone and oestrogens DHEA=dehydroepiandrosterone; DHEA-S=dehydroepiandrosterone-sulphate; 3β-HSD=3β-hydroxysteroid dehydrogenase; 17β-HSD=17β-hydroxysteroid dehydrogenase; CRH=corticotrophin-releasing hormone; ACTH=adrenocorticotropic hormone; 16α-OH-DHEA-S=16α-hydroxy-dehydroepiandrosterone-sulphate[71, 72, 95, 96]. Colour scheme: pink=enzyme; yellow=molecule; green=organ.

1.4.7 Uterine oestrogen and progesterone receptors

A potential mechanism for the functional withdrawal of progesterone at the time of human labour is a change in the functional availability of uterine progesterone and oestrogen receptors[35, 97]. Progesterone receptors (PR) belong to the nuclear receptor superfamily which act as transcription factors when activated by binding of progesterone[35]. Upon activation, PRs dimerise and are transported to the cell nuclei where they bind to specific promotor regions of progesterone-responsive genes[35]. The two predominant isoforms of the nuclear PRs are Progesterone Receptor type A (PRA) and Progesterone Receptor type B (PRB)[35], both of which are formed from the same gene along with a further isoform, Progesterone Receptor C (PRC). PRA and PRC are both structurally N-terminally truncated PRB[35, 98, 99]. PRA, PRB and PRC isoforms have been shown to be expressed in human myometrium. Experimental studies indicate that PRA has less activity than PRB, and PRA additionally acts to repress the transcriptional activity of PRB through transrepression, thereby blocking progesterone activation via PRB[100]. PRC is predominantly present in the cytosol and does not bind to DNA, although it can bind progesterone. It is suggested that PRC may also reduce progesterone-PR function by sequestering progesterone away from PRB. In addition, PRC has been shown to bind to PRB thereby further reducing the ability of PRB to bind to promoter regions[98].

In animal studies, both total PR-knockout and PRA-knockout mice are infertile, whereas PRB-knockout mice are fertile and have normal pregnancies, indicating that PRA is essential for and can independently affect all the reproductive actions of progesterone in the mouse[97]. These studies indicate that the functional activity of progesterone within the myometrium depends not only on the level of progesterone and its receptors, but also on the ratio of PRAs, PRBs and potentially PRCs[32, 35, 98].

Oestrogen receptors in humans have two main subtypes, ERα and ERβ. These are produced from different genes and differ in ligand binding affinity and in how they are distributed among different tissues[97]. ERα appears to be the receptor through which the uterotonic actions of oestrogen are driven. Recently the spliced ERα receptor variant ERΔ7 has been shown to dominantly suppress the uterotonic actions of oestrogen, and this receptor withdraws at term in an oestrogen-dependent manner. This mechanism is suggested to be involved in the loss of myometrial quiescence at term[101], and that changing concentrations of oestriol and oestradiol may be responsible for the change in oestrogen receptors[102].

Oestriol is an inhibitor of the action of oestradiol at low concentrations but becomes an agonist when the ratio of oestriol to oestradiol exceeds 10 to 1. One group investigated changes in the ratios of progesterone, oestriol and oestradiol

in spontaneously labouring women at 26 weeks' gestation and in the last month of pregnancy. Oestriol was found to increase, resulting in an increase in the oestriol/oestradiol ratio, a decrease in the progesterone/oestriol ratio, and an unchanged progesterone/oestradiol ratio. The authors suggested that this created an oestrogenic environment for the onset of labour[103].

Investigation of ER and PR receptor immunoactivity and binding has been conducted in human labouring and non-labouring myometrium, and ER activity was found to be consistently low, with no changes according to labour-status; conversely, PR activity was consistently higher than that of ER and its activity increased with labour[97, 104]. However, these experiments did not distinguish between the PRA and PRB isoforms. Further experiments showed PRA levels within the myometrium specifically increased with labour onset with a corresponding decrease in progesterone responsiveness and an overexpression of PRA in cell culture experiments using human myometrial cells[100, 104]. Western blot analysis of human myometrium indicated that PRA protein is not present before, but is present after, the onset of labour[100]. It is suggested that the increase in PRA at the time of labour could be the mechanism of the functional withdrawal of progesterone in humans, and thereby potentially involved in the trigger for human labour[97, 100].

Interestingly, Mesiano *et al.* (2002)[97] showed that PRA, PRB and ERα mRNA levels were higher in myometrium taken from 12 women who were in labour when compared with myometrium taken from 12 women not in labour, and while PRA levels were higher than PRB in both the labouring and non-labouring samples, the ratio of PRA/PRB mRNA increased 2-3-fold in the labouring group (see Figure 1.4)[97]. This group went on to investigate whether the responsiveness of the myometrium to oestrogen was associated with a change in the ERα gene expression between labouring and non-labouring myometrium through comparison of levels of ERα mRNA, Oxytocin receptor (OTr) mRNA, and COX-2 mRNA. Both *OTr* and *COX-2* are contraction-associated genes that are known to be activated within the myometrium during labour, with *OTr* upregulated by oestrogen. *COX-2* encodes for the form of cyclooxygenase that is inducible and is thought to cause an increase in prostaglandin production by gestational tissues at the time of parturition. *OTr* encodes the oxytocin receptor, which is essential to enhance the response of myometrium to oxytocin [32]. OTr mRNA was shown to increase 6-fold during labour, and COX-2 mRNA 115-fold. Both correlated positively with ERα mRNA levels in myometrium taken from women not in labour (elective caesarean section); and this correlation was not shown in myometrium taken from women in labour. The authors suggest this could be because at this stage one or all these genes are already fully expressed. ERβ

mRNA expression was found to be low in both labouring and non-labouring myometrium, and not affected by labour[32]. While these findings are in concordance with the previously described work which found increased protein abundance of PRA with labour, the authors emphasise that the findings do not necessarily translate to equivalent changes in receptor function as proteins can undergo a variety of modifications following translation such as phosphorylation[97].

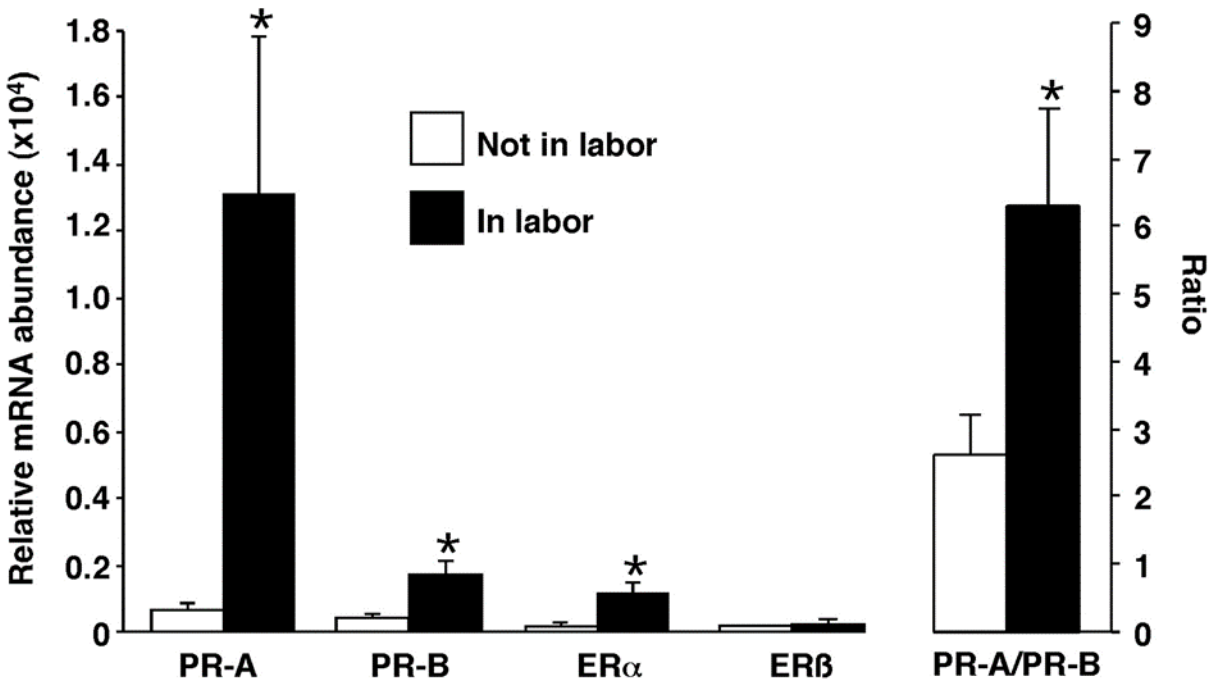

Figure 1.6: Relative abundance of mRNA encoding PRA, PRB, ERα, ERβ, and the PRA/PRB ratio in labouring and non-labouring myometrium. * = p≤0.05 (Figure extracted from Mesiano et al. (2002)[97])

A potential aetiology for the mechanism of a functional withdrawal of progesterone at term is that an increased expression of PRA results in decreased myometrial responsiveness to progesterone and an increase in ERα expression. This crosses a threshold where decreased progesterone responsiveness and

increased oestrogen responsiveness results in an increase in the production of contractile proteins leading to increased myometrial contractility and labour [32, 35]. However, other researchers argue against this. For example, Condon *et al.* (2006)[98] investigated myometrial protein levels rather than mRNA, and found that while PRB protein was present in the nuclei of lower uterine segment and fundal myometrium in both labour and non-labour, levels only increased in the fundus with labour, not in the lower segment[98]. Further, that PRA protein was found neither in the nuclei nor cytoplasm of myometrium taken from labouring and non-labouring women. While this contrasts with findings of previous studies, the authors suggest these previous studies used experimental techniques which would have incorrectly identified PRC as PRA[98]. This study counters the hypothesis that functional progesterone withdrawal is secondary to PR isoform expression, as the inhibitory PRA protein was not found in any of the myometrial samples, although PRC could play a role[35, 98]. It is noted that in all of these studies there are inconsistencies and that this is in part due to the variability of performing experiments on human cell lines and tissues *in vitro*[35].

Of note, if functional progesterone withdrawal does not occur at the myometrium, then it is possible that it occurs within the decidua or fetal tissues including the amnion and chorion[35]. For example, one group investigated the expression and possible localisation of PRA and PRB within the amnion, chorion

and decidua sampled at the time of caesarean section at term from women who had laboured (n=7) and those who had not (n=7). Nuclear expression of the nuclear progesterone receptor mRNA was quantified using reverse transcriptase polymerase chain reaction, and localisation determined via immunohistochemistry. It was determined that nuclear PR mRNA was highest in the chorio-decidua, but that this did not change with labour, and that these mRNA were very low in the amnion samples. Further, immunohistochemistry indicated that the PRs were only present in the decidual cell nuclei, which suggests that the decidua is a more likely target of progesterone actions in pregnancy[105].

1.4.8 Corticotrophin-releasing hormone and preterm birth

Maternal corticotrophin-releasing hormone (CRH) has been shown to be associated with the timing of parturition, with increased levels in the maternal plasma occurring as pregnancy progresses. CRH is found to be high in women who have a preterm delivery in some situations, but notably not if the preterm delivery is associated with infection. In addition, towards the end of pregnancy, levels of the CRH binding protein have been shown to reduce, thereby increasing the bioactivity of circulating CRH. CRH production is stimulated by glucocorticoids by the placenta; and CRH results in corticotrophin production by the pituitary then cortisol from the adrenal cortex. Progesterone, oestrogen and

nitric oxide inhibit CRH production by the placenta. It is suggested that the increased levels of placental CRH during pregnancy results in increased production by the maternal adrenal glands of cortisol and DHEA-S, the latter of which is a substrate for the synthesis of oestrogen[7].

1.4.9 Inflammatory pathways and parturition

Another potential trigger for parturition involves inflammatory pathways[106], with a change in the balance between inflammatory and non-inflammatory responses[50]. Previous studies have shown that the transcriptome of labour is associated with inflammatory pathways, including those for cytokines[32]. One group used computer programming techniques to integrate three datasets of gene expression in labouring compared with non-labouring myometrium. The researchers showed that labour at term was associated with differential expression of cytokines and of genes regulating pathways related to cell differentiation, proliferation and apoptosis[32]. This was in contrast to the pathways of muscle relaxation and cyclic AMP signalling that they found were enriched in non-labour (elective caesarean section)[32, 56, 107].

However, it has been questioned whether inflammation is a cause or a result of labour. For example, one group performed myometrial studies to test the activation of two pro-inflammatory pathways, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway (described below) and the

Activator Protein 1 (AP-1) pathway, at different points of pregnancy. They found that the NF-kB declined towards term but was increased in early and established labour. Further, that while flow cytometry indicated that neutrophil numbers increased with labour, immunohistochemistry showed that these were located within the blood vessels rather than within the myometrium itself[108]. This suggests that inflammation may be a result of labour rather than the trigger, and may also be seen in work which compares not in labour myometrium sampled from caesarean section at term with in labour myometrium sampled at caesarean section, as if frozen at the time of sampling[3], the inflammatory effects of the labour immediately preceding the caesarean are likely to bias the results. This is supported by a study which investigated leucocyte infiltration in myometrial and decidual samples from 648 women at the time of caesarean section, including in women with signs or symptoms of infection. Two-hundred and fifty-three women had elective caesarean section with no preceding labour or rupture of membranes; 48 had a caesarean section in labour with in-tact membranes; and 347 had a caesarean section following rupture of membranes. Eighty-nine of the pregnancies were delivered preterm (less than 37 weeks' gestation). Overall, inflammation was detected in 240 (42.9%) of 559 decidual samples, and 155 (24.0%) of 646 of the myometrial samples. The three main factors associated with an increased finding of a moderate to marked infiltration

of leucocytes were being in labour, chorioamnionitis and endometritis. Fifty-three women were diagnosed with chorioamnionitis and 58 with endometritis. Chorioamnionitis and endometritis were both associated with increased presence of myometrial infilatration of leucocytes for both preterm (odds ratio 5.1 ($p = 0.02$) and 12.8 ($p = 0.007$), respectively) and term (odds ratio 3.8 ($p = 0.0001$) and 2.6 ($p = 0.002$), respectively). The researchers found that in both the preterm and the term group, for those without clinical signs of infection, the findings of a moderate infiltrate of leucocytes was associated with the onset of labour. In women who were delivered at term, the odds ratio for having a moderate to marked myometrial inflammation was 3.2 ($p = 0.05$) in women who were 3-5 cm dilated, and 3.8 ($p = 0.02$) in women who were 6-10 cm dilated. In women who delivered at term the odds ratio for myometrial inflammatory infiltrate was 7.8 ($p = 0.002$). There was no association found between myometrial infiltration and the prediction of development of post-operative endometritis[109].

Regarding the myometrial work in this thesis where myometrium is compared between those with elective caesarean section and emergency (in labour) caesarean section, the potential of inflammatory reactions from the labour were attempted to be removed by allowing the myometrium to equilibrate in buffer prior to experimentation.

1.4.9.1 Nuclear Factor kappa-light-chain-enhancer of activated B cells

The NF-kB signalling pathway is rooted in activation of NF-kB by pro-inflammatory cytokines including TNFα and IL-1, resulting in expression of genes that code for inflammatory factors such as chemokines, cytokines, and adhesion molecules[110]. NF-kB has been associated with inflammatory conditions such as rheumatoid arthritis, asthma and multiple sclerosis[110]. One group observed that NF-kB was activated in samples of fundal myometrium sampled from women in labour but was not activated in fundal myometrium sampled from women not in labour. In myometrium taken from the lower segment, NF-kB was neither activated in labour nor when not in labour. The authors also determined that activation of the NF-kB pathway via stimulation with IL-1β in hTERT myometrial cells (which do not express PRA or PRC) in culture resulted in an increase in PRB expression[98]. The authors suggest that their findings indicate that changes in the expression of different PR isoforms with labour may be due to stimulation of inflammatory response pathways within the uterus.

1.4.9.2 Amniotic fluid and surfactant

Increased presence of inflammatory factors such as interleukins within the amniotic fluid and infiltration of the myometrium by macrophages and neutrophils are associated with labour in humans[106]. Interestingly, such observations appear to be consistent in both women who deliver preterm and

women who deliver at term. While it is feasible that a proportion of PTB results from infection leading to an inflammatory response, the majority of PTB and term deliveries are not known to be associated with infection and other aetiologies for activation of these inflammatory pathways have been sought[106].

An early suggestion was that maturation of the fetal lung and increased production of surfactant would result in surfactant being present in the amniotic fluid as term approached, and that this could trigger a type of inflammatory response[106, 111]. One group found that fetal pulmonary surfactant isolated from amniotic fluid stimulated the release of prostaglandin E from incubated discs of human amnion; and that arachidonic acid made up 2% of the surfactant lecithin fatty acids[111]. The researchers suggested that phospholipids from surfactant that is secreted into the amniotic fluid from the matured fetal lung are a source of the arachidonic precursor for prostaglandin synthesis by the amnion[106, 111]. However, if this were the case it would not necessarily explain all preterm labour as the fetal lungs are not matured until later in the third trimester[112].

1.4.9.3 Macrophages

Tissue macrophages and other inflammatory cells within the decidua may also play a role in such an inflammatory pathway for the initiation of labour. The decidua is in contact with both the myometrium and the amnion/chorion, and it has been observed that during labour uterine decidual macrophages produce

increased quantities of prostaglandin F2α[6, 113]. It has also been shown that tissue macrophages can produce pro-inflammatory cytokines such as TNF-α and IL-1, and therefore some argue that this could be the mechanism responsible for PTB that is associated with infection, as presented pictorially in Figure 1.5[6].

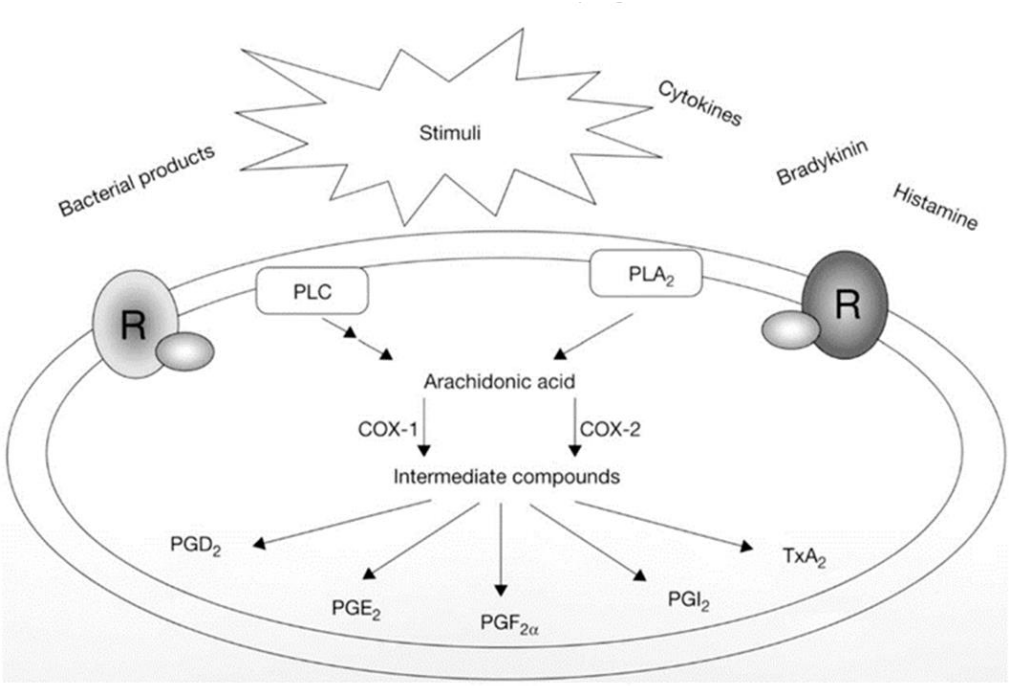

Figure 1.7: Illustration showing proposed actions of macrophages at the fetal membranes . Macrophages synthesise prostaglandin and thromboxane and proposed to be involved in onset of preterm labour associated with infection. The release of prostaglandins and inflammatory markers likely cause contractions in neighbouring myometrium. PG = prostaglandin; Tx = thromboxane; PLC, PLA2=phospholipases; R=receptors. Adapted from López Bernal (2007)[2].

1.4.9.4 Lipid profile and preterm birth

Epidemiological studies indicate that women who experience PTB are at increased risk for developing cardiovascular disease in later life[114-116]. It has also been shown that risk factors for PTB are similar to those for cardiovascular disease, such as smoking, dyslipidaemia and previous PTB[115]. One group investigated placental vascular integrity and PTB, and found that PTB was

significantly associated with placental vascular pathology including atherosis, thrombosis and infarcts of villi[115, 117]. A systematic review investigating a potential association between maternal lipids and PTB found that the results from nine cohort studies and five case-control studies were heterogenous, but that there was a possible increased risk of spontaneous PTB in women who had high triglyceride levels, but no association between low or high density lipoprotein cholesterol and PTB. In addition, elevated homocysteine levels were also associated with PTB in the second trimester. The authors concluded that the potential association between triglycerides and homocysteine and PTB warranted further investigation[115]. This is important as it is possible that modification of some of these factors could reduce the risk for PTB in some women. Chapter 4 of this thesis involves analysis of NMR metabolomics and gestational age at delivery, including analysis of maternal triglyceride and lipoprotein concentrations.

1.5 Preterm birth

While this thesis is focused mainly on improving our understanding of spontaneous labour at term, it is also hypothesised that this would improve our understanding of some causes of PTB. Much previous research has been conducted on PTB as it is this condition which has such high morbidity and mortality worldwide. It is, therefore, worthwhile discussing the main findings

from research into PTB in order to determine if there may be some factors identified in the diagnosis of PTB that can inform the investigation and experimentation into term labour. For example, there have been many large randomised controlled trials into progesterone administered to the mother (described below) with no reduction in PTB rate. The studies which will be outlined in this thesis indicate that this may be because maternal administration of progesterone does not prevent changes in fetal signals that trigger labour.

1.5.1 The cervix and preterm birth

Spontaneous PTB is a heterogenous condition, and for approximately 50% of cases there is no obvious cause. In some cases premature cervical dilatation has been noted prior to the onset of any symptoms such as uterine contractions[31, 50, 118]. Researchers have investigated potential pathways involving cervical dilatation as a trigger for spontaneous labour. Where once it was presumed that preterm remodelling and dilatation of the cervix followed the same pattern as that of the cervix at term, studies, including those on mice, indicate that there are independent pathways in PTB that seem to involve an acceleration of the cervical events at term, and another in response to infection[50]. For example, PTB in the mouse can be induced through administration of the progesterone receptor antagonist Mifepristone, effectively resulting in a decrease in progesterone and acceleration of the processes involved in cervical dilatation at

term. In contrast, the premature cervical ripening and dilatation in an infection mouse model, induced through administration of LPS into the mouse uterine horn, results in a proinflammatory response with an influx of neutrophils to the cervix and an increased expression of COX-2[50, 119]. Further, genetically engineered mice without the C5a complement receptor do not respond to either mifepristone or LPS with PTB, suggesting that the complement system is involved in both the preterm and term physiology of these mice models[50]. Gonzalez *et al.* (2009)[119] performed microarray analysis of cervical tissue taken from the following mouse models: induced PTB following administration of mifepristone; induced PTB following administration of LPS (inflammatory model); and term labour in the mouse. This identified distinct gene pathways between the three mouse models, where there was increased expression of genes associated with inflammation and immunity in the cervix of the LPS-induced labour but not with term labour[119]. Cytokine expression was also reported to increase postnatally, but not at the time of term labour. The authors suggest that this could indicate that activation of immune pathways are sufficient for cervical ripening but are not essential; and that the molecular mechanisms within the cervix associated with PTB and term labour are distinct, according to the aetiology and timing of the labour; with corresponding independent mechanisms for remodelling of ECM and change in cervical tensile

strength[50, 119]. While these experiments are not ethical in humans, women with conditions such as Ehlers-Danlos and Marfan syndrome, where there is a genetic defect in collagen or elastin synthesis, have increased incidence of premature rupture of membranes and/or cervical insufficiency[120]. It has been suggested that polymorphisms or failure to activate certain genes associated with connective tissue metabolism and synthesis may predispose individuals to preterm or post-term deliveries, respectively[50].

1.5.2 Randomised controlled trials with progesterone for prevention of pregnancy loss

The high burden of complications of labour such as PTB on society described above has motivated researchers to investigate potential treatments for prevention and treatment of pregnancy loss, including miscarriage and PTB. If such treatments were found to be effective, they could cast light on the pathway for spontaneous labour in humans. For this reason, such studies will be explored here.

Because of the quiescent effect of progesterone on the myometrium, and in part because of the effect of progesterone withdrawal in other mammals in triggering labour, progesterone has long been administered to women considered at risk of miscarriage or preterm labour. However, the efficacy of this treatment has been debated. A Cochrane meta-analysis investigating the

effectiveness of progesterone on preventing miscarriage in women with recurrent (defined variably as two-or-more or three-or-more miscarriages) miscarriage included thirteen Randomised Controlled Trials (RCT). Nine of these RCTs compared progesterone to placebo and four compared progesterone with standard care. Data from eleven of these trials were used for analysis (n=2359 women), considered to be of low risk for bias according to the Cochrane standards. The meta-analysis indicated that there was a reduction in the number of women having miscarriages in the progesterone treatment group (Risk Ratio (RR) 0.69, 95% confidence interval (95%CI) 0.51 to 0.92), and a more pronounced effect was observed for women with three previous miscarriages compared with two previous. The authors concluded that for women with unexplained recurrent miscarriages, progesterone supplementation is likely to reduce the rate of miscarriage in subsequent pregnancies, however the trials included were small and therefore larger trials were recommended. [121]

Larger RCTs have since been published, with varying results. The PROMISE (Progesterone in Recurrent Miscarriages) trial was a multicentre, double-blind, placebo-controlled, randomised trial comparing women with a history of recurrent miscarriage (defined as three or more consecutive or non-consecutive losses in the first trimester) treated with twice-daily vaginal suppositories containing either 400 milligrams of micronized progesterone (n=404) or a

matched placebo (n=432). Treatment commenced soon after a positive pregnancy test. The authors found no difference between the rate of live births between the treatment and placebo arm (RR 1.04, 95%CI 0.94 to 1.15), and the authors concluded that progesterone therapy in the first trimester of pregnancy does not improve live birth rate among women with unexplained recurrent miscarriage[122]. The PRISM (Progesterone in spontaneous miscarriage) trial was a multi-centre, randomised, double-blind, placebo-controlled trial compared active treatment with progesterone to placebo in comparing women who presented before 12 completed weeks' gestation with bleeding. Women were again randomised to the same treatment (n=2079) and matched placebo (n=2074) groups as for the PROMISE trial. The primary outcome was live birth rate, and the authors found no strong evidence of a clinically relevant difference in live birth rates between the two groups (Relative Rate 1.03, 95%CI 1.00 to 1.07)[123]. However, in a subsequent publication using data from the PRISM trial a sub-group analysis, that was not prespecified, has been reported. It suggested that for women with bleeding in early pregnancy and who have a history of three or more previous miscarriages (n=137 treatment vs n=148 placebo), there was a possible benefit to treatment (RR for live birth 1.28, 95%CI 1.08 to 1.51)[124], with a difference in live birth rate of 72% in the treatment arm and 52% in the placebo. The authors recommended that women with a history of miscarriage

presenting with bleeding in the first trimester may benefit from progesterone treatment following discussion with the women regarding the uncertainty of the evidence. However, as some of these findings were post-hoc subgroups, these findings should be considered exploratory and the findings would need to be replicated.

OPPTIMUM (Does progesterone prophylaxis to prevent preterm labour improve outcome?) was a multicentre, randomised, double-blind placebo-controlled trial comparing treatment with 200mg daily of Progesterone vaginal pessary (n=618) or a matched placebo (n=610), administered between 22-24 completed weeks to 34 completed weeks of gestation to women who were at risk of preterm birth (previous spontaneous birth at less than 34 weeks gestation, or a cervical length less than 25mm, or a positive fetal fibronectin test with other positive history). The primary outcomes were fetal death or birth before 34 weeks gestation, a composite of brain injury, death or bronchopulmonary dysplasia in the neonatal period, and a standardised cognitive score measured in the child at 2 years of age. Progesterone did not change the rate of these outcomes when compared with placebo. The odds ratio (OR) for preterm birth was 0.86, with a 95%CI of 0.61 to 1.22. The authors recommended that OPPTIMUM should encourage those who use progesterone in the treatment for preterm birth prophylaxis to

review their practise in order to identify women who may benefit. Further, that alternative strategies are required to prevent preterm birth[125].

These large, well-designed, multi-centre RCTs into the effect of treatment with progesterone on pregnancy loss seem to indicate that there is either none or very limited benefit in treatment of PTB or recurrent miscarriage with progesterone pessaries. Although there may be some improved outcomes for women with at least three previous miscarriages and bleeding in early pregnancy, this finding would require replication in a larger pre-defined study. This indicates that exogenous progesterone administered via the mother is not likely to prevent pregnancy loss in most women at risk of preterm birth or recurrent miscarriage. Further research is required to better understand the mechanism of labour so that other potential treatments can be designed, and studies in this thesis are designed to investigate whether there may be more local changes in progesterone and oestrogen, including possible communication between the fetus and placenta.

1.5.3 Induction of labour

With regards to induction of labour, the prostaglandin $F_2\alpha$ was found to increase in the utero-ovarian vein of sheep at the time of labour; and accordingly labour in ewes can be induced with administration of $F_2\alpha$ in later gestation, although not as effective as glucocorticoid administration[33]. A reduction in progesterone

through inhibition of 3β-hydroxysteroid dehydrogenase also induced labour and delivery in sheep[33]. In humans, maternal oestrogen levels are high throughout pregnancy, and despite this the myometrium remains in relative quiescence until term. An hypothesis for this apparent insensitivity of the myometrium to oestrogen during human pregnancy is suppression of the oestrogen receptor ERα by progesterone[32], and administration of the progesterone antagonist RU486 to Rhesus monkeys induces both labour and the expression of ERα by the myometrium[32]. Induction of labour with current methods are unsuccessful approximately 33% of the time[126], and it is likely that an improved understanding of the mechanisms of spontaneous labour would allow better targeting of drugs to improve the efficiency of IOL. In addition, a better understanding of pathways associated with failed IOL would help to inform research into what is required for successful IOL.

1.6 New approaches to understanding the mechanisms of human parturition: the potential of omics data

As described, treatment options for prevention of pregnancy loss, including PTB, and IOL' remain limited; and for prevention of pregnancy loss in particular, few options have proven efficacy. A large part of this problem is that we do not fully understand the mechanism for labour in humans. Advances in this area of research have been restricted because animal models do not replicate what

happens in humans; and experimental techniques such as myometrial cell culture do not always replicate what occurs to the uterus and myometrium during human pregnancy and labour *in vivo*[127]. Over the past decades many advances have been made in our understanding of reproduction and pregnancy. Recently, new techniques have been developed that may allow us to further elaborate and gain a better understanding of these biological pathways, capitalising on high-dimensional omics data and large-scale cohort studies, as will be discussed here.

1.6.1 The omics techniques

The omics techniques include genomics, transcriptomics, proteomics, and metabolomics. These technologies and their uses are rapidly developing and allow a fine-grained snapshot of a person's molecular profile. These techniques can be used as a systematic approach to generate and validate hypotheses which can be tested in future studies[128], including understanding of normal physiological as well as disease processes and biomarker discovery[128]. Excitingly, because omics studies allow investigation of global metabolites and proteins in a relatively non-invasive manner, including in human pregnancy, they provide a methodology by which human labour can potentially be investigated more directly and systematically. Possible pitfalls in analysis of omics studies include technical and biological variation, including tissue specificity and replication. In

observational studies in humans it is also important to collect, and include in the analysis, background data in order to control for confounding factors[128]. This thesis presents studies involving metabolomics and proteomics and these will be discussed here. Genomics and transcriptomics will also be described briefly.

1.6.2 Genomics and transcriptomics

Genomics is the study of the genome, the total DNA contained within a cell or organism. The human genome is comprised of 3.2 billion bases with approximately 30 000 – 40 000 genes, although only half appear to code for proteins[129]. DNA microarrays are used to compare and contrast the expression of thousands of genes between individuals simultaneously[130]. The transcriptome is the set of all RNA molecules generated from genes in the genome of a cell or collection of cells. DNA microarrays are used to compare and contrast the expression levels of each gene, with expression of a gene measured in terms of the number of mRNA molecules for that gene[128]. While this can be used to analyse gene activity within disease, mRNA abundance does not always reflect protein abundance and this has to be taken into account when interpreting results[128].

1.6.3 Metabolomics

The study of complex but interrelated biochemical pathways has been facilitated by metabolomics platforms that allow the measurement of simultaneous

changes in multiple metabolites in small samples of bodily fluids or tissues[131]. Metabolites are intermediate and final products of metabolism which include lipids, carbohydrates, peptides, nucleotides, hormones, and signalling molecules[131-133]. Importantly, the metabolome is the final product of the transcriptome and proteome, and therefore is likely to be the most analogous to the phenotype of a system[128, 131], although the metabolome is also affected by environmental input such as medications and diet[131-133].

Generally, the process of metabolomics involves two stages. The first stage includes experimental analytical techniques to quantify concentrations of multiple metabolites within a sample of interest, most commonly utilising mass spectrometry (MS) or nuclear magnetic resonance (NMR). The second stage is data processing using advanced computational programmes[131, 134]. Liquid chromatography (LC) or gas chromatography (GC) coupled to MS results in excellent separation of molecules within a sample according to their mass-to-charge ratio. Different MS platforms include: LC coupled with single-stage MS (LC-MS) or tandem MS (LC-MS/MS); GC coupled to MS (GC-MS); and high or ultrahigh performance LC coupled to ultraviolet or fluorescence detection (HPLC/UPLC)[135]. LC-MS, GC-MS and NMR are the most commonly used metabolomics analytical techniques, and 80% of published metabolomics studies are reported to use MS platforms[135]. There are advantages and

disadvantages to each of the different platforms, and decisions regarding this are often based on the study and sample type, as well as the cost and availability of a platform [135].

The benefits of MS platforms over NMR platforms have been reported to be a potential for increased sensitivity, and that MS allows detection of a greater number of metabolites[134] including molecules with nanomolar (nm) concentrations. This potentially allows for closer analysis of physiological pathways. MS platforms can be more selective and considered a better method for targeted analysis whereas NMR has often been used for nonselective analysis although improving techniques, such as with the Nightingale Platform (used by Born in Bradford), are reducing this difference[136]. In contrast, NMR is typically more reproducible than MS, and because of the problem of peak overlaps between multiple metabolites NMR achieves faster throughput. With NMR, all the metabolites that are at a level that is detectable can be measured at one time, whereas different ionisation methods are required to increase the number of metabolites measured with MS. Further, NMR involves minimal sample preparation, whereas MS requires more including chromatography; and NMR is quantitative as metabolite concentrations are proportional to the signal intensity, whereas for MS the ionisation efficiency also needs to be considered.

NMR can usually measure up to 200 metabolites in one measurement, whereas with MS techniques several hundred can be detected and quantified[135].

In addition, NMR is particularly good for large scale metabolomics studies as it is highly automatable and reproducible[135], and advances in these technologies have relatively recently allowed extensive metabolomic profiling within large cohort studies[136]. This has propelled us into a potential for discovering and uncovering pathways and biomarkers which previously have been hidden, and this could include the trigger for human labour. In order to get the most comprehensive results it is often recommended to use multiple platforms[135].

As blood samples, including serum and plasma, are useful and practical samples to measure during pregnancy, it is useful that this is where much of the acute changes in the metabolome are likely to be found[136]. Previous studies have included use of metabolomics to investigate pregnancy related changes in maternal and cord blood, fetal membranes, cervico-vaginal secretions, urine and amniotic fluid; with the potential of identifying metabolic profiles or biomarkers associated with different outcomes, such as PTB[137, 138], missed miscarriage[139], diagnosis of exposure to chorioamnionitis in the neonate[140], and the effect of maternal diet on amniotic fluid composition[137, 138, 141]. Some of these will be discussed further within the upcoming chapters of this thesis. Prior to commencement of this thesis there were no published studies identified

which specifically used metabolomics and phosphoproteomics to investigate potential associations with gestational age at delivery and the trigger for human labour[5].

1.6.4 Proteomics

As described above, the functional content of genes may be best analysed from the proteome[142, 143]. However, the investigation of protein function within eukaryotic cells is complicated by post-translational modifications such as phosphorylation, methylation, lipid attachment and glycosilation[142], as it is not always known how these modifications may affect protein function. Proteomic analysis generally involves the following steps: protein separation and isolation; techniques to determine structural characteristics of these proteins; and use of databases to identify the individual proteins[143]. Prior to the development of techniques for global proteomic analysis, proteins were most typically isolated using chromatography-based techniques such as affinity chromatography, size exclusion chromatography and ion-exchange chromatography. Enzyme-linked immunosorbent assays (ELISA) and western blotting techniques were used for analysis of selected proteins. Further, for separation of complex proteins, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis are often used[142]. These techniques allow

detailed quantification of proteins present, however only a limited number of proteins are detected with each experiment.

Development of MS techniques in the proteomics process allows measuring proteomes with high coverage in one experiment[143, 144]. Site-specific enzymatic proteases, for example trypsin, are used to digest proteins to peptides and these are fragmented within the mass spectrometer. The resulting spectra are used to identify the corresponding peptide sequence from databases[144]. Protein expression proteomics investigates the expression of different proteins in samples which differ by a variable, for example disease or non-disease, and such studies can help to identify proteins associated with a certain disease[143]. As such, proteomics can provide an insight into the dynamic relationship between genes and the cell environment, and such studies have been used to identify disease biomarkers for clinical use[128].

Phosphoproteomics allows analysis of total proteins and phosphorylation of proteins within tissues, and has recently been used in experimentation of the myometrium in humans[145]. Using phosphoproteomics, one group detected 22 phosphopeptides where the relative ratio either doubled or halved between spontaneous or oxytocin-driven phasic contractions *in vitro*. The largest changes were seen with phosphorylation of myosin light chain kinase on serine 1760, which is known to be associated with reduced calmodulin binding and kinase

activity. The authors found that phosphoproteomics analysis of myometrium in this setting presents a novel platform for assessment changes in proteins during spontaneous and oxytocin-induced contractions[145]. The specific phosphoproteomics methodology used in this thesis are described in more detail in Chapter 5.

1.7 Summary

Complications of PTB and IOL can cause long term disabilities and increased psychological stress and worry to women and their families, as well as increased financial cost to the National Health Service (NHS)[4, 36,43, 44]. While advances have been made in prediction models for PTB, there are still limited treatment options available. Currently management of preterm labour involves trying to reduce contractions for up to 24 hours in order to administer steroids and magnesium sulphate for maturation of the fetal lungs and stabilisation of the fetal neurological membranes for neuroprotection, respectively. There are no current treatments available that stop or prevent PTB once preterm labour has commenced[31, 146]. There are still large gaps in understanding of the mechanisms for labour in humans which include the timing of gestational length, and whether this relates to an interaction between the maternal/placental/fetal circulations, potentially driven by the fetus. Given maternal progesterone remains high until the end of the third stage (delivery of the placenta)[72, 87], it is

very possible that the mechanisms involved in the trigger of labour are locally driven, and involve local signals between the fetus, placenta, decidua and myometrium.

As discussed, while the mechanisms of labour in mammals such as sheep and rodents are relatively well understood, the elucidated mechanisms do not seem to be directly replicated in humans; and *in vitro* models do not always adequately reflect what happens *in vivo* to the uterus during pregnancy and labour. Moreover, while it is hypothesised that there may be a functional withdrawal of progesterone in humans that results in myometrial contractions and labour, exogenous administration of progesterone to women does not appear to prevent preterm birth or miscarriage in the majority of scenarios.

The recent advances in omics technologies bring with them potential to be able to analyse direct cellular pathways that are occurring during pregnancy, labour and at delivery, and without causing risk to the mother or baby. The overarching aim of this thesis is to use these technologies to better understand human labour and to develop hypotheses to test in future studies.

1.8 Thesis aim and objectives

Aim 1: To further knowledge and understanding of the mechanisms and trigger of human parturition using omics technologies.

Objective 1.1: To further our understanding of the signals between the mother, placenta and fetus at the time of term labour by elucidating the metabolome of maternal blood, the cord vein, and cord artery, respectively, at the time of delivery.

Objective 1.2: To determine if there are differences in these signals between spontaneous labour, induction of labour and no labour (caesarean section). Any differences would indicate further areas to investigate with regards to the process of spontaneous labour at term.

Aim 2: To investigate whether the maternal metabolome during pregnancy is associated with gestational length of pregnancy.

Objective 2.1: Investigate maternal metabolome at different time points during pregnancy and determine if there is an association with gestational age at spontaneous labour.

Objective 2.2: Investigate an association between maternal metabolites sampled during pregnancy and gestational age at delivery for women included in the Born in Bradford cohort study.

Aim 3: Given the myometrium generates the contractions of labour and expulsion of the fetus, the third aim of the thesis is to investigate what signals are necessary within the myometrium for successful labour.

Objective 3.1: to investigate the phosphoproteome changes with myometrial contraction and relaxation.

Objective 3.2: To investigate whether there are differences in the phosphoproteome between myometrium sampled from women where the cervix has previously dilated to 10 cm (full dilatation) with myometrium sampled from women who have an emergency caesarean section for failure to progress.

The next four chapters of this thesis present studies undertaken by Katherine Birchenall (thesis author) to answer the above aim and objectives: Chapter 2 and Chapter 3 present two clinical studies which utilised MS metabolomics to investigate human pregnancy; Chapter 4 presents a study that used NMR metabolomics data from the Born in Bradford cohort study; and Chapter 5 presents a study which investigated phosphoproteomics in human myometrium. There is no separate methodology chapter, and instead each of the studies in Chapters 2-5 are presented independently, all containing sections on background, methodology, results, discussion and conclusions. Finally, Chapter 6 presents an overall discussion of the findings.

CHAPTER 2. INVESTIGATING METABOLITE CHANGES IN MATERNAL AND FETAL PLASMA FOLLOWING SPONTANEOUS LABOUR AT TERM IN HUMANS USING UNTARGETED METABOLOMICS

2.1 Background

This pilot study was designed to investigate the effect of labour on the human metabolome in pregnancies delivered at term. It was hypothesised that a better understanding of any differences in the human metabolome within cord and maternal blood at term between those who laboured and delivered vaginally (VD) and those who did not labour and delivered via elective caesarean section (CS) would help identify potential target pathways for metabolites involved in the spontaneous initiation of labour. This study was also intended to demonstrate the potential of mass spectrometry (MS) metabolomics analysis of maternal plasma in human pregnancy, which had not been extensively performed previously.

The results of this study demonstrate that even with a relatively small sample size, significant and robust changes can be identified providing new insights into the metabolic responses of the human maternal-feto-placental unit in spontaneous labour.

2.2 Disclaimer

The findings from the study presented in this chapter have been published in a peer-reviewed journal[5], the reference for which is:

Birchenall, K.A., Welsh, G.I. & Lopez Bernal, A. (2019). Metabolite Changes in Maternal and Fetal Plasma Following Spontaneous Labour at Term in Humans Using Untargeted Metabolomics Analysis: A Pilot Study.
International Journal of Environmental Research and Public Health: 16(9), 1527; doi:10.3390/ijerph16091527.

This thesis chapter is adapted from that publication, which was written in full by the thesis author (Katherine Birchenall) with support from supervisors Professor Andrés López Bernal and Dr Gavin Welsh (co-authors). The full paper is attached in the Appendices (2A1).

2.3 Study responsibilities

The thesis author (Katherine Birchenall) designed this study, wrote the protocol, recruited all participants, collected, and processed all samples, and analysed the results. The MS metabolomics described, along with some initial statistical analysis, was performed by Metabolon, Inc., with support from Dr Gregg Michelotti at Metabolon, Inc.. This will be described further below. All reference to "the researcher" refer to the author of this thesis, Katherine Birchenall.

2.4 Funding

This part of the PhD was funded by the Mother and Baby Trust, an Above and Beyond charity [grant number G109481-101].

2.5 Ethical approval

The study was conducted in accordance with the Declaration of Helsinki, and ethical approval for the study was gained from the National Research Ethics Service Committee-South West, Bristol (reference number: E5431).

2.6 Background

Our understanding of the mechanisms of human parturition remains incomplete and consequently management options for complications of pregnancy such as preterm birth (PTB), and common interventions such as induction of labour (IOL)[2, 36], remain clinically unsatisfactory[4].

A better understanding of human labour at term (defined as 37–42 weeks' gestation) would allow development of methods for improved prediction and prevention of PTB, as well as more effective strategies for IOL[29, 30, 47]. The aetiology of PTB is heterogeneous and frequently pathological, however it may result in stimulation of the same triggers for parturition as spontaneous labour at term[2, 147]. The proportion of pregnancies with IOL in England has increased from 20.3% in 2006–2017 to 29.4% in 2016–2017[30, 45-48]. IOL can be inefficient, with potentially severe complications for the mother and neonate, including

lengthy hospital stays at high cost to the National Health Service and a negative impact on the birth experience[4, 43, 44]. There is a need to identify the metabolic changes underlying the physiological onset of spontaneous human labour and to develop new clinically useful predictive markers and better drug targets.

Metabolomics allows the investigation of a wide range of metabolites across the main biochemical pathways[148]. The advantage of studying the metabolome or metabolic profile of a system is that it reveals the current activity rather than a prediction of what may happen, as is the case with genomic studies[132, 133, 149]. The most commonly used metabolomic techniques are mass spectrometry (MS) and nuclear magnetic resonance (NMR), paired with data processing using advanced computational programmes[134, 149]. NMR can measure a broad range of metabolites however it is less sensitive than MS. Gas chromatography (GC) or liquid chromatography (LC) coupled to MS provides excellent separation of molecules within a sample according to their mass-to-charge ratio and analysis at a wide range of concentrations. As MS is more sensitive, it can be utilised to measure more metabolites, but it has a longer analysis time than NMR[134]. Metabolomics has been used to investigate pregnancy related changes in maternal and cord blood, fetal membranes, cervico-vaginal secretions, urine and amniotic fluid; with the potential of identifying metabolic profiles or biomarkers associated with different outcomes, such as PTB[137, 138], missed

miscarriage[139], diagnosis of exposure to chorioamnionitis in the neonate[140], and the effect of maternal diet on amniotic fluid composition[137, 138, 141]. However, studies focusing on parturition are scarce.

This chapter presents novel MS metabolomics data from a pilot study designed to investigate the effect of parturition on plasma metabolites in the fetal and maternal circulation at the time of delivery. The findings demonstrate that even with a relatively small sample size significant and robust changes can be identified providing new insights into the metabolic responses of the maternal-feto-placental unit in spontaneous labour.

2.7 Aims and objectives

The aim of this study was to investigate differences in the cord and maternal plasma metabolic profiles between women who spontaneously labour and deliver vaginally and those who deliver via elective caesarean section and do not labour.

The specific objectives were:

1. Determine feasibility of recruiting women to this type of study.
2. To identify potential pathways using MS metabolomics in a small cohort of women (numbers limited by time and available funds), and assess the robustness and adequacy of the methodology.

3. To select relevant pathways to investigate with larger follow-on studies if appropriate.

2.8 Methodology

2.8.1 Participants

Women with low-risk pregnancies were recruited at term who planned either a spontaneous labour and vaginal delivery (VD) or an elective caesarean section (CS) for reasons not related to maternal or fetal disease, for example breech presentation. It was intended to recruit ten women to each group. The target number of ten was chosen as this was a pilot study and it was not known how useful the MS metabolomics technology would be in this setting. Such a study design had not previously been published (according to a literature search by the researcher) and therefore ten per group was deemed a reasonable target following discussion with the researcher and supervisors.

The inclusion criteria were as follows:

- women with uncomplicated singleton term pregnancies.
- age 18-40 years.
- no significant past medical history.

The exclusion criteria were as follows:

- multiple pregnancies.

- age under 18 years or over 41 years.
- taking medications likely to affect metabolomics.
- Diabetes.
- pre-eclampsia.
- other metabolic conditions.
- raised temperature.
- signs of fetal distress during labour.

The following demographic information was collected:

- ethnicity.
- age.
- Body Mass Index (BMI).
- smoking status.
- time last eaten.
- time of delivery.
- duration of delivery.
- drugs given during hospital stay.

- Apgar scores of the baby at delivery.
- cord gases if taken.

Forty women were approached at St Michael's Hospital, Bristol, between October 2016 and January 2017. The women were asked if they may be interested in taking part in the study and given an information leaflet (Appendix 3A1) and the study explained by the researcher. For those women who were happy to consent to the study, the researcher returned, and the women signed the consent form (Appendix 3A2) (Figure 2.1). Thirty-eight women gave informed, written consent to take part in the study. 17 women were excluded for the following reasons: one had their labour induced, one had increased maternal age, two developed pyrexia during their labour, one developed a pyrexia and pre-eclampsia during their labour, two had an emergency CS, and ten delivered when the researcher was not present in the hospital. Twelve women delivered via elective CS and ten of these were included in the study (as ten was the intended number to include in each group), and nine women delivered via spontaneous VD and were included in the study.

2.8.2 Collection of samples

Cord blood and maternal (intervillous) blood samples were obtained within 30 minutes of delivery of the baby and placenta. Delayed cord clamping is routinely carried out at St Michael's Hospital for both vaginal and caesarean section

deliveries, and this occurred for all the deliveries in this study. Intervillous samples were collected through making a cut in the placenta using a scalpel and extracting blood using a 10 ml syringe, as described previously[150]. This method of maternal blood sampling was used to limit the intrusion of the study on the important bonding time for the family at delivery, negating the need to physically take blood from the mother immediately following delivery and meant that the researcher did not have to enter the delivery room at all during this significant time. The midwives brought the placenta to the researcher (who was standing just outside the door of the delivery room) immediately. The researcher collected the mixed cord sample from both the cord vein and cord artery using a sterile 21 Gauge needle and syringe, transferred into a Vacutainer tube containing EDTA, then centrifuged at 1000 x g for ten minutes. Two hundred microlitres of the clear upper plasma layer was transferred into chilled propylene tubes and immediately stored at -80 degrees Celsius prior to transportation. All samples were frozen within an hour of delivery.

Samples were collected between October 2016 and February 2017, and were transported to Metabolon, Inc. in April 2017. Samples were labelled with a unique study identifier, with no patient identifying information. All patient identifying information and matching study identifier were kept on an encrypted ExCel spreadsheet file, kept on a secure NHS computer. Consent forms were

kept in a study file, locked in a filing cabinet, in a specified locked research office within St Michael's Hospital. The Human Tissue Act was adhered to at all times.

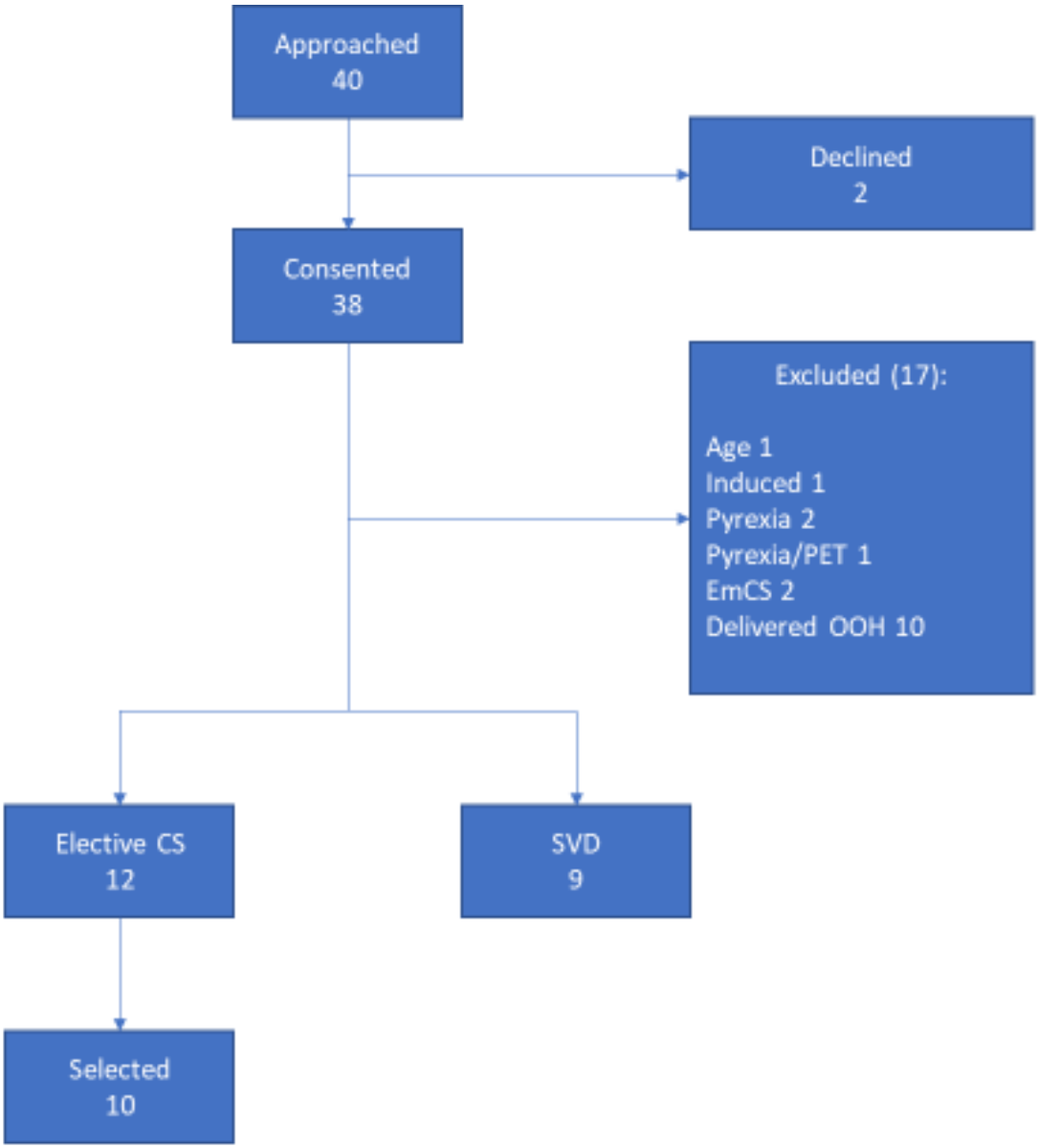

Figure 2.1: Flow diagram for women approached to join the study

2.8.3 Metabolomics analysis

The samples were transported on dry ice to Metabolon, Inc., (North Carolina, United States of America) for ultrahigh performance liquid chromatography-

tandem mass spectrometry (UHPLC/MS)[151-153]. All samples were analysed together to reduce risk of batch bias. This included the measurement of 826 known metabolites for each sample, grouped into 108 sub-pathways and the following eight super-pathways: Lipid, Amino Acid, Peptide, Energy, Nucleotide, Cofactors and Vitamins, and Xenobiotics. Briefly, plasma samples were subjected to methanol extraction then split into aliquots for analysis by UHPLC/MS in the positive (involving two methods, one optimized for hydrophilic, and the other hydrophobic compounds), negative or polar ion mode. Metabolites were identified by automated comparison of ion features to a reference library of chemical standard, followed by visual inspection for quality control[154]. For quality assurance and quality control (QC), pooled QC plasma replicates, as well as several internal standards, were assessed to determine instrument variability, with representative relative standard deviation (RSD) = 3% for internal standards and 7% for endogenous biochemicals.

2.8.4 Statistical analysis

The two experimental groups were labelled VC/EC and VM/EM. VC/EC represents the difference in the means between VC, the metabolite quantity identified in cord plasma from women who laboured and delivered vaginally, and EC, the metabolite quantity identified in cord plasma from women who did not labour and delivered via elective CS. VM/EM represents the difference in the

means between VM, the metabolite quantity identified in intervillous plasma from women who laboured and delivered vaginally, and EM, the metabolite quantity contained in intervillous plasma from women who did not labour and delivered via elective CS. Welch's two-sample t-test was used to identify metabolites for which the means were significantly different between VC and EC or VM and EM. Analysis was performed on log-transformed data, considered significant if $p \leq 0.05$. A q-value was also calculated to control for false discovery rate and account for the multiple comparisons which occur in metabolomic-based studies. Metabolon, Inc., estimate that ten metabolites will be shown to be significant by chance for every 200 compounds analysed, and a q-value of <0.10 gives high confidence that a significant difference is not due to chance. The statistical analysis plan was designed by the researcher, and Metabolon, Inc. performed the initial statistical analysis in ArrayStudio and R, as part of their collaboration in the project.

2.8.5 Over-representation analysis

Overrepresentation analysis (ORA) software has been utilised to enhance and guide analysis of transcriptomics and proteomic analysis (discussed further in Chapter 5), and similar software has been developed for metabolomics analyses.

Previous work has been conducted to compare the effectiveness of different ORA programmes in the analysis of metabolomics data, including

MetaboAnalyst, REACTOME, MetExplore, and KEGGREST. ORA analyses were found to be consistent regardless of the analytic approach used[155]. For the metabolomics analysis in this thesis, MetaboAnalyst (version 4.0) was selected as it is specifically designed for use with metabolomics and has been used successfully by other researchers[156, 157]. The MetaboAnalyst software is based on the statistical package R and maps common compound names to a range of database identifiers, including KEGG, Human Metabolome Database (HMDB), METLIN, and PubChem[158]. There are some limitations to ORA analysis, including that there is the potential for some bias towards those diseases and pathways which have previously been investigated, as only a small proportion of the metabolome is currently elucidated. On the other hand, examining and explaining results according to biological knowledge from the literature can also be biased according to the prior background knowledge or interest of the researcher[157]. Therefore, for this thesis, both ORA enrichment pathway analysis and investigation of individual metabolite changes will be performed to aid understanding of the results observed. In addition, such standardised pathway analysis can help to compare outcomes of the different studies included in this thesis.

For the MS analysis in this thesis, HMDB identifiers were put inputted into the MetaboAnalyst programme online (www.metaboanalyst.ca) and enrichment

analysis performed. The pathway enrichment analysis selected implemented the hypergeometric test to evaluate whether a specific metabolite group is represented more than would be expected by chance within the given compound list. Raw one-tailed p values are calculated, as well as a p-value calculated following adjustment for multiple testing using the Holm method[157, 158]. For the present chapter, MetaboloAnalyst was used for those metabolites which had a significant p-value (≤0.05) for VM/EM and VC/EC.

2.9 Results

2.9.1 Demographics

Table 2.1 presents the characteristics of the labouring and non-labouring groups. All nine women in the vaginal delivery group had confirmed labours of spontaneous onset. All women were of white ethnicity and there were no significant differences in age, parity, or Apgar scores at birth between the two groups. None of the women had experienced medical complications during the pregnancy, nor had there been concern for any of the fetuses. The mean BMI at booking was 21.6 in the VD group and 25.6 in the elective CS group (p=0.02), due to an outlier in the elective CS group with BMI 38.3. Further analysis was performed and confirmed her inclusion did not affect the spread of results. The median gestation at delivery was 40 weeks in the VD group and 39 weeks and 2 days in the elective CS group (p=0.04), an expected difference as planned CSs

are routinely booked between 39 and 40 weeks' gestation whereas spontaneous deliveries may occur up to three weeks later.

2.9.2 Metabolomics

All the results refer to ten women in the elective CS group and nine women in the VD group. It was not possible to obtain intervillous blood from one placenta, reducing the VM samples to eight. The numbers of metabolites with significant ($p \leq 0.05$) differences between VM and EM, and VC and EC, in the eight super-pathways are presented in Table 2.2. Of the 108 metabolite sub-pathways measured, 83 contained metabolites with statistically significant VC/EC and/or VM/EM differences. For the following analysis, xenobiotic metabolites will be considered separately.

2.9.3 Changes in cord plasma metabolome

Table 2.3 shows the changes in cord plasma (VC/EC) of specific metabolites within the sub-pathways ordered according to the magnitude of fold-change. Of these, 120 metabolites significantly increased in the cord plasma of women who laboured, 102 of which also had a significant q value (<0.10), indicating these differences are unlikely to have occurred due to chance. These comprised 58 lipid metabolites, 14 amino acid metabolites, nine carbohydrate metabolites, eight nucleotide metabolites, six energy metabolites, three cofactors and vitamins metabolites, two peptide metabolites and two partially characterised

molecules. The metabolite with the largest VC/EC fold-change was heme, which had an average 14.30-fold increase in the cord plasma ($p=0.024$) taken from women who laboured.

39 metabolites significantly decreased in the cord plasma of women who laboured (VC/EC), and 27 of these had a significant q value: eleven amino acids, six lipids, four peptides, three cofactors and vitamins and three nucleotides.

Table 2.1: Characteristics of included human participants

Demographic	VD	Elective CS	p value
	(n = 9)	(n = 10)	(at ≤0.05)
Age (years)	32.4 (24-39)[a]	34.6 (30-40) [a]	NS
BMI at pregnancy booking	21.6 (19.9-23.1) [a]	25.56 (21.7-38.3) [a]	0.02
Ethnicity			
White European	9	10	
Smoking history:			NS
Never	8	4	
Ex	0	5	
Current	1 (4/day)	1 (10/day)	
Maternal past medical history			
	Eczema: 1 ICSI pregnancy (own sperm/egg): 1	Previous postnatal depression: 1 Well controlled asthma: 1 MTHFR gene 1: 1	NA
Gravida	1.2 (0-2) [a]	2.4 (0-5) [a]	NS
Parity	0.6 (0-2) [a]	1.7 (0-7) [a]	NS
Significant pregnancy complications	Nil	Nil	NA

Indication for elective CS			
Breech	NA	3	NA
Previous CS	NA	3	NA
Maternal request	NA	1	NA
Previous 3rd degree vaginal tear	NA	1	NA
Previous traumatic delivery	NA	1	NA
Tocophobia	NA	1	NA
Duration of labour in minutes	312 minutes (70 - 650) [a]	NA	NA
Gestation at delivery (weeks+days)	40 (38+1-41+6) [a]	39+2 (38+4-40+3) [a]	0.04
Apgar scores at 1, 5 and 10 minutes:			NS
8,9,10	1	0	
9,10,10	8	10	
Management of third stage:			NS
Syntometrine or Carbetocin	8	10	
Physiological	1	0	
Interval between delivery and freezing of sample (minutes)	50 (36-60) [a]	30.7 (24-45) [a]	0.02

[a] Mean (range); VD = Vaginal Delivery; CS = Caesarean Section; BMI = Body Mass Index; NS = not significant; NA = non-applicable; ICSI = intracytoplasmic sperm injection

Table 2.2: Number of metabolites which significantly increased or decreased at the time of delivery between women who spontaneously laboured and delivered vaginally and women who did not labour and delivered via elective caesarean section in the maternal (intervillous) plasma (VM/EM) and cord plasma (VC/EC) for each of the super pathways.

Super pathway	Number metabolites measured	Number significant changes VM/EM			Number significant changes VC/EC		
		↑	↓	Total (%)	↑	↓	Total (%)
Lipid	405	139	14	153 (37.8)	73	12	85 (21.0)
Amino Acid	177	9	15	24 (13.6)	15	14	29 (16.4)
Xenobiotics	108	18	3	21 (19.4)	16	5	21 (19.4)
Carbohydrate	29	7	0	7 (24.1)	9	0	9 (31.0)
Cofactors and Vitamins	31	3	4	7 (22.6)	3	5	8 (25.8)
Energy	11	3	0	3 (27.3)	6	0	6 (54.6)
Nucleotide	38	2	2	4 (10.5)	9	3	12 (31.6)
Peptide	24	0	1	1 (4.2)	3	5	8 (33.3)
Partially characterised	3	2	0		2	0	

VM = Maternal (intervillous) plasma from women who laboured and delivered vaginally (n=8); EM = maternal plasma from women who did not labour and delivered via caesarean section (n=10); VC = cord plasma from women who laboured and delivered vaginally (n=9); EC= cord plasma from women who did not labour and delivered via elective caesarean section (n=10); ↑ = Number of metabolites significantly elevated; ↓ = Number of metabolites significantly decreased.

2.9.4 Changes in maternal plasma metabolome

Table 2.4 shows the corresponding changes in maternal plasma metabolites (VM/EM). Overall, 165 metabolites statistically significantly increased in labouring women, all of which had a significant q value. These included: nine amino acids, seven carbohydrates, three cofactors and vitamins, three energy metabolites, 139 lipids, two nucleotides, and two partially characterised

molecules. The metabolite with the greatest significant-fold increase in maternal plasma of women who laboured was the partially characterised molecule glucuronide of C10H18O2 (8), with an average 10.85-fold increase in VM plasma. The dicarboxylate fatty acids maleate and adipate (C6-DC) increased 10.42-fold and 8.06-fold, respectively.

36 metabolites were significantly decreased in the maternal plasma of women who laboured when compared with women who did not labour (VM/EM), with a corresponding significant q value, comprising fifteen amino acids, four cofactors and vitamins, fourteen lipids, two nucleotides and one peptide. Among these were steroid sulphates, including 3-dehydrocholate (p=0.002), 16a-hydroxy DHEA 3-sulfate (0=0.007) and andro steroid monosulfate C19H28O4S (p=0.017), with fold-changes 0.13, 0.22 and 0.26, respectively.

Table 2.3: Fold-change in cord plasma (VC/EC) for metabolites (xenobiotics excluded) with significant p (≤0.05) and q (≤0.10), ordered according to fold-change (greatest to smallest), with corresponding sub-pathway and super-pathway.

Metabolite	Sub-Pathway	Super-Pathway	Fold-change (VC/EC)	p-value	q-value
Heme	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	**14.30**	0.0239	0.0918
Fibrinopeptide A, Phosphono-Ser	Fibrinogen Cleavage Peptide	Peptide	**11.71**	0.0144	0.0689
Glucuronide Of C10H18O2	Partially Characterized Molecules	Partially Characterised Molecules	**9.98**	0.0159	0.0734
Spermidine	Polyamine Metabolism	Amino Acid	**7.26**	0.0172	0.0764
Adenosine 5'-Diphosphoribose (Adp-Ribose)	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**7.25**	0.0180	0.0784
Glycerol 3-Phosphate	Glycerolipid Metabolism	Lipid	**7.13**	0.0064	0.0435
Glucuronide Of C10H18O2	Partially Characterized Molecules	Partially Characterised Molecules	**7.07**	0.0214	0.0887
Inosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**6.01**	0.0016	0.0194
Cysteine-Glutathione Disulfide	Glutathione Metabolism	Amino Acid	**5.13**	0.0035	0.0312
Cortisol	Corticosteroids	Lipid	**3.57**	0.0000	0.0015
Adenosine 3',5'-Cyclic Monophosphate (Camp)	Purine Metabolism, Adenine containing	Nucleotide	**3.53**	0.0009	0.0141
Maltose	Glycogen Metabolism	Carbohydrate	**3.39**	0.0254	0.0925
Trans-Urocanate	Histidine Metabolism	Amino Acid	**3.23**	0.0012	0.0148
Corticosterone	Corticosteroids	Lipid	**3.09**	0.0002	0.0070

Cortisone 21-Sulfate	Corticosteroids	Lipid	**3.02**	0.0001	0.0051
Hypoxanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**2.96**	0.0001	0.0049
Androstenediol (3Alpha, 17Alpha) Monosulfate	Androgenic Steroids	Lipid	**2.80**	0.0110	0.0583
3-Phosphoglycerate	Glycolysis, Gluconeogenesis and Pyruvate Metabolism	Carbohydrate	**2.77**	0.0233	0.0918
5Alpha-Androstan-3Alpha,17Beta-Diol Monosulfate	Androgenic Steroids	Lipid	**2.63**	0.0141	0.0677
N-Formylphenylalanine	Tyrosine Metabolism	Amino Acid	**2.56**	0.0056	0.0403
Xanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**2.55**	0.0000	0.0008
Palmitoleate (16:1N7)	Long Chain Monounsaturated Fatty Acid	Lipid	**2.52**	0.0190	0.0805
1,2-Dipalmitoyl-Gpe (16:0/16:0)	Phosphatidylethanolamine (PE)	Lipid	**2.51**	0.0269	0.0955
Margaroylcarnitine (C17)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**2.47**	0.0105	0.0579
Eicosenoylcarnitine (C20:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**2.43**	0.0045	0.0362
5-Dodecenoate (12:1N7)	Medium Chain Fatty Acid	Lipid	**2.41**	0.0018	0.0209
3-Hydroxylaurate	Fatty Acid, Monohydroxy	Lipid	**2.40**	0.0000	0.0020
S-Adenosylhomocysteine (Sah)	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**2.34**	0.0223	0.0898
Gamma-Glutamylglutamate	Gamma-glutamyl Amino Acid	Peptide	**2.33**	0.0286	0.0988

Galactonate	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**2.31**	0.0185	0.0796
Androsterone Sulfate	Androgenic Steroids	Lipid	**2.27**	0.0007	0.0128
Cysteinylglycine	Glutathione Metabolism	Amino Acid	**2.21**	0.0123	0.0625
Lactate	Glycolysis, Gluconeogenesis and Pyruvate Metabolism	Carbohydrate	**2.13**	0.0000	0.0018
Alpha-Ketoglutarate	TCA Cycle	Energy	**2.13**	0.0011	0.0148
Adenosine 5'-Monophosphate (Amp)	Purine Metabolism, Adenine containing	Nucleotide	**2.12**	0.0246	0.0918
Hexadecadienoate (16:2N6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.09**	0.0004	0.0094
Stearoylcarnitine (C18)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**2.08**	0.0256	0.0925
Myristoleate (14:1N5)	Long Chain Monounsaturated Fatty Acid	Lipid	**2.03**	0.0165	0.0745
3-Hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid	**2.01**	0.0002	0.0066
Glycerophosphoinositol	Phospholipid Metabolism	Lipid	**1.98**	0.0134	0.0653
(16 Or 17)-Methylstearate (A19:0 Or I19:0)	Fatty Acid, Branched	Lipid	**1.96**	0.0041	0.0353
Fructose	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**1.96**	0.0186	0.0796
Palmitoleoylcarnitine (C16:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.95**	0.0009	0.0141
1-Palmitoyl-2-Oleoyl-Gpe (16:0/18:1)	Phosphatidylethanolamine (PE)	Lipid	**1.93**	0.0095	0.0554
Oleoylcarnitine (C18:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.90**	0.0010	0.0145

Laurate (12:0)	Medium Chain Fatty Acid	Lipid	**1.90**	0.0010	0.0146
Acetylcarnitine (C2)	Fatty Acid Metabolism (Acyl Carnitine, Short Chain)	Lipid	**1.89**	0.0004	0.0094
10-Heptadecenoate (17:1N7)	Long Chain Monounsaturated Fatty Acid	Lipid	**1.89**	0.0026	0.0282
Pantothenate	Pantothenate and CoA Metabolism	Cofactors and Vitamins	**1.89**	0.0241	0.0918
N-Acetylglucosamine/N-Acetylgalactosamine	Aminosugar Metabolism	Carbohydrate	**1.88**	0.0033	0.0304
Malate	TCA Cycle	Energy	**1.86**	0.0012	0.0148
Ribulonate/Xylulonate	Pentose Metabolism	Carbohydrate	**1.85**	0.0009	0.0141
Cortisone	Corticosteroids	Lipid	**1.85**	0.0010	0.0145
Dihomo-Linoleoylcarnitine (C20:2)	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	Lipid	**1.84**	0.0059	0.0413
Oleate/Vaccenate (18:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**1.84**	0.0067	0.0438
Linolenate [Alpha Or Gamma; (18:3N3 Or 6)]	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.83**	0.0067	0.0438
Xanthosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**1.82**	0.0005	0.0104
10-Nonadecenoate (19:1N9)	Long Chain Monounsaturated Fatty Acid	Lipid	**1.81**	0.0012	0.0151
Linoleate (18:2N6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.81**	0.0032	0.0301
Laurylcarnitine (C12)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	**1.80**	0.0084	0.0518
Caprate (10:0)	Medium Chain Fatty Acid	Lipid	**1.79**	0.0031	0.0299

Succinate	TCA Cycle	Energy	**1.78**	0.0098	0.0565
2-Hydroxyglutarate	Fatty Acid, Dicarboxylate	Lipid	**1.76**	0.0024	0.0275
Myristate (14:0)	Long Chain Saturated Fatty Acid	Lipid	**1.75**	0.0071	0.0450
Oestrone 3-Sulfate	Estrogenic Steroids	Lipid	**1.74**	0.0132	0.0651
Myristoleoylcarnitine (C14:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.72**	0.0077	0.0483
Palmitoylcarnitine (C16)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.72**	0.0115	0.0597
Eicosenoate (20:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**1.69**	0.0030	0.0298
Glycerol	Glycerolipid Metabolism	Lipid	**1.68**	0.0000	0.0003
N-Acetyltaurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.68**	0.0016	0.0194
Caprylate (8:0)	Medium Chain Fatty Acid	Lipid	**1.67**	0.0160	0.0734
Dihomo-Linoleate (20:2N6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.65**	0.0045	0.0362
Linoleoylcarnitine (C18:2)	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	Lipid	**1.64**	0.0003	0.0087
(14 Or 15)-Methylpalmitate (A17:0 Or I17:0)	Fatty Acid, Branched	Lipid	**1.64**	0.0029	0.0298
Uracil	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.64**	0.0109	0.0583
Myristoylcarnitine (C14)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.63**	0.0010	0.0146
Pyruvate	Glycolysis, Gluconeogenesis and Pyruvate Metabolism	Carbohydrate	**1.62**	0.0031	0.0298

(S)-3-Hydroxybutyrylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**1.61**	0.0236	0.0918
Glutamate, Gamma-Methyl Ester	Glutamate Metabolism	Amino Acid	**1.59**	0.0287	0.0988
Decanoylcarnitine (C10)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	**1.58**	0.0131	0.0651
5-Dodecenoylcarnitine (C12:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.57**	0.0245	0.0918
Arachidonoylcarnitine (C20:4)	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	Lipid	**1.55**	0.0006	0.0105
Palmitate (16:0)	Long Chain Saturated Fatty Acid	Lipid	**1.54**	0.0065	0.0435
Isocitric Lactone	TCA Cycle	Energy	**1.54**	0.0221	0.0898
Margarate (17:0)	Long Chain Saturated Fatty Acid	Lipid	**1.53**	0.0049	0.0373
5,6-Dihydrouracil	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.53**	0.0094	0.0554
(12 Or 13)-Methylmyristate (A15:0 Or I15:0)	Fatty Acid, Branched	Lipid	**1.53**	0.0100	0.0570
1-Stearoyl-2-Oleoyl-Gpi (18:0/18:1)	Phosphatidylinositol (PI)	Lipid	**1.51**	0.0264	0.0943
Phenyllactate (Pla)	Phenylalanine Metabolism	Amino Acid	**1.50**	0.0054	0.0392
Vanillactate	Tyrosine Metabolism	Amino Acid	**1.49**	0.0005	0.0099
Imidazole Lactate	Histidine Metabolism	Amino Acid	**1.47**	0.0256	0.0925
Stearoyl-Arachidonoyl-Glycerol (18:0/20:4)	Diacylglycerol	Lipid	**1.44**	0.0161	0.0734
Docosadienoate (22:2N6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.43**	0.0053	0.0391
Isocitrate	TCA Cycle	Energy	**1.43**	0.0202	0.0850

Creatine	Creatinine Metabolism	Amino Acid	**1.41**	0.0029	0.0298
Fumarate	TCA Cycle	Energy	**1.41**	0.0043	0.0358
2-Hydroxy-3-Methylvalerate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.41**	0.0130	0.0650
Stearate (18:0)	Long Chain Saturated Fatty Acid	Lipid	**1.31**	0.0068	0.0440
Glucose	Glycolysis, Gluconeogenesis and Pyruvate Metabolism	Carbohydrate	**1.31**	0.0105	0.0579
Oleoyl Ethanolamide	Endocannabinoid	Lipid	**1.31**	0.0226	0.0904
Pentadecanoate (15:0)	Long Chain Saturated Fatty Acid	Lipid	**1.30**	0.0043	0.0358
5-Oxoproline	Glutathione Metabolism	Amino Acid	**1.18**	0.0035	0.0312
Serine	Glycerine, Serine and Threonine Metabolism	Amino Acid	**0.85**	0.0113	0.0591
Tryptophan	Tryptophan Metabolism	Amino Acid	**0.83**	0.0243	0.0918
Arginine	Urea cycle, Arginine and Proline Metabolism	Amino Acid	**0.82**	0.0103	0.0579
Histidine	Histidine Metabolism	Amino Acid	**0.80**	0.0047	0.0368
Citrulline	Urea cycle, Arginine and Proline Metabolism	Amino Acid	**0.80**	0.0213	0.0887
Gamma-Glutamylleucine	Gamma-glutamyl Amino Acid	Peptide	**0.80**	0.0257	0.0925
N1-Methylinosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.79**	0.0275	0.0967
Indole-3-Carboxylic acid	Tryptophan Metabolism	Amino Acid	**0.74**	0.0086	0.0526
Carnosine	Histidine Metabolism	Amino Acid	**0.73**	0.0232	0.0918

Gamma-Glutamyl-Alpha-Lysine	Gamma-glutamyl Amino Acid	Peptide	**0.72**	0.0180	0.0784
Picolinate	Tryptophan Metabolism	Amino Acid	**0.69**	0.0047	0.0368
2'-Deoxyuridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.69**	0.0251	0.0925
1-Methylnicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.68**	0.0239	0.0918
5-Methyluridine (Ribothymidine)	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.66**	0.0037	0.0324
Xanthurenate	Tryptophan Metabolism	Amino Acid	**0.66**	0.0152	0.0719
N-Acetyltyrosine	Tyrosine Metabolism	Amino Acid	**0.62**	0.0053	0.0391
N1-Methyl-4-Pyridone-3-Carboxamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.60**	0.0286	0.0988
N1-Methyl-2-Pyridone-5-Carboxamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.56**	0.0110	0.0583
1-Stearoyl-Gpi (18:0)	Lysophospholipid	Lipid	**0.54**	0.0170	0.0759
Isoleucylglycine	Dipeptide	Peptide	**0.51**	0.0003	0.0081
N-Acetylkynurenine	Tryptophan Metabolism	Amino Acid	**0.51**	0.0119	0.0611
N-Acetyl-2-Aminooctanoate	Fatty Acid, Amino	Lipid	**0.47**	0.0110	0.0583
Cholate	Primary Bile Acid Metabolism	Lipid	**0.44**	0.0221	0.0898
Valylglycine	Dipeptide	Peptide	**0.38**	0.0003	0.0081
3-Dehydrocholate	Secondary Bile Acid Metabolism	Lipid	**0.29**	0.0001	0.0065
Palmitoylcholine	Fatty Acid Metabolism (Acyl Choline)	Lipid	**0.18**	0.0093	0.0554
Arachidonoylcholine	Fatty Acid Metabolism (Acyl Choline)	Lipid	**0.08**	0.0029	0.0298

Table 2.4: Fold-change in maternal (intervillous) plasma (VM/EM) for metabolites (xenobiotics excluded) with significant p (≤0.05) and q (≤0.10), ordered according to fold-change (greatest to smallest), with corresponding sub-pathway and super-pathway.

Metabolite	Sub-Pathway	Super-Pathway	Fold-change (VM/EM)	p-value	q-value
Glucuronide Of C10H18O2	Partially Characterized Molecules	Partially Characterized Molecules	**10.85**	0.0113	0.053
Maleate	Fatty Acid, Dicarboxylate	Lipid	**10.42**	0.0359	0.0857
Adipate (C6-Dc)	Fatty Acid, Dicarboxylate	Lipid	**8.06**	0.0178	0.0625
N-Acetylaspartate (Naa)	Alanine and Aspartate Metabolism	Amino Acid	**7.14**	0.0007	0.0113
Caproate (6:0)	Medium Chain Fatty Acid	Lipid	**6.71**	0.012	0.0539
Glucuronide Of C10H18O2	Partially Characterized Molecules	Partially Characterized Molecules	**6.44**	0.0267	0.0759
Corticosterone	Corticosteroids	Lipid	**5.41**	0.0001	0.0076
Cortisol	Corticosteroids	Lipid	**5.02**	0.0003	0.0079
1-Oleoylglycerol (18:1)	Monoacylglycerol	Lipid	**4.49**	0.0069	0.0417
Arachidoylcarnitine (C20)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**4.24**	0.0128	0.0559
Androsterone Sulfate	Androgenic Steroids	Lipid	**3.99**	0.0042	0.0325
Epiandrosterone Sulfate	Androgenic Steroids	Lipid	**3.7**	0.0132	0.0565
Androstenediol (3Alpha, 17Alpha) Monosulfate	Androgenic Steroids	Lipid	**3.45**	0.0089	0.0476
Glucuronate	Aminosugar Metabolism	Carbohydrate	**3.31**	0.0314	0.08

12-Hete	Eicosanoid	Lipid	**3.21**	0.0053	0.0357
5Alpha-Androstan-3Beta,17Beta-Diol Disulfate	Androgenic Steroids	Lipid	**3.09**	0.0347	0.0839
Oleoyl-Linoleoyl-Glycerol (18:1/18:2)	Diacylglycerol	Lipid	**2.93**	0.0407	0.0919
9,10-Dihome	Fatty Acid, Dihydroxy	Lipid	**2.9**	0.0091	0.0476
Lactosyl-N-Nervonoyl-Sphingosine (D18:1/24:1)	Ceramides	Lipid	**2.83**	0.0024	0.0261
1-Stearoyl-2-Oleoyl-Gpe (18:0/18:1)	Phosphatidylethanolamine (PE)	Lipid	**2.81**	0.0211	0.0693
Androsterone Glucuronide	Androgenic Steroids	Lipid	**2.74**	0.0138	0.0565
1-Linoleoyl-Gpi (18:2)	Lysophospholipid	Lipid	**2.72**	0.0005	0.0109
Palmitoleoyl-Linoleoyl-Glycerol (16:1/18:2)	Diacylglycerol	Lipid	**2.7**	0.0358	0.0857
3-Methyladipate	Fatty Acid, Dicarboxylate	Lipid	**2.68**	0.0295	0.078
Eicosenoylcarnitine (C20:1)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**2.58**	0.0003	0.0079
Erucate (22:1N9)	Long Chain Fatty Acid	Lipid	**2.58**	0.001	0.0144
P-Cresol-Glucuronide	Tyrosine Metabolism	Amino Acid	**2.57**	0.0461	0.0942
Palmitoyl-Linoleoyl-Glycerol (16:0/18:2)	Diacylglycerol	Lipid	**2.57**	0.0265	0.0759
Oleoyl-Linoleoyl-Glycerol (18:1/18:2)	Diacylglycerol	Lipid	**2.49**	0.0121	0.0539

2-Hydroxybehenate	Fatty Acid, Monohydroxy	Lipid	**2.49**	0.0304	0.0792
Oleoyl-Oleoyl-Glycerol (18:1/18:1)	Diacylglycerol	Lipid	**2.47**	0.0449	0.0934
Pristanate	Fatty Acid, Branched	Lipid	**2.45**	0.0439	0.0934
Xanthosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**2.4**	0.0171	0.0615
Oleoyl-Oleoyl-Glycerol (18:1/18:1)	Diacylglycerol	Lipid	**2.38**	0.0066	0.0414
Maltose	Glycogen Metabolism	Carbohydrate	**2.36**	0.0178	0.0625
Lignoceroylcarnitine (C24)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**2.35**	0.0044	0.0338
Ribulonate/Xylulonate	Pentose Metabolism	Carbohydrate	**2.34**	0.0003	0.0079
5Alpha-Pregnan-3Beta,20Alpha-Diol Monosulfate (1)	Progestin Steroids	Lipid	**2.31**	0.0049	0.0353
12,13-Dihome	Fatty Acid, Dihydroxy	Lipid	**2.31**	0.043	0.0934
Fructose	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**2.28**	0.0326	0.0809
Diacylglycerol (16:1/18:2, 16:0/18:3)	Diacylglycerol	Lipid	**2.26**	0.0094	0.0477
Ximenoylcarnitine (C26:1)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**2.25**	0.0025	0.0261
5Alpha-Pregnan-3Beta,20Alpha-Diol Monosulfate (2)	Progestin Steroids	Lipid	**2.25**	0.0039	0.0318
Eicosenoate (20:1)	Long Chain Fatty Acid	Lipid	**2.23**	0.002	0.024

Diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1)	Diacylglycerol	Lipid	**2.21**	0.0424	0.0934
Campesterol	Sterol	Lipid	**2.18**	0.0113	0.053
1-Palmitoyl-2-Oleoyl-Gpe (16:0/18:1)	Phosphatidylethanolamine (PE)	Lipid	**2.18**	0.0225	0.0728
Dihomo-Linoleoylcarnitine (C20:2)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**2.17**	0.0307	0.0792
Laurate (12:0)	Medium Chain Fatty Acid	Lipid	**2.16**	0.0001	0.0065
5Alpha-Pregnan-3Beta,20Beta-Diol Monosulfate (1)	Progestin Steroids	Lipid	**2.13**	0.0053	0.0357
Palmitoyl-Oleoyl-Glycerol (16:0/18:1)	Diacylglycerol	Lipid	**2.12**	0.0168	0.0614
13-Hode + 9-Hode	Fatty Acid, Monohydroxy	Lipid	**2.11**	0.0146	0.057
5,6-Dihydrouracil	Pyrimidine Metabolism, Uracil containing	Nucleotide	**2.06**	0.0278	0.076
Alpha-Ketoglutarate	TCA Cycle	Energy	**2.05**	0.0106	0.0517
3Beta-Hydroxy-5-Cholestenoate	Sterol	Lipid	**2.03**	0.0147	0.057
1-Stearoyl-2-Docosahexaenoyl-Gpe (18:0/22:6)	Phosphatidylethanolamine (PE)	Lipid	**2.03**	0.0363	0.0858
Behenate (22:0)	Long Chain Fatty Acid	Lipid	**2**	0.003	0.0271
Diacylglycerol (14:0/18:1, 16:0/16:1)	Diacylglycerol	Lipid	**2**	0.0271	0.076

Pyruvate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**1.98**	0.0002	0.0079
1-Palmitoleoyl-2-Linolenoyl-Gpc (16:1/18:3)	Phosphatidylcholine (PC)	Lipid	**1.98**	0.0323	0.0809
Lactate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**1.96**	0	0.0015
Myristoyl-Linoleoyl-Glycerol (14:0/18:2)	Diacylglycerol	Lipid	**1.96**	0.0138	0.0565
Lactosyl-N-Palmitoyl-Sphingosine (D18:1/16:0)	Ceramides	Lipid	**1.93**	0.0013	0.0177
10-Nonadecenoate (19:1N9)	Long Chain Fatty Acid	Lipid	**1.92**	0.0051	0.0357
Glycerol	Glycerolipid Metabolism	Lipid	**1.91**	0.0002	0.0079
Ceramide (D18:1/17:0, D17:1/18:0)	Ceramides	Lipid	**1.88**	0.0443	0.0934
Cystine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.87**	0.0054	0.0357
10-Heptadecenoate (17:1N7)	Long Chain Fatty Acid	Lipid	**1.87**	0.0027	0.0265
Decanoylcarnitine (C10)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**1.86**	0.0006	0.0113
Lactosyl-N-Behenoyl-Sphingosine (D18:1/22:0)	Sphingolipid Metabolism	Lipid	**1.86**	0.0311	0.0797
2,3-Dihydroxy-2-Methylbutyrate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.84**	0.0211	0.0693
Docosadienoate (22:2N6)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.84**	0.0002	0.0079

1-Palmitoyl-2-Docosahexaenoyl-Gpe (16:0/22:6)	Phosphatidylethanolamine (PE)	Lipid	**1.83**	0.0332	0.0811
Nervonate (24:1N9)	Long Chain Fatty Acid	Lipid	**1.81**	0.0015	0.0195
Margarate (17:0)	Long Chain Fatty Acid	Lipid	**1.81**	0.0026	0.0261
Linolenate [Alpha Or Gamma; (18:3N3 Or 6)]	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.8**	0.0145	0.057
Tricosanoyl Sphingomyelin (D18:1/23:0)	Sphingolipid Metabolism	Lipid	**1.8**	0.0241	0.074
Laurylcarnitine (C12)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**1.78**	0.0009	0.0144
Dihomo-Linoleate (20:2N6)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.78**	0.0048	0.0353
Palmitoleate (16:1N7)	Long Chain Fatty Acid	Lipid	**1.78**	0.0084	0.0461
N-Stearoyl-Sphingosine (D18:1/18:0)	Ceramides	Lipid	**1.78**	0.009	0.0476
1-Palmitoleoyl-2-Linoleoyl-Gpc (16:1/18:2)	Phosphatidylcholine (PC)	Lipid	**1.78**	0.0116	0.0532
Caprylate (8:0)	Medium Chain Fatty Acid	Lipid	**1.78**	0.0236	0.0734
5-Dodecenoate (12:1N7)	Medium Chain Fatty Acid	Lipid	**1.78**	0.0244	0.0745
Hexadecadienoate (16:2N6)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.78**	0.0321	0.0809
1-Stearoyl-Gpi (18:0)	Lysophospholipid	Lipid	**1.76**	0.0031	0.0273
Cholesterol	Sterol	Lipid	**1.76**	0.0049	0.0353
Octadecenedioate (C18:1-Dc)	Fatty Acid, Dicarboxylate	Lipid	**1.75**	0.0201	0.0685

Docosapentaenoate (N3 Dpa; 22:5N3)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.74**	0.0026	0.0261
Sphingomyelin (D18:2/23:0, D18:1/23:1, D17:1/24:1)	Sphingolipid Metabolism	Lipid	**1.74**	0.0056	0.0362
1-Palmitoyl-Gpi (16:0)	Lysophospholipid	Lipid	**1.74**	0.0166	0.061
Caprate (10:0)	Medium Chain Fatty Acid	Lipid	**1.74**	0.0389	0.09
N-Stearoyl-Sphingadienine (D18:2/18:0)	Ceramides	Lipid	**1.73**	0.0087	0.0471
Nonadecanoate (19:0)	Long Chain Fatty Acid	Lipid	**1.71**	0.001	0.0148
Sphingomyelin (D18:1/21:0, D17:1/22:0, D16:1/23:0)	Sphingolipid Metabolism	Lipid	**1.71**	0.0162	0.0601
17-Methylstearate (I19:0)	Fatty Acid, Branched	Lipid	**1.7**	0.0003	0.0079
Oleate/Vaccenate (18:1)	Long Chain Fatty Acid	Lipid	**1.7**	0.0041	0.0325
15-Methylpalmitate (I17:0)	Fatty Acid, Branched	Lipid	**1.7**	0.005	0.0356
Oleoylcarnitine (C18:1)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**1.7**	0.0068	0.0416
Glycosyl-N-Behenoyl-Sphingadienine (D18:2/22:0)	Ceramides	Lipid	**1.69**	0.0449	0.0934
13-Methylmyristate (I15:0)	Fatty Acid, Branched	Lipid	**1.67**	0.0076	0.0435
Linoleate (18:2N6)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.67**	0.0104	0.0513
Carotene Diol	Vitamin A Metabolism	Cofactors and Vitamins	**1.66**	0.027	0.076
5-Dodecenoylcarnitine (C12:1)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**1.66**	0.0011	0.015

Arachidate (20:0)	Long Chain Fatty Acid	Lipid	**1.66**	0.0037	0.0318
Myristate (14:0)	Long Chain Fatty Acid	Lipid	**1.65**	0.0141	0.0565
1-(1-Enyl-Palmitoyl)-2-Linoleoyl-Gpc (P-16:0/18:2)	Plasmalogen	Lipid	**1.65**	0.014	0.0565
1-Stearoyl-2-Arachidonoyl-Gpe (18:0/20:4)	Phosphatidylethanolamine (PE)	Lipid	**1.65**	0.0207	0.0693
Pregnanediol-3-Glucuronide	Progestin Steroids	Lipid	**1.65**	0.045	0.0934
Imidazole Lactate	Histidine Metabolism	Amino Acid	**1.64**	0.0115	0.0532
1-Myristoyl-2-Arachidonoyl-Gpc (14:0/20:4)	Phosphatidylcholine (PC)	Lipid	**1.64**	0.0201	0.0685
Oleoyl-Arachidonoyl-Glycerol (18:1/20:4)	Diacylglycerol	Lipid	**1.64**	0.0283	0.076
Carotene Diol	Vitamin A Metabolism	Cofactors and Vitamins	**1.63**	0.0266	0.0759
3-Hydroxylaurate	Fatty Acid, Monohydroxy	Lipid	**1.63**	0.0274	0.076
Ceramide (D18:1/20:0, D16:1/22:0, D20:1/18:0)	Ceramides	Lipid	**1.61**	0.033	0.0811
N-Behenoyl-Sphingadienine (D18:2/22:0)	Sphingolipid Metabolism	Lipid	**1.61**	0.0435	0.0934
Glycosyl Ceramide (D18:1/20:0, D16:1/22:0)	Ceramides	Lipid	**1.61**	0.0457	0.0942
1-Palmitoyl-2-Arachidonoyl-Gpe (16:0/20:4)	Phosphatidylethanolamine (PE)	Lipid	**1.58**	0.0231	0.0734

Sphingomyelin (D18:2/21:0, D16:2/23:0)	Sphingolipid Metabolism	Lipid	**1.56**	0.0007	0.0113
Sphingomyelin (D18:1/19:0, D19:1/18:0)	Sphingolipid Metabolism	Lipid	**1.56**	0.0006	0.0113
Octanoylcarnitine (C8)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**1.56**	0.0281	0.076
Glycosyl-N-Stearoyl-Sphingosine (D18:1/18:0)	Ceramides	Lipid	**1.55**	0.0464	0.0942
Pentadecanoate (15:0)	Long Chain Fatty Acid	Lipid	**1.54**	0.0029	0.027
Ceramide (D18:2/24:1, D18:1/24:2)	Ceramides	Lipid	**1.53**	0.0397	0.091
5Alpha-Pregnan-3Beta-Ol,20-One Sulfate	Progestin Steroids	Lipid	**1.52**	0.0405	0.0919
Sphingomyelin (D17:2/16:0, D18:2/15:0)	Sphingolipid Metabolism	Lipid	**1.51**	0.0254	0.075
Glycosyl-N-Palmitoyl-Sphingosine (D18:1/16:0)	Ceramides	Lipid	**1.51**	0.0305	0.0792
N-Palmitoyl-Sphinganine (D18:0/16:0)	Sphingolipid Metabolism	Lipid	**1.51**	0.0447	0.0934
2-Hydroxynervonate	Fatty Acid, Monohydroxy	Lipid	**1.5**	0.0111	0.0529
Ceramide (D16:1/24:1, D18:1/22:1)	Ceramides	Lipid	**1.5**	0.0429	0.0934
Beta-Hydroxyisovalerate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.49**	0.0252	0.075
Aconitate [Cis Or Trans]	TCA Cycle	Energy	**1.47**	0.0059	0.0377

1-Palmitoyl-2-Linoleoyl-Gpc (16:0/18:2)	Phosphatidylcholine (PC)	Lipid	**1.46**	0.0287	0.0766
4-Hydroxy-2-Oxoglutaric Acid	Fatty Acid, Dicarboxylate	Lipid	**1.46**	0.0325	0.0809
Stearate (18:0)	Long Chain Fatty Acid	Lipid	**1.45**	0.0022	0.0259
Palmitate (16:0)	Long Chain Fatty Acid	Lipid	**1.45**	0.0084	0.0461
Cis-4-Decenoylcarnitine (C10:1)	Fatty Acid Metabolism (Acyl Carnitine)	Lipid	**1.45**	0.0161	0.0601
3-Hydroxy-2-Ethylpropionate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.44**	0.0003	0.0079
Glucose	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**1.44**	0.0025	0.0261
Alpha-Tocopherol	Tocopherol Metabolism	Cofactors and Vitamins	**1.41**	0.0219	0.0714
Sphingomyelin (D18:2/14:0, D18:1/14:1)	Sphingolipid Metabolism	Lipid	**1.41**	0.014	0.0565
1-Palmitoyl-2-Gamma-Linolenoyl-Gpc (16:0/18:3N6)	Phosphatidylcholine (PC)	Lipid	**1.41**	0.0204	0.0688
1-Linoleoyl-2-Arachidonoyl-Gpc (18:2/20:4N6)	Phosphatidylcholine (PC)	Lipid	**1.39**	0.0363	0.0858
Thyroxine	Tyrosine Metabolism	Amino Acid	**1.38**	0.015	0.0572
1-Stearoyl-2-Arachidonoyl-Gpi (18:0/20:4)	Phosphatidylinositol (PI)	Lipid	**1.37**	0.043	0.0934
Sphingomyelin (D18:2/18:1)	Sphingolipid Metabolism	Lipid	**1.36**	0.002	0.024
9-Hydroxystearate	Fatty Acid, Monohydroxy	Lipid	**1.36**	0.0303	0.0792

Sphingomyelin (D17:1/16:0, D18:1/15:0, D16:1/17:0)	Sphingolipid Metabolism	Lipid	**1.34**	0.0134	0.0565
Behenoyl Sphingomyelin (D18:1/22:0)	Sphingolipid Metabolism	Lipid	**1.33**	0.0237	0.0734
1-Stearoyl-2-Linoleoyl-Gpc (18:0/18:2)	Phosphatidylcholine (PC)	Lipid	**1.33**	0.0325	0.0809
Lignoceroyl Sphingomyelin (D18:1/24:0)	Sphingolipid Metabolism	Lipid	**1.32**	0.017	0.0615
Sphingomyelin (D18:2/23:1)	Sphingolipid Metabolism	Lipid	**1.3**	0.0039	0.0318
1-Palmitoyl-2-Oleoyl-Gpc (16:0/18:1)	Phosphatidylcholine (PC)	Lipid	**1.28**	0.0013	0.0177
Sphingomyelin (D18:1/24:1, D18:2/24:0)	Sphingolipid Metabolism	Lipid	**1.28**	0.0028	0.027
Sphingomyelin (D18:1/14:0, D16:1/16:0)	Sphingolipid Metabolism	Lipid	**1.27**	0.0124	0.0547
Sphingomyelin (D18:1/17:0, D17:1/18:0, D19:1/16:0)	Sphingolipid Metabolism	Lipid	**1.24**	0.004	0.0318
1-Oleoyl-2-Docosahexaenoyl-Gpc (18:1/22:6)	Phosphatidylcholine (PC)	Lipid	**1.24**	0.0066	0.0414
Palmitoyl Sphingomyelin (D18:1/16:0)	Sphingolipid Metabolism	Lipid	**1.23**	0.0138	0.0565
3-Methyl-2-Oxobutyrate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.21**	0.0236	0.0734

Sphingomyelin (D18:1/22:2, D18:2/22:1, D16:1/24:2)	Sphingolipid Metabolism	Lipid	**1.21**	0.0331	0.0811
Citrate	TCA Cycle	Energy	**1.2**	0.0249	0.0749
Sphingomyelin (D18:1/20:0, D16:1/22:0)	Sphingolipid Metabolism	Lipid	**1.2**	0.0254	0.075
Palmitoyl Ethanolamide	Endocannabinoid	Lipid	**1.19**	0.0093	0.0477
Sphingomyelin (D18:2/24:1, D18:1/24:2)	Sphingolipid Metabolism	Lipid	**1.14**	0.0073	0.0424
1-Palmitoyl-2-Palmitoleoyl-Gpc (16:0/16:1)	Phosphatidylcholine (PC)	Lipid	**1.14**	0.04	0.0912
Arginine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.8**	0.0444	0.0934
Trans-4-Hydroxyproline	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.77**	0.0289	0.0768
Histidine	Histidine Metabolism	Amino Acid	**0.76**	0.0156	0.0587
Serine	Glycine, Serine and Threonine Metabolism	Amino Acid	**0.75**	0.0201	0.0685
Citrulline	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.73**	0.0081	0.0457
Threonine	Glycine, Serine and Threonine Metabolism	Amino Acid	**0.72**	0.0275	0.076
Ornithine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.69**	0.0348	0.0839

Tryptophan	Tryptophan Metabolism	Amino Acid	**0.69**	0.0493	0.0997
Indole-3-Carboxylic Acid	Tryptophan Metabolism	Amino Acid	**0.66**	0.0078	0.0445
Tetradecanedioate (C14-Dc)	Fatty Acid, Dicarboxylate	Lipid	**0.66**	0.0281	0.076
Lysine	Lysine Metabolism	Amino Acid	**0.64**	0.0053	0.0357
S-1-Pyrroline-5-Carboxylate	Glutamate Metabolism	Amino Acid	**0.63**	0.0393	0.0905
Picolinate	Tryptophan Metabolism	Amino Acid	**0.61**	0.0135	0.0565
Hexadecenedioate (C16:1-Dc)	Fatty Acid, Dicarboxylate	Lipid	**0.61**	0.0098	0.0491
Gamma-Glutamyl-Alpha-Lysine	Gamma-glutamyl Amino Acid	Peptide	**0.59**	0.0039	0.0318
Indolebutyrate	Tryptophan Metabolism	Amino Acid	**0.58**	0.0444	0.0934
5-Methyluridine (Ribothymidine)	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.58**	0.0005	0.0104
Bilirubin (Z,Z)	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	**0.57**	0.015	0.0572
N-Palmitoylserine	Endocannabinoid	Lipid	**0.56**	0.0419	0.0934
Homoarginine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.51**	0.021	0.0693
Phosphoethanolamine	Phospholipid Metabolism	Lipid	**0.5**	0.0255	0.075
Glycohyocholate	Secondary Bile Acid Metabolism	Lipid	**0.5**	0.0443	0.0934
Bilirubin (E,Z Or Z,E)	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	**0.46**	0.0261	0.0759
Bilirubin (E,E)	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	**0.39**	0.0095	0.0479

Androstenediol (3Beta,17Beta) Disulfate (2)	Androgenic Steroids	Lipid	**0.37**	0.0422	0.0934
Pyridoxal	Vitamin B6 Metabolism	Cofactors and Vitamins	**0.34**	0.0453	0.0937
Adenosine	Purine Metabolism, Adenine containing	Nucleotide	**0.33**	0.0228	0.0731
Oestriol 3-Sulfate	Estrogenic Steroids	Lipid	**0.32**	0.0189	0.0657
17Alpha-Hydroxypregnenolone 3-Sulfate	Pregnenolone Steroids	Lipid	**0.31**	0.0266	0.0759
Androstenediol (3Beta,17Beta) Disulfate (1)	Androgenic Steroids	Lipid	**0.31**	0.0464	0.0942
N-Acetylkynurenine	Tryptophan Metabolism	Amino Acid	**0.3**	0.0145	0.057
21-Hydroxypregnenolone Monosulfate	Pregnenolone Steroids	Lipid	**0.3**	0.0278	0.076
N-Acetyl-2-Aminooctanoate	Fatty Acid, Amino	Lipid	**0.27**	0.0031	0.0273
Andro Steroid Monosulfate C19H28O6S	Androgenic Steroids	Lipid	**0.26**	0.0174	0.0619
16A-Hydroxy Dhea 3-Sulfate	Androgenic Steroids	Lipid	**0.22**	0.0071	0.042
3-Dehydrocholate	Secondary Bile Acid Metabolism	Lipid	**0.13**	0.0022	0.0259

2.9.5 Xenobiotics

Metronidazole is given to all non-allergic women having elective caesarean sections at St Michael's Hospital, and in this case all ten women included who had an elective caesarean section were administered it at the time of knife-to-skin, while none of the women included who laboured and went on to have a vaginal delivery received it, and this was reflected in the results. There were significant increases in both maternal and cord plasma of the following analgesics: 4-acetamidophenol, 3-(N-acetyl-L-cystein-S-yl) acetaminophen, 4-acetaminophen sulfate, 4-acetamidophenylglucuronide, 2-hydroxyacetaminophen sulfate, 2-methoxyacetaminophen sulfate, 2-methoxyacetaminophen glucuronide, 3-(cystein-S-yl) acetaminophen and ibuprofen. There was a large increase of triethanolamine in cord and maternal blood from women who were delivered vaginally. Ranitidine was significantly decreased in cord plasma of labouring vs non-labouring women, but there were no changes in maternal plasma. Ranitidine is given to all women who have an elective caesarean section to take the night before and then the morning of the operation. For women in labour ranitidine is given if they have an epidural when they are in active labour. This may explain the difference observed in these levels (Tables 2.5 and 2.6).

Table 2.5: Fold-change in cord plasma (VC/EC) for xenobiotics with significant p (≤0.05) and q (≤0.10), ordered according to fold-change (greatest to smallest), with corresponding sub-pathway and super-pathway (of note, the fold-change for Metronidazole is recorded as 0 as there was 0 quantity recorded in the VC group, however the p-value is the smallest).

Metabolite	Sub-Pathway	Super-Pathway	Fold-change (VC/EC)	p-value	q-value
Triethanolamine	Chemical	Xenobiotics	**39.65**	0.0028	0.0298
4-Acetamidophenol	Drug - Analgesics, Anesthetics	Xenobiotics	**16.03**	0.0000	0.0031
4-Acetaminophen Sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**7.73**	0.0004	0.0093
4-Acetamidophenylglucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**5.60**	0.0003	0.0093
2-Hydroxyacetaminophen Sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**5.60**	0.0005	0.0100
2-Methoxyacetaminophen Sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**5.09**	0.0002	0.0081
2-Methoxyacetaminophen Glucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**4.13**	0.0004	0.0093
3-(Cystein-S-Yl) Acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics	**3.99**	0.0005	0.0099
3-(N-Acetyl-L-Cystein-S-Yl) Acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics	**3.71**	0.0087	0.0526
Ibuprofen	Drug - Analgesics, Anesthetics	Xenobiotics	**2.71**	0.0011	0.0146
Benzoate	Benzoate Metabolism	Xenobiotics	**2.26**	0.0158	0.0734
Ranitidine	Drug - Gastrointestinal	Xenobiotics	**0.54**	0.0059	0.0413
Acesulfame	Food Component/Plant	Xenobiotics	**0.31**	0.0061	0.0424
Saccharin	Food Component/Plant	Xenobiotics	**0.04**	0.0000	0.0000
Metronidazole	Drug - Antibiotic	Xenobiotics	**0.00**	0.0000	0.0000

Table 2.6: Fold-change in maternal (intervillous) plasma (VM/EM) for xenobiotics with significant p (≤0.05) and q (≤0.10), ordered according to fold-change (greatest to smallest) (of note, the fold-change for Metronidazole is recorded as 0 as there was 0 quantity recorded in the VC group, however the p-value is the smallest).

Metabolite	Sub-Pathway	Super-Pathway	Fold-change (VM/EM)	p-value	q-value
Triethanolamine	Chemical	Xenobiotics	**19.54**	0.0121	0.0539
4-Acetamidophenol	Drug - Analgesics, Anesthetics	Xenobiotics	**18.20**	0.0000	0.0039
3-(Cystein-S-Yl) Acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics	**10.15**	0.0007	0.0113
4-Acetaminophen Sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**10.09**	0.0002	0.0079
2-Methoxyacetaminophen Sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**9.91**	0.0001	0.0066
4-Acetamidophenylglucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**8.84**	0.0002	0.0078
3-(N-Acetyl-L-Cystein-S-Yl) Acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics	**8.27**	0.0070	0.0417
2-Hydroxyacetaminophen Sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**8.06**	0.0003	0.0079
2-Methoxyacetaminophen Glucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**7.39**	0.0001	0.0070
4-Hydroxybenzoate	Benzoate Metabolism	Xenobiotics	**6.14**	0.0107	0.0517
Morphine-6-Glucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**3.91**	0.0370	0.0870
Morphine-3-Glucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**3.05**	0.0380	0.0885
Ibuprofen	Drug - Analgesics, Anesthetics	Xenobiotics	**2.90**	0.0004	0.0082
Benzoate	Benzoate Metabolism	Xenobiotics	**2.78**	0.0444	0.0934
Salicyluric Glucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**2.75**	0.0000	0.0003

Phytanate	Food Component/Plant	Xenobiotics	**2.51**	0.0025	0.0261
3-Methylxanthine	Xanthine Metabolism	Xenobiotics	**2.19**	0.0233	0.0734
7-Methylxanthine	Xanthine Metabolism	Xenobiotics	**1.78**	0.0247	0.0748
Umbelliferone Sulfate	Food Component/Plant	Xenobiotics	**0.32**	0.0376	0.0878
Saccharin	Food Component/Plant	Xenobiotics	**0.14**	0.0007	0.0113
Metronidazole	Drug - Antibiotic	Xenobiotics	**0.00**	0.0000	0.0000

2.9.6 Lipid super-pathway

The lipid super-pathway contained the most differences between labouring and non-labouring women (Figure 2.2 and Table 2.2). Most of these changes were significant increases in maternal plasma (VM/EM). 405 metabolites were identified in this super-pathway: 34.3% (139/405) of the metabolites significantly increased and 3.5% (14/405) significantly decreased in the maternal plasma of women who laboured when compared to non-labour; correspondingly, 18.0% (73/405) of the metabolites significantly increased in the cord plasma of women who laboured, and 3.0% (12/405) significantly decreased. Of note, 22 sphingolipids (Table 2.7) significantly increased in maternal plasma of labouring women with only one corresponding increase found in cord plasma; whereas the sub-pathway with the most significant changes seen in the cord plasma within the lipid super-pathway were fatty acid metabolism (acyl carnitine), long chain fatty acids and polyunsaturated fatty acids (Figure 2.2). There were significant increases in the medium and long chain fatty acids in both cord and maternal plasma and there were selective changes in the maternal and fetal circulation for acyl carnitines, acyl cholines, and eicosanoids (12-HETE and 23-HHTrE) (Tables 2.2 and 2.3).

Table 2.7: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for sphingolipids, with corresponding p and q values (significant when p≤0.05 and q≤0.10).

Sphingolipid	Fold-change (VC/EC)	p-value	q-value	Fold-change (VM/EM)	p-value	q-value
Sphinganine	**1.54**	NS	0.3393	**1.28**	NS	0.362
Sphinganine-1-Phosphate	**1.28**	NS	0.2858	**1.53**	NS	0.197
N-Palmitoyl-Sphinganine (D18:0/16:0)	**1.42**	NS	0.1602	**1.51**	0.045	0.093
N-Palmitoyl-Sphingadienine (D18:2/16:0)	**1.21**	NS	0.494	**1.28**	NS	0.213
N-Behenoyl-Sphingadienine (D18:2/22:0)	**1.06**	NS	0.5461	**1.61**	0.044	0.093
Myristoyl Dihydrosphingomyelin (D18:0/14:0)	**1.17**	NS	0.216	**1.02**	NS	0.498
Palmitoyl Dihydrosphingomyelin (D18:0/16:0)	**1.04**	NS	0.4674	**0.98**	NS	0.452
Behenoyl Dihydrosphingomyelin (D18:0/22:0)	**1.06**	NS	0.5635	**0.9**	NS	0.261
Palmitoyl Sphingomyelin (D18:1/16:0)	**1.06**	NS	0.2737	**1.23**	0.014	0.057
Stearoyl Sphingomyelin (D18:1/18:0)	**0.9**	NS	0.3165	**1.02**	NS	0.466
Behenoyl Sphingomyelin (D18:1/22:0)	**0.94**	NS	0.4308	**1.33**	0.024	0.073
Tricosanoyl Sphingomyelin (D18:1/23:0)	**1.05**	NS	0.5663	**1.8**	0.024	0.074
Lignoceroyl Sphingomyelin (D18:1/24:0)	**1.06**	NS	0.5133	**1.32**	0.017	0.062
Sphingomyelin (D18:1/14:0, D16:1/16:0)	**1.05**	NS	0.4497	**1.27**	0.012	0.055
Sphingomyelin (D18:2/14:0, D18:1/14:1)	**1.08**	NS	0.4942	**1.41**	0.014	0.057
Sphingomyelin (D17:1/16:0, D18:1/15:0, D16:1/17:0)	**1.12**	NS	0.2597	**1.34**	0.013	0.057

Sphingomyelin (D18:2/16:0, D18:1/16:1)	**1.03**	NS	0.5712	**1.09**	NS	0.118
Sphingomyelin (D18:1/17:0, D17:1/18:0, D19:1/16:0)	**1.09**	NS	0.3643	**1.24**	0.004	0.032
Sphingomyelin (D18:1/18:1, D18:2/18:0)	**0.98**	NS	0.5218	**0.88**	NS	0.335
Sphingomyelin (D18:1/20:0, D16:1/22:0)	**0.92**	NS	0.3642	**1.2**	0.025	0.075
Sphingomyelin (D18:1/20:1, D18:2/20:0)	**0.93**	NS	0.4066	**0.91**	NS	0.388
Sphingomyelin (D18:1/21:0, D17:1/22:0, D16:1/23:0)	**1.14**	NS	0.4942	**1.71**	0.016	0.06
Sphingomyelin (D18:1/22:1, D18:2/22:0, D16:1/24:1)	**1**	NS	0.5663	**1.09**	NS	0.351
Sphingomyelin (D18:2/23:0, D18:1/23:1, D17:1/24:1)	**1.28**	NS	0.1998	**1.74**	0.006	0.036
Sphingomyelin (D18:1/24:1, D18:2/24:0)	**1.06**	NS	0.4865	**1.28**	0.003	0.027
Sphingomyelin (D18:2/24:1, D18:1/24:2)	**1**	NS	0.579	**1.14**	0.007	0.042
Sphingosine	**1.83**	NS	0.1642	**0.98**	NS	0.539
Sphingosine 1-Phosphate	**1.27**	NS	0.1783	**1.38**	NS	0.109
Sphingomyelin (D18:2/23:1)	**1.12**	NS	0.3657	**1.3**	0.004	0.032
Sphingomyelin (D18:2/21:0, D16:2/23:0)	**1.23**	NS	0.2532	**1.56**	0.001	0.011
Sphingomyelin (D18:1/20:2, D18:2/20:1, D16:1/22:2)	**1.06**	NS	0.5133	**1.18**	NS	0.208
Sphingomyelin (D18:2/24:2)	**0.89**	NS	0.2953	**0.94**	NS	0.468
Sphingomyelin (D18:1/25:0, D19:0/24:1, D20:1/23:0, D19:1/24:0)	**1.64**	0.046	0.1299	**1.14**	NS	0.314
Sphingomyelin (D18:1/22:2, D18:2/22:1, D16:1/24:2)	**0.92**	NS	0.3643	**1.21**	0.033	0.081
Sphingomyelin (D18:0/20:0, D16:0/22:0)	**1.16**	NS	0.5509	**0.92**	NS	0.309
Sphingomyelin (D18:0/18:0, D19:0/17:0)	**1.01**	NS	0.5748	**0.81**	NS	0.22

Sphingomyelin (D17:2/16:0, D18:2/15:0)	**1.09**	NS	0.4276	**1.51**	0.025	0.075
Sphingomyelin (D18:2/18:1)	**1.07**	NS	0.4308	**1.36**	0.002	0.024
Sphingomyelin (D18:1/19:0, D19:1/18:0)	**1.09**	NS	0.4352	**1.56**	0.001	0.011
N-Palmitoyl-Heptadecasphingosine (D17:1/16:0)	**1.15**	NS	0.4012	**1.54**	NS	0.188
N-Stearoyl-Sphinganine (D18:0/18:0)	**1.12**	NS	0.5295	**1.16**	NS	0.49
Sphingadienine	**1.35**	NS	0.5274	**0.58**	NS	0.264
Lactosyl-N-Behenoyl-Sphingosine (D18:1/22:0)	**1.94**	NS	0.2146	**1.86**	0.031	0.08

Table 2.8: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for pregnenolone steroids, with corresponding p and q values (significant when p≤0.05 and q≤0.10).

Pregnenolone Steroids	Fold-change (VC/EC)	p-value	q-value	Fold-change (VM/EM)	p-value	q-value
Pregnenolone Sulfate	**1.10**	NS	0.5121	**0.39**	0.055	0.108
17alpha-Hydroxypregnenolone 3-Sulfate	**1.23**	NS	0.5178	**0.31**	0.027	0.076
17alpha-Hydroxypregnanolone Glucuronide	**0.85**	NS	0.2681	**0.9**	NS	0.435
21-Hydroxypregnenolone Monosulfate	**0.78**	NS	0.2146	**0.3**	0.028	0.076
21-Hydroxypregnenolone Disulfate	**0.94**	NS	0.5039	**0.44**	NS	0.15
21-Hydroxypregnanolone Disulfate	**1.12**	NS	0.4033	**1**	NS	0.417
Pregnen-Diol Disulfate C21H34O8S2	**1.37**	NS	0.5635	**1.01**	NS	0.47
Pregn Steroid Monosulfate C21H34O5S	**1.41**	NS	0.3514	**0.86**	NS	0.451

Table 2.9: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for progestin steroids, with corresponding p and q values (significant when p≤0.05 and q≤0.10)

Progestin Steroids	Fold-change (VC/EC)	p-value	q-value	Fold-change (VM/EM)	p-value	q-value
Progesterone	**1.42**	NS	0.1545	**1.38**	NS	0.532
5alpha-Pregnan-3beta-Ol,20-One Sulfate	**1.11**	NS	0.4856	**1.52**	0.041	0.092
5alpha-Pregnan-3beta,20beta-Diol Monosulfate	**1.34**	NS	0.3011	**2.13**	0.005	0.036
5alpha-Pregnan-3beta,20alpha-Diol Monosulfate 1	**1.36**	NS	0.2510	**2.31**	0.005	0.035
5alpha-Pregnan-3beta,20alpha-Diol Monosulfate 2	**1.52**	NS	0.3514	**2.25**	0.004	0.032
5alpha-Pregnan-3beta,20alpha-Diol Disulfate	**1.12**	NS	0.3907	**1.34**	NS	0.309
5alpha-pregnan-diol disulfate	**1.05**	NS	0.4194	**1.69**	NS	0.3101
5alpha-Pregnan-3alpha,20beta-Diol Disulfate	**1.43**	NS	0.1853	**0.86**	NS	0.314
Pregnanediol-3-Glucuronide	**0.95**	NS	0.5413	**1.65**	0.045	0.093
Pregnanolone/Allopregnanolone Sulfate	**1.08**	NS	0.5635	**1.67**	0.059	0.111

Table 2.10: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for endocannabinoid steroids, with corresponding p and q values (significant when p≤0.05 and q≤0.10).

Endocannabinoid Steroids	Fold-change (VC/EC)	p-value	q-value	Fold-change (VM/EM)	p-value	q-value
Palmitoyl Ethanolamide (PEA)	**1.19**	0.0357	0.1158	**1.19**	0.009	0.048
N-Palmitoylserine	**0.93**	NS	0.4460	**0.56**	0.042	0.093
Oleoyl Ethanolamide (OEA)	**1.31**	0.0226	0.0904	**1.39**	NS	0.209
Arachidonoyl Ethanolamide (AEA/Andamide)	**1.48**	0.0296	0.1015	**1.25**	NS	0.277
Linoleoyl Ethanolamide	**1.34**	0.0707	0.1642	**1.32**	NS	0.226
N-Stearoyltaurine	**0.96**	NS	0.5605	**1.22**	NS	0.413
N-Palmitoyltaurine	**0.97**	NS	0.5321	**0.81**	NS	0.255
N-Linoleoyltaurine	**0.98**	NS	0.5461	**0.83**	NS	0.196
N-Oleoylserine	**0.94**	NS	0.5133	**0.77**	NS	0.311

Table 2.11: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for ceramides, with corresponding p and q values (significant when p≤0.05 and q≤0.10).

Ceramide	Fold-change (VC/EC)	p-value	q-value	Fold-change (VM/EM)	p-value	q-value
N-Palmitoyl-Sphingosine (D18:1/16:0)	**1.38**	NS	0.1455	**1.49**	NS	0.135
N-Stearoyl-Sphingosine (D18:1/18:0)	**1.27**	NS	0.351	**1.78**	0.009	0.048
N-Stearoyl-Sphingadienine (D18:2/18:0)	**1.49**	NS	0.1491	**1.73**	0.009	0.047
Ceramide (D16:1/24:1, D18:1/22:1)	**1.26**	NS	0.3228	**1.5**	0.043	0.093
Ceramide (D18:1/14:0, D16:1/16:0)	**1.28**	NS	0.3303	**1.53**	NS	0.119
Ceramide (D18:1/17:0, D17:1/18:0)	**1.79**	NS	0.195	**1.88**	0.044	0.093
Ceramide (D18:1/20:0, D16:1/22:0, D20:1/18:0)	**1.05**	NS	0.5688	**1.61**	0.033	0.081
Ceramide (D18:2/24:1, D18:1/24:2)	**1.34**	NS	0.2513	**1.53**	0.04	0.091
Glycosyl-N-Palmitoyl-Sphingosine (D18:1/16:0)	**1.35**	NS	0.1674	**1.51**	0.031	0.079
Glycosyl-N-Stearoyl-Sphingosine (D18:1/18:0)	**1.21**	NS	0.4308	**1.55**	0.046	0.094
Glycosyl-N-Behenoyl-Sphingadienine (D18:2/22:0)	**1.38**	NS	0.4688	**1.69**	0.045	0.093
Glycosyl-N-(2-Hydroxynervonoyl)-Sphingosine (D18:1/24:1(2OH))	**0.57**	NS	0.5276	**1.99**	NS	0.121
Lactosyl-N-Palmitoyl-Sphingosine (D18:1/16:0)	**1.73**	NS	0.1461	**1.93**	0.001	0.018
Lactosyl-N-Nervonoyl-Sphingosine (D18:1/24:1)	**2.99**	NS	0.1762	**2.83**	0.002	0.026
Glycosyl Ceramide (D18:1/20:0, D16:1/22:0)	**1.22**	NS	0.5114	**1.61**	0.046	0.094
Glycosyl Ceramide (D18:1/23:1, D17:1/24:1)	**1.67**	NS	0.3228	**1.36**	NS	0.324
Glycosyl Ceramide (D18:2/24:1, D18:1/24:2)	**1.33**	NS	0.3755	**1.5**	NS	0.112

Of the steroid metabolites, cholesterol was significantly increased in the maternal plasma of women who laboured (VM/EM) (Table 2.4) but did not change in the cord plasma (VC/EC). 17α-hydroxypregnenolone 3-sulfate and 21-hydroxypregnenolone monosulfate were significantly decreased in the maternal plasma of women who laboured (VM/EM),

with no corresponding changes seen in the cord plasma (Table 2.8). There were significant increases in 5α-pregnan-3β-ol,20-one sulfate, 5α-pregnan-3β,20β-diol monosulfate, 5α-pregnan-3β,20α-diol monosulfate, 5α-pregnan-3β,20α-diol monosulfate and pregnanediol-3-glucuronide in maternal plasma, which were not seen in the cord (Table 2.9).

Of the steroid metabolites, cholesterol was significantly increased in the maternal plasma of women who laboured (VM/EM) (Table 2.4) but did not change in the cord plasma (VC/EC). 17α-hydroxypregnenolone 3-sulfate and 21-hydroxypregnenolone monosulfate were significantly decreased in the maternal plasma of women who laboured (VM/EM), with no corresponding changes seen in the cord plasma (Table 2.8). There were significant increases in 5α-pregnan-3β-ol,20-one sulfate, 5α-pregnan-3β,20β-diol monosulfate, 5α-pregnan-3β,20α-diol monosulfate, 5α-pregnan-3β,20α-diol monosulfate and pregnanediol-3-glucuronide in maternal plasma, which were not seen in the cord (Table 2.9).

Of the endocannabinoid metabolites, three were significantly increased in the cord plasma of women who laboured when compared with those who did not (VC/EC); and one significantly increased, and one significantly decreased in the maternal plasma of women who laboured (VM/EM) (Table 2.10). Twelve of seventeen ceramide metabolites significantly increased in the maternal plasma (VM/EM), however there were no such significant differences seen in the cord plasma samples (VC/EC) (Table 2.11).

Corticosterone significantly increased in maternal plasma (VM/EM) and in cord plasma (VC/EC) (5.41 and 3.09-fold, respectively) of women who laboured, and the corresponding increases for cortisol were 5.02-fold and 3.57-fold. There

were also significant increases in cortisone and cortisone 21-sulfate in cord plasma but not in maternal plasma (Tables 2.3 and 2.4).

Androstenediol (3β,17β) disulfate and andro steroid monosulfate C19H28O4S significantly decreased in maternal plasma of women who laboured (VM/EM). 16a-hydroxy DHEA 3-sulfate significantly decreased in both the cord and maternal plasma. Androsterone glucuronide, epiandrosterone sulfate, 5α-androstan-3β and 17β-diol disulfate significantly increased in the maternal plasma, and androsterone sulfate and androstenediol (3α, 17α) monosulfate significantly increased in both maternal and cord plasma (Tables 2.3 and 3.4).

Of the oestrogenic steroids, oestrone 3-sulfate significantly increased in the cord plasma with no change in the maternal plasma, while oestriol 3-sulfate significantly decreased in the maternal plasma with no change in the cord (Tables 2.3 and 2.4).

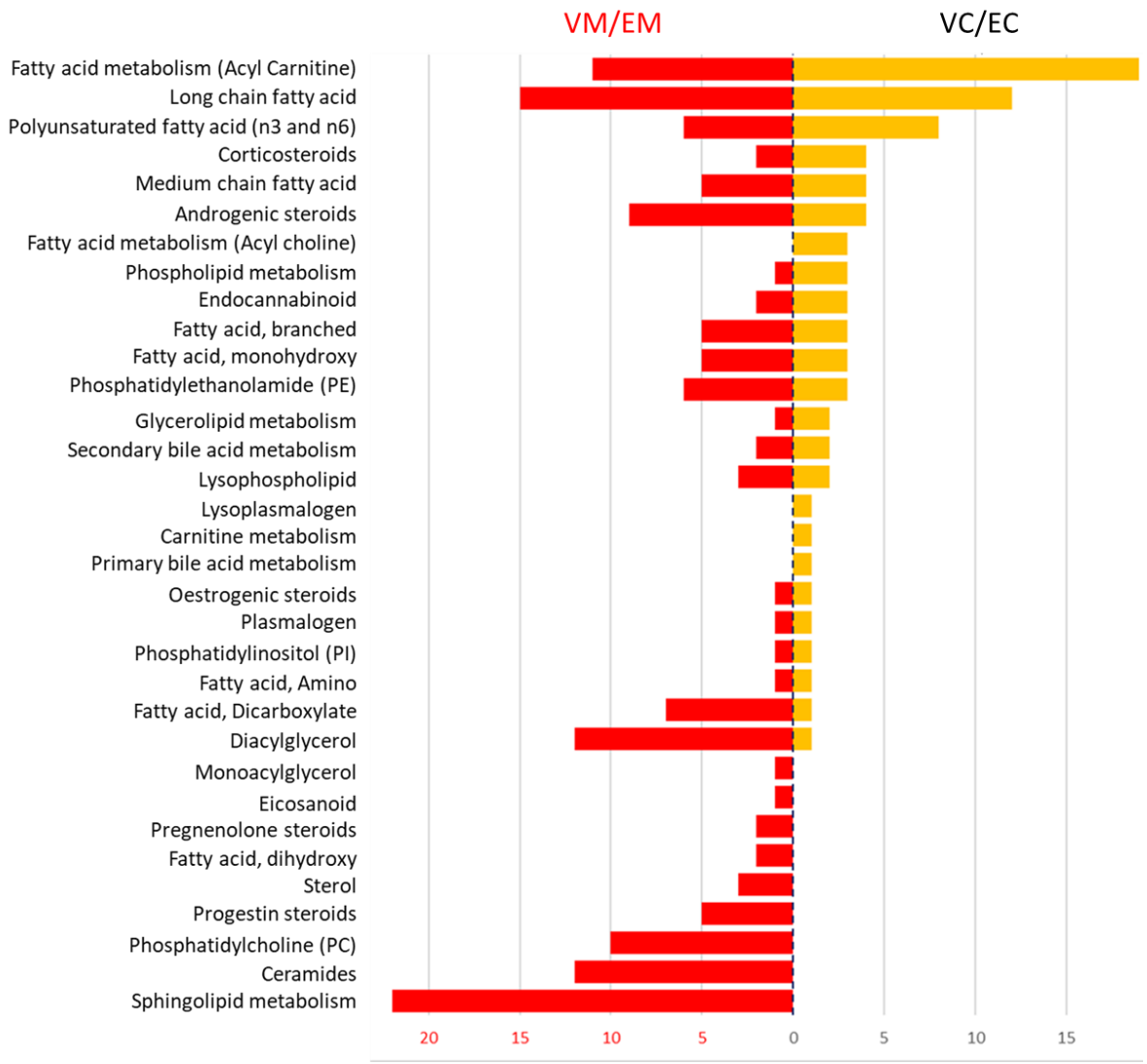

Figure 2.2: Differences in the lipid superpathway between the maternal and cord blood. Comparison of number of metabolites with significant changes between maternal (intervillous) plasma taken from women who laboured and maternal (intervillous) plasma taken from women who did not labour (VM/EM) compared with cord plasma taken from women who laboured and cord plasma taken from women who did not labour (VC/EC) for each of the sub-pathways within the Lipids super-pathways.

2.9.7 Nucleotides

The most significant changes seen within the nucleotide super-pathway were within VC/EC (Figure 2.3), including significant increases in inosine, hypoxanthine, and xanthine, and a significant decrease in N1-methylinosine. 5-methyluridine (ribothymidine) significantly decreased in both cord (VC/EC) and

maternal plasma (VM/EM), while 5,6-dihydrouracil and xanthosine significantly increased (Tables 2.3 and 2.4).

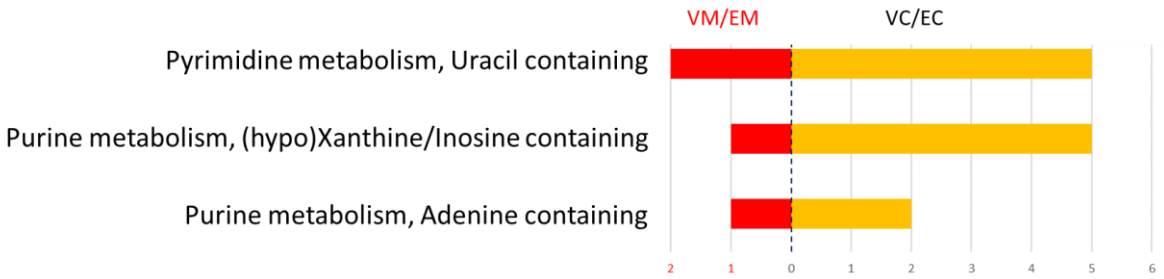

Figure 2.3: Differences in the nucleotides super-pathway between the maternal and cord blood. Comparison of number of metabolites with significant changes between maternal (intervillous) plasma taken from women who laboured and maternal (intervillous) plasma taken from women who did not labour (VM/EM) compared with cord plasma taken from women who laboured and cord plasma taken from women who did not labour (VC/EC) for each of the sub-pathways within the Nucleotides super-pathway.

2.9.8 Amino acids

Unlike the other super-pathways, most of the significant changes within the amino-acid super-pathway were decreases in VC/EC and VM/EM (Figure 2.4). Serine, histidine, tryptophan, N-acetylkynurenine, picolinate, indole-3-carboxylic acid, citrulline and arginine significantly decreased in both VC/EC and VM/EM. Carnosine, N-acetyltyrosine and xanthurenate significantly decreased in VC/EC only, while S-1-pyrroline-5-carboxylate, indolebutyrate, ornithine, homoarginine, threonine, lysine and trans-4-hydroxyproline decreased in VM/EM only. By contrast, N-acetylaspartate increased 7.14-fold in VM/EM only, and cysteine-glutathione disulfide increased 5.21-fold in VC/EC (Tables 2.3 and 2.4).

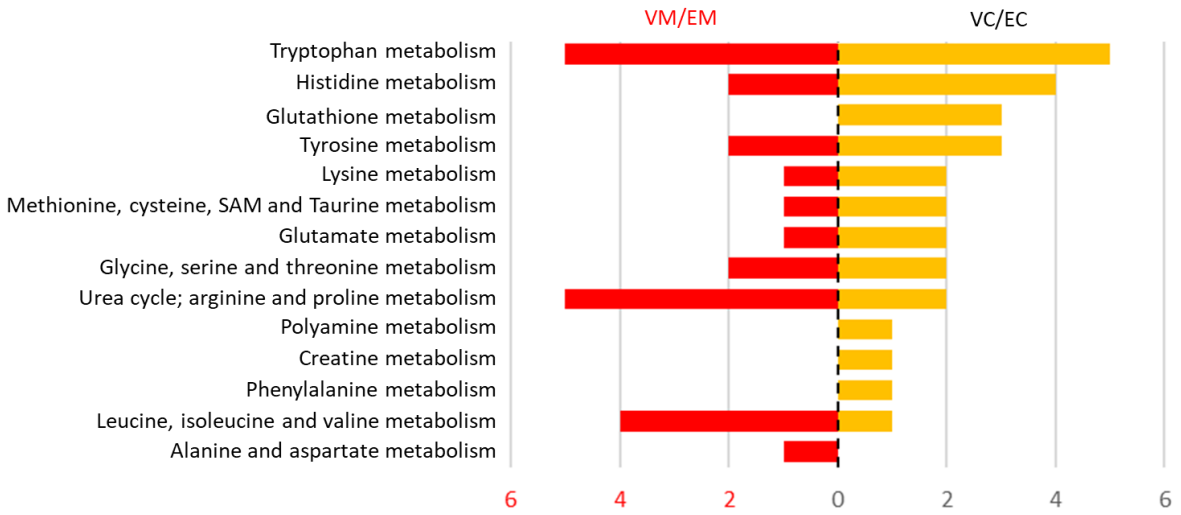

Figure 2.4: Differences in the amino acids super-pathway between the maternal and cord blood. Comparison of number of metabolites with significant changes between maternal (intervillous) plasma taken from women who laboured and maternal (intervillous) plasma taken from women who did not labour (VM/EM) compared with cord plasma taken from women who laboured and cord plasma taken from women who did not labour (VC/EC) for each of the sub-pathways within the Amino Acids super-pathway.

2.9.9 Peptides

24 metabolites were measured in this super-pathway (Figure 2.5 and Table 2.2). Two of the four fibrinogen cleavage peptide metabolites measured were significantly increased in cord plasma of women who laboured compared with non-labour: fibrinopeptide A des-ala increased 12.12-fold and fibrinopeptide A phosphono-ser increased 11.34-fold. There were no corresponding changes in maternal plasma.

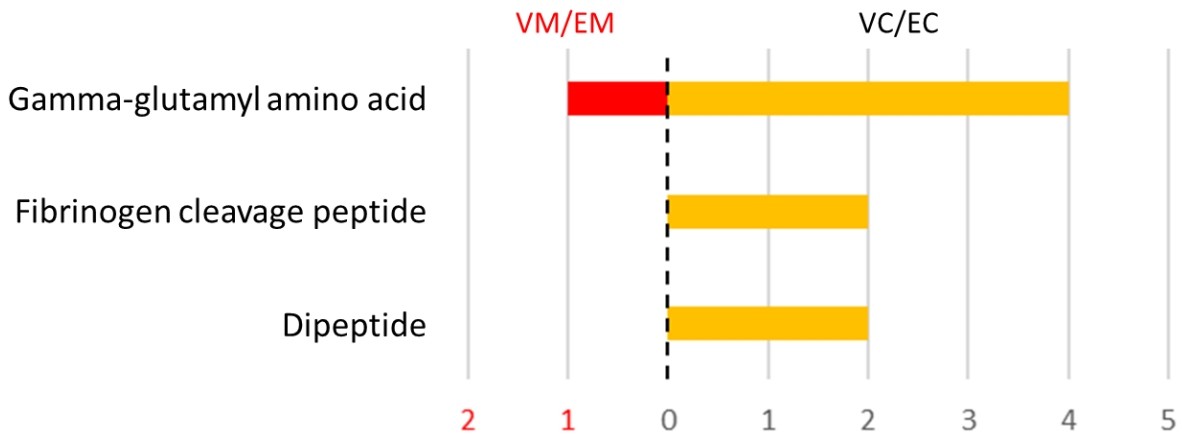

Figure 2.5: Differences in the peptides super-pathway between the maternal and cord blood. Comparison of number of metabolites with significant changes between maternal (intervillous) plasma taken from women who laboured and maternal (intervillous) plasma taken from women who did not labour (VM/EM) compared with cord plasma taken from women who laboured and cord plasma taken from women who did not labour (VC/EC) for each of the sub-pathways within the Peptides super-pathway.

2.9.10 Carbohydrate metabolism

29 metabolites were measured in this super-pathway (Figure 2.6 and Table 2.2), and of these glucose, maltose, fructose, ribulonate/xylulonate, pyruvate and lactate were significantly increased in both the cord and maternal plasma taken from women who laboured compared with non-labour. 3-phosphoglycerate was significantly increased in cord plasma.

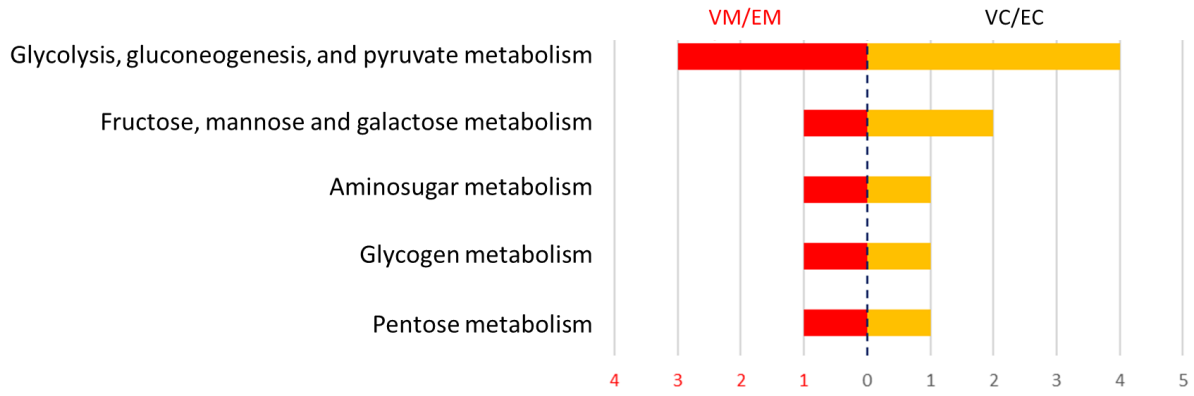

Figure 2.6: Differences in the carbohydrates super-pathway between the maternal and cord blood. Comparison of number of metabolites with significant changes between maternal (intervillous) plasma taken from women who laboured and maternal (intervillous) plasma taken from women who did not labour (VM/EM) compared with cord plasma taken from women who laboured and cord plasma taken from women who did not labour (VC/EC) for each of the sub-pathways within the Carbohydrates super-pathway.

2.9.11 Energy super-pathway

11 metabolites were measured in this super-pathway (Table 2.2). Six metabolites of the TCA cycle were significantly increased in cord plasma of labouring women, but only three increased in maternal plasma (Figure 2.7, Tables 2.3 and 2.4). There were no significant changes in oxidative phosphorylation.

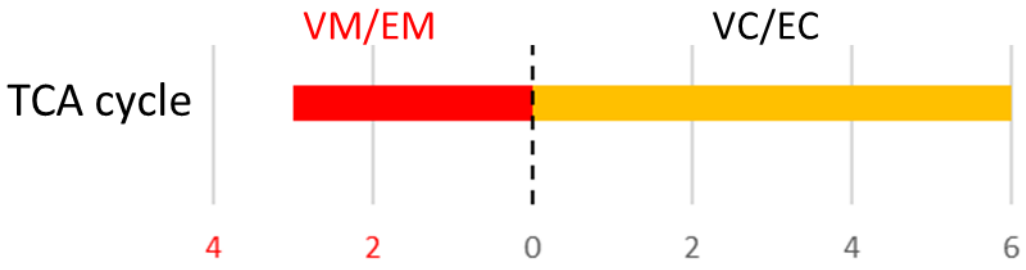

Figure 2.7: Differences in the energy super-pathway between the maternal and cord blood. Comparison of number of metabolites with significant changes between maternal (intervillous) plasma taken from women who laboured and maternal (intervillous) plasma taken from women who did not labour (VM/EM) compared with cord plasma taken from women who laboured and cord plasma taken from women who did not labour (VC/EC) for each of the sub-pathways within the Energy super-pathway.

2.9.12 Co-factors and vitamins

31 metabolites were measured in this super-pathway (Figure 2.8 and Table 2.2). Of these, 1-methylnicotinamide, N1-methyl-2-pyridone-5-carboxamide and N1-methyl-4-pyridone-3-carboxamide from nicotinate and nicotinamide metabolism were significantly decreased in the cord plasma taken from women who laboured (VC/EC), and adenosine 5'-diphosphoribose (ADP-ribose) significantly increased (Table 2.2). There were no such changes in the maternal plasma.

Heme significantly increased 14.3-fold in cord plasma (VC/EC), with no change seen in the maternal plasma, and two metabolites of vitamin A metabolism and one metabolite of vitamin B6 metabolism were significantly increased with labour in maternal plasma (VM/EM), with no corresponding changes in the cord (Tables 2.3 and 2.4).

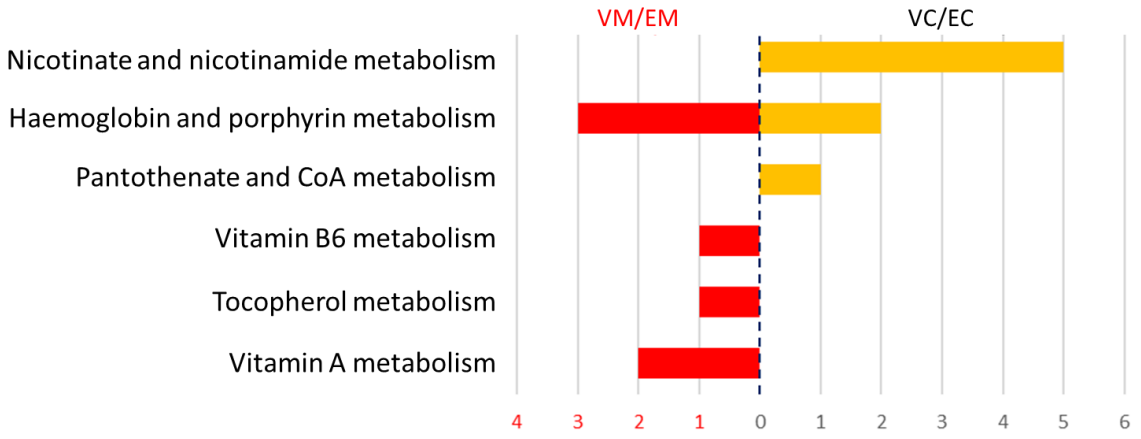

Figure 2.8: Differences in the cofactors and vitamins super-pathway between the maternal and cord blood. Comparison of number of metabolites with significant changes between maternal (intervillous) plasma taken from women who laboured and maternal (intervillous) plasma taken from women who did not labour (VM/EM) compared with cord plasma taken from women who laboured and cord plasma taken from women who did not labour (VC/EC) for each of the sub-pathways within the Cofactors and Vitamins super-pathway.

2.9.13 Over-representation analysis results

Table 2.12 and Figure 2.9 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for those metabolites which were significant at $p<0.05$. Beta oxidation of very long chain fatty acids, glucose-alanine cycle, and fatty acid biosynthesis were the most over-represented pathways, with more than the expected number of hits, although the over-representation did not reach statistical significance for any of these pathways.

Table 2.12: Results of the over-representation enrichment analysis for metabolites with significant p (≤0.05) and p (<0.1) for VM/EM, where total is the total number of metabolites in the original pathway, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathway	Total	Expected	Hits	Raw p	Holm p
Beta Oxidation of Very Long Chain Fatty Acids	17	2.32	4	0.193	1
Glucose-Alanine Cycle	13	1.78	3	0.258	1
Fatty Acid Biosynthesis	35	4.79	6	0.342	1
Methylhistidine Metabolism	4	0.547	1	0.445	1
Alpha Linolenic Acid and Linoleic Acid Metabolism	19	2.6	3	0.493	1
Phosphatidylethanolamine Biosynthesis	12	1.64	2	0.504	1
Urea Cycle	29	3.96	4	0.576	1
Transfer of Acetyl Groups into Mitochondria	22	3.01	3	0.597	1
Ammonia Recycling	32	4.38	4	0.658	1
Biotin Metabolism	8	1.09	1	0.693	1
Alanine Metabolism	17	2.32	2	0.699	1
Oxidation of Branched Chain Fatty Acids	26	3.55	3	0.713	1
Phytanic Acid Peroxisomal Oxidation	26	3.55	3	0.713	1
Aspartate Metabolism	35	4.79	4	0.728	1
Gluconeogenesis	35	4.79	4	0.728	1
Homocysteine Degradation	9	1.23	1	0.735	1
Lactose Degradation	9	1.23	1	0.735	1
Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	27	3.69	3	0.738	1
Malate-Aspartate Shuttle	10	1.37	1	0.772	1
Pyruvaldehyde Degradation	10	1.37	1	0.772	1

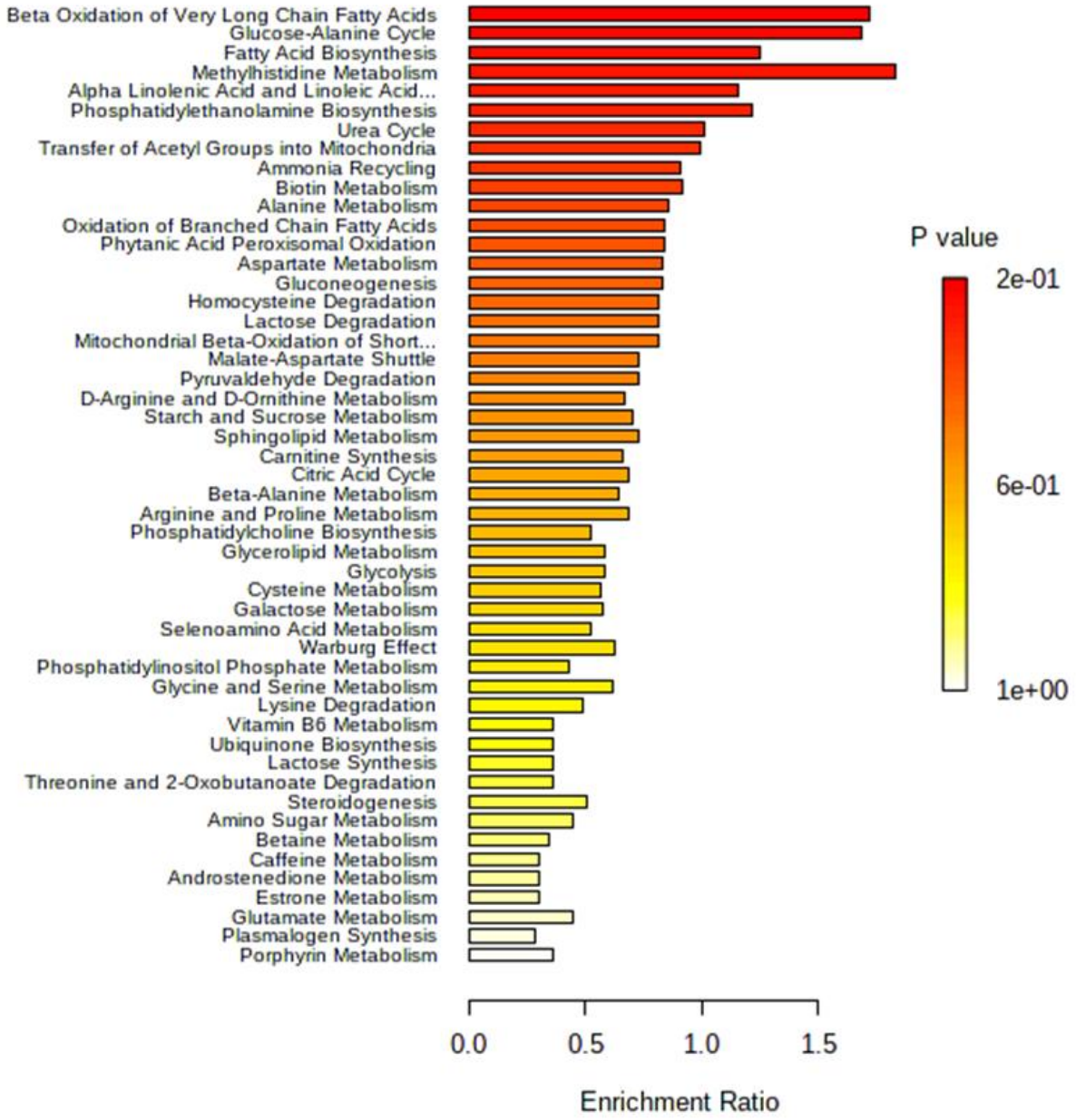

Figure 2.9: overview of the results of the over-representation analysis for VM/EM, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

Table 2.13 and Figure 2.10 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for those metabolites which were significant at p≤0.05 for VC/EC. These show that for VC/EC, methylhistidine metabolism, the urea cycle and beta oxidation of very long chain fatty acids were the most over-represented, although again the over-representation did not reach statistical significance.

Table 2.13: Results of the over-representation enrichment analysis for metabolites with significant p (≤0.05) and p (<0.1) for VC/EC, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathways	Total	Expected	Hits	Raw p	Holm p
Methylhistidine Metabolism	4	0.434	2	0.0604	1
Urea Cycle	29	3.14	6	0.0842	1
Beta Oxidation of Very Long Chain Fatty Acids	17	1.84	4	0.103	1
Arginine and Proline Metabolism	53	5.75	9	0.109	1
Ammonia Recycling	32	3.47	6	0.123	1
Citric Acid Cycle	32	3.47	6	0.123	1
Mitochondrial Electron Transport Chain	19	2.06	4	0.141	1
Beta-Alanine Metabolism	34	3.69	6	0.153	1
Glucose-Alanine Cycle	13	1.41	3	0.159	1
Warburg Effect	58	6.29	9	0.166	1
Nicotinate and Nicotinamide Metabolism	37	4.01	6	0.204	1
Alanine Metabolism	17	1.84	3	0.277	1
Glycerolipid Metabolism	25	2.71	4	0.283	1
Glycine and Serine Metabolism	59	6.4	8	0.303	1
Aspartate Metabolism	35	3.79	5	0.327	1
Gluconeogenesis	35	3.79	5	0.327	1
Fatty Acid Biosynthesis	35	3.79	5	0.327	1
Purine Metabolism	74	8.02	9	0.41	1
Carnitine Synthesis	22	2.38	3	0.433	1
Transfer of Acetyl Groups into Mitochondria	22	2.38	3	0.433	1

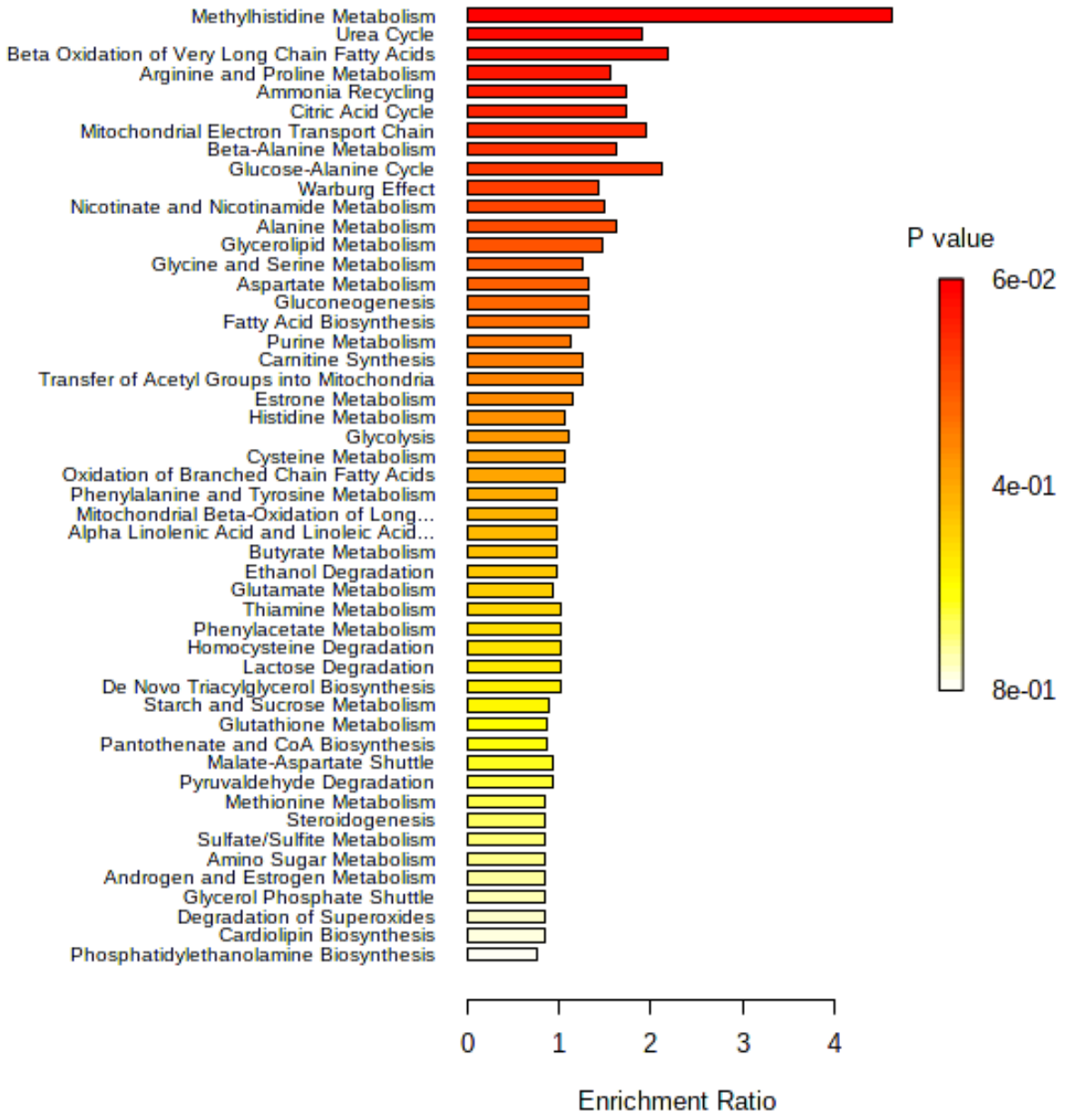

Figure 2.10: overview of the results of the over-representation analysis for VC/EC, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

2.10 Discussion

This study is unique in its comparison of the metabolomic profile of cord and maternal plasma at the time of delivery between women who have laboured and delivered vaginally and women who have not laboured and delivered via elective caesarean section. The clear-cut differences in metabolite levels between the two groups confirm the strong impact that active labour has on both the mother and neonate. A proportion of these changes may be due to the stress of labour itself, but it seems plausible that within there are clues as to which pathways trigger spontaneous parturition in women. In addition, the observed changes in xenobiotics such as metronidazole were as expected, supporting the robustness of metabolomic methodology in this setting. While it cannot be ruled out that the intervillous blood samples had some degree of fetal blood contamination, the very distinct metabolic profiles that were obtained in cord and intervillous plasma confirms the fetal and maternal circulations were sampled separately[150], thereby confirming the reliability of this non-invasive method for experimental sampling of maternal blood after delivery. These maternal and fetal differences are maintained, at least in part, by placental transport, as described in Chapter 1[60-62].

It could be argued that the stress of labour resembles strenuous exercise, and it would be useful to look to studies which investigate metabolic changes due to exercise to see if they match any of the changes observed here. Medium- and long-chain acetylcarnitines increase up to 9-fold following intense exercise in men [159]. A major source of acetylcarnitine is acetyl Co-A, formed from pyruvate oxidation, and this increase following exercise is likely due to a switch to increased fatty acid oxidation in skeletal muscle instead of glycolysis during exercise[160, 161]. The significant increases in acylcarnitine fatty acid metabolism observed in the present study within both the cord and maternal plasma of labouring women (VC/EC and VM/EM) (Tables 2.3 and 2.4) could in part be explained by fatty acid oxidation for energy used by the woman and neonate during labour and delivery. However, while the source of this may be from working skeletal muscle in the labouring mother, this explanation is less likely in the fetus/neonate who is not thought to be using their skeletal muscles during labour and delivery. The increased levels of stress metabolites observed in labour, including cortisol, are likely to be generating fatty acid mobilisation[162]. The increase in sphingomyelins observed in labouring maternal plasma and the increase in acylcarnitine fatty acid metabolism in both the cord and maternal plasma of labouring women could indicate a considerable shift in intermediary

metabolism at the time of labour following the relative decrease of these metabolites in pregnancy[163].

Labour and vaginal delivery would be expected to affect metabolites of fetal and maternal adrenal origin, particularly glucocorticoids, as part of the physiological stress response[151, 164]. In support of this theory, cortisol and its major metabolites cortisone, cortisone 21-sulfate and corticosterone were significantly increased in the cord plasma of women who spontaneously laboured and delivered vaginally (VC) when compared with those who did not labour (EC). Similarly, cortisol and corticosterone were significantly increased in the maternal plasma taken from women who laboured and delivered vaginally (VM) when compared with those who did not labour (EM) (Tables 2.3 and 2.4). This data confirms that there is activation of the fetal and maternal adrenal cortex during labour and vaginal delivery, and while this may be part of the mechanism initiating spontaneous labour, further studies are needed to investigate this hypothesis.

Recent studies also suggest that the effect of the intrapartum fetal environment may alter epigenetics, potentially having life-long effects[165-167]. Epigenetics are heritable changes in gene expression which are independent of the underlying DNA sequence. It is suggested that environment factors can alter epigenetics, and that such changes could form a link between early life exposures and

subsequent life events, including health[165-168]. For example, it has been reported that there is an increased risk for type 1 diabetes[167], atopy[169] and obesity[168, 170] in children and young adults born via elective caesarean section when compared with those born via vaginal delivery. However, more recently a large cohort study has disputed the claim regarding obesity[171]. While this area of research remains controversial, the results from the present study illustrate how different the intrapartum fetal environments are between elective caesarean section and spontaneous vaginal delivery.

Within cord plasma (VC/EC), heme was the metabolite with the greatest fold-change (Table 2.2). Heme is a complex of iron with protoporphyrin IX, present in haemoglobin and myoglobin. Its levels in the plasma can increase rapidly following haemolysis or tissue injury[152]. It is possible that the increased heme present in the cord taken from women who laboured in this study is a marker of the increased trauma and tissue damage which a neonate experiences during vaginal delivery when compared with delivery via caesarean section.

2.10.1 Endocannabinoid-related pathways

Endocannabinoids are formed from membrane phospholipids and are described as non-classical neurotransmitters[153]. The two characterised cannabinoid receptors are CB1 and CB2. CB1 is present in areas of the brain (where activation leads to psychoactivity), peripheral nerve terminals and extraneural sites

including the uterus. CB2 is largely limited to cells and organs of the immune system[172]. AEA is one of the most well described endocannabinoids and is a bioactive lipid which binds to CB1 and CB2. Fatty acid amide hydrolase (FAAH) is the primary enzyme which breaks down AEA[172]. Interestingly, cannabis stimulates the endocannabinoid system, and its use has been associated with pregnancy complications such as preterm birth, placental abruption, fetal growth restriction and spontaneous miscarriage[5, 173].

Endocannabinoids are considered potential biomarkers for reproductive events, with both predictive and diagnostic uses[153]. For example, previous studies show that maternal plasma AEA declines throughout pregnancy before a sharp increase at the time of labour[173], and AEA has been found to be 3.7-fold higher in women labouring at term than in non-labouring women at term. One group measured the AEA levels in plasma in women who went through induction of labour (IOL), comparing AEA in blood taken before IOL and again when the women were in active labour. The researchers found a 1.5-fold increase in mean plasma AEA levels, with a negative correlation between "the percentage change in plasma AEA level and the induction-to-delivery interval"[174]. Another group found that AEA levels in the placenta were significantly higher than in fetal membranes. Further, that AEA concentrations were significantly higher in the umbilical vein when compared with the artery, although this was not a

consistent finding, suggesting either transport across the placenta or placental production of AEA[173]. It is known that the uterus can produce its own AEA[172], and myometrial studies show that AEA exerts a concentration-dependent relaxation effect on human myometrial contraction *in vitro*[175]. Elevated plasma AEA in early pregnancy (<8 weeks) has been shown to be predictive of miscarriage, and lower peripheral lymphocyte expression of FAAH was found in women who miscarried compared with those whose pregnancy continued[173, 176]. The CB1-knock out mouse has elevated corticotrophin-releasing hormone (CRH) and spontaneous preterm labour[174]. It has been previously suggested that increased AEA could lead to an increase in prostaglandins, as FAAH converts AEA to ethanolamine and arachidonic acid, which is necessary for the production of prostaglandins, and that this could be the link between elevated AEA and labour[5, 174].

Ceramide is a ubiquitous sphingolipid second messenger and an important signal effector molecule, and is mainly produced *de novo* in the endoplasmic reticulum but can be produced within minutes from the breakdown of sphingolipids, for example in lysosomes, in the cellular response to apoptosis and stress[177]. It has been reported that the increase in oxidative stress resulting from maternal pre-eclampsia (PET) increases *de novo* production of ceramides and decreased ASAH1 activity and expression, resulting in an accumulation of

ceramides. The authors suggested that this may cause the increased trophoblast cell autophagy that is observed in women with PET[177]. CB1 activation has been shown to induce sphingomyelin hydrolysis and acute production of ceramide within minutes in primary astrocytes and C6 glioma cells, and it is also thought that CB2 activation leads to *de novo* sustained ceramide production[172]. In tumour cells, CB1 activation leading to ceramide production has been shown to lead to apoptosis[172], and TNF-α appears to be involved in the link between CB1 and CB2 activation and ceramide production[172,177,5, 178], whereby inflammatory events may stimulate the onset of labour.

In this study, AEA, OEA, and PEA were significantly increased in the cord plasma taken from the women who laboured (VC/EC) (Table 2.10). PEA was significantly increased in the corresponding maternal plasma (VM/EM), while N-oleoylserine significantly decreased in maternal plasma[173, 174]. Interestingly, there are reports of increased ceramide levels and expression of serine palmitoyl transferase, the rate-limiting enzyme for ceramide synthesis de novo, in the placentas of women who laboured compared with those who delivered by elective CS[179]. The data presented here support the view that the endocannabinoid/ceramide/sphingolipid pathway has the potential to stimulate labour[173, 174, 179].

2.10.2 Progesterone and pregnenolone

Parturition has long been associated with changes in progesterone and pregnenolone metabolism in many species[180, 181], and as discussed in the introduction, many researchers believe that the functional progesterone withdrawal is involved in the initiation of labour in humans[174]. Although there were no significant differences in progesterone levels in the cord (VC/EC) or maternal (VM/EM) plasma found in the present study, there was a significant increase in metabolites of the progestin sub-pathway in the maternal plasma of labouring women (VM/EM). It has previously been reported that there is a strong positive correlation of 3β and 16α hydroxyl-steroids with gestational age[182], reflecting maturation of the fetal adrenal zone and potential involvement of these metabolites in the preparation for labour.

Further, the data from this pilot study uncovers a rich and complex pattern of steroid metabolites in spontaneous labour and vaginal delivery reflected in both maternal and fetal circulations (Table 2.8 and 2.9). Of interest is the relative increase in VC/EC of pregnenolone and hydroxypregnenolone sulphates which are mirrored by changes in the opposite direction in the maternal circulation (Table 2.8). Moreover, progesterone and pregnandiol metabolites increased in both the maternal and fetal circulations (Table 2.9). These changes indicate increased turnover of C21 compounds, which are the first steroids derived from

cholesterol through the P450scc and 3-β-hydroxysteroid dehydrogenase pathway during labour. Phillips *et al.* (2014)[183] have previously demonstrated changes in cholesterol-transporting lipoproteins in the maternal circulation in women in spontaneous labour[183], confirming the need for high cholesterol turnover during parturition. The results of the present study show for the first time a comprehensive picture of C21 metabolites in the maternal and fetal circulations in relation to labour and vaginal delivery that can be used to guide future studies investigating steroid changes at parturition.

2.10.3 Potential link with lymphocytes

Reviewing the literature and the results from this pilot study has led to the development of the hypothesis that activation of the endocannabinoid system may be involved in the trigger of parturition in humans, and that this activation may be driven by lymphocytes and progesterone. In early pregnancy, decreased levels of FAAH in peripheral lymphocytes is associated with miscarriage[184]. It has been suggested that peripheral lymphocytes could therefore be involved in control of pregnancies through the regulation of AEA in the uterus[176], which could in turn regulate lymphocyte-dependent cytokines[184]. Progesterone is known to upregulate lymphocyte FAAH activity via the transcription factor Ikaros, resulting in increased breakdown and decreased levels of AEA[153,176,184]. Therefore, it would be expected that lower progesterone levels would result in

an increase in AEA. Progesterone has also been shown to favour t-cells producing th2 cytokines (IL-4 and IL-10), thus inhibiting th1 cytokines (IL-12 and INF-γ) and therefore allowing successful pregnancy. Progesterone, via IL-4, has also been shown to stimulate the release of Leukaemia inhibitory factor (LIF) from t-lymphocytes, and low LIF production has been found in women with recurrent miscarriages. Further, macrophages express both CB1 and CB2, and can produce AEA and 2-AG, as well as producing LIF (pro-inflammatory), and therefore may also be involved[184]. One group found that stimulating human lymphocytes with progesterone enhanced FAAH activity in a dose-response. The researchers also found that AEA reduced LIF release from leucocytes, but that PEA did not affect the amount released. The suggestion is that reduced LIF leads to miscarriage in early pregnancy[184]. Endocannabinoids have been found to increase IL-4, IL-6, and IL-10 production; and to inhibit the release from lymphocytes of TNFα and INFγ[5, 153]. As functional progesterone withdrawal has been linked to the onset of labour[174], it could be that this is the mechanism behind the increase in AEA observed in previous studies immediately prior to labour[5].

2.10.4 Over-representation analysis

Although the ORA indicated that none of the metabolite pathways were significantly over-represented, the top five pathways appear to be biologically

relevant. The pathways with the greatest enrichment ratio for VM/EM included fatty acid biosynthesis pathways and methylhistidine metabolism; and for VC/EC, methylhistidine metabolism, urea cycle, and beta oxidation of very long chain fatty acids were the most over-represented pathways.

Methylhistidine is positively associated with a high protein diet[185], and the urinary excretion of 3-methylhistidine has been found to be increased in later pregnancy, suggesting that the protein that is held in the maternal muscles in early pregnancy is later catabolised, providing a source of amino acids, potentially for fetal protein synthesis[186]. One group found that 3-methylhistidine, creatinine, acetyl groups and acetate were independent predictors of pre-eclampsia in serum taken from 143 women between 26 and 41 weeks of gestation, 17 of whom developed pre-eclampsia; by contrast 3-methylhistidine was negatively associated with development of pre-eclampsia[187]. In Chapter 3 of this thesis, maternal serum 3-methylhistidine is shown to increase throughout pregnancy, being highest in latent phase (Appendix 3A3) and 1-methylhistidine was lower in the cord artery of those who were induced than the cord artery of those who spontaneously laboured (0.76-fold change, p=0.029) (Appendix 3A4). It is possible that methylhistidine metabolism may be associated with vascular changes of pre-eclampsia and in the triggering of labour, as will be discussed later in this thesis. Moreover, the

increase observed throughout pregnancy may reflect increasing catabolism of muscle for provision of amino acids, and the increase observed in labouring women may be a result of increased muscle activation with labour. The over-representation of fatty acid metabolism may also represent increased breakdown of fatty acids.

2.11 Conclusions

This pilot study demonstrates that there are a wide range of metabolic changes at the time of delivery between women who laboured spontaneously and women who did not labour. The data will be useful to investigate the biochemical pathways involved in the physiological mechanism of labour in normal term pregnancy and provide a pattern against which pathological changes in preterm labour can be compared. These results indicate an interplay between metabolites in the maternal and fetal circulations which point to the involvement of the endocannabinoid, sphingolipid, ceramide and steroid systems in the mechanism of active labour. Figure 2.9 illustrates this hypothesis, including the suggested link with fatty acid amide hydrolase (FAAH). FAAH is the primary enzyme which breaks down the endocannabinoids anandamide (AEA)[172], oleoyl-ethanolamide (OEA) and palmitoyl-ethanolamide (PEA)[188]. AEA is the most studied endocannabinoid, and elevated plasma AEA in early pregnancy (<8 weeks) has been shown to be predictive of miscarriage. Further, a lower

peripheral lymphocyte expression of FAAH was found in women who miscarried compared with those in whom pregnancy was ongoing[173, 176]. It has been previously suggested that higher concentrations of AEA could lead to an increase in prostaglandins as FAAH converts AEA to ethanolamine and arachidonic acid. Arachidonic acid is necessary for the production of prostaglandins, and therefore this could be a link between elevated AEA and labour[174]. Stimulating human lymphocytes in cell culture with progesterone enhanced FAAH activity in a dose-response[184], and therefore if there was a functional withdrawal of progesterone that also reduced the sensitivity of lymphocytes to progesterone, this could in turn reduce FAAH activity and therefore result in increased AEA concentrations immediately prior to labour[5]. Further studies are now warranted, where serial maternal samples are taken throughout pregnancy to determine when these changes take place, especially focusing on the weeks and days immediately prior to spontaneous labour.

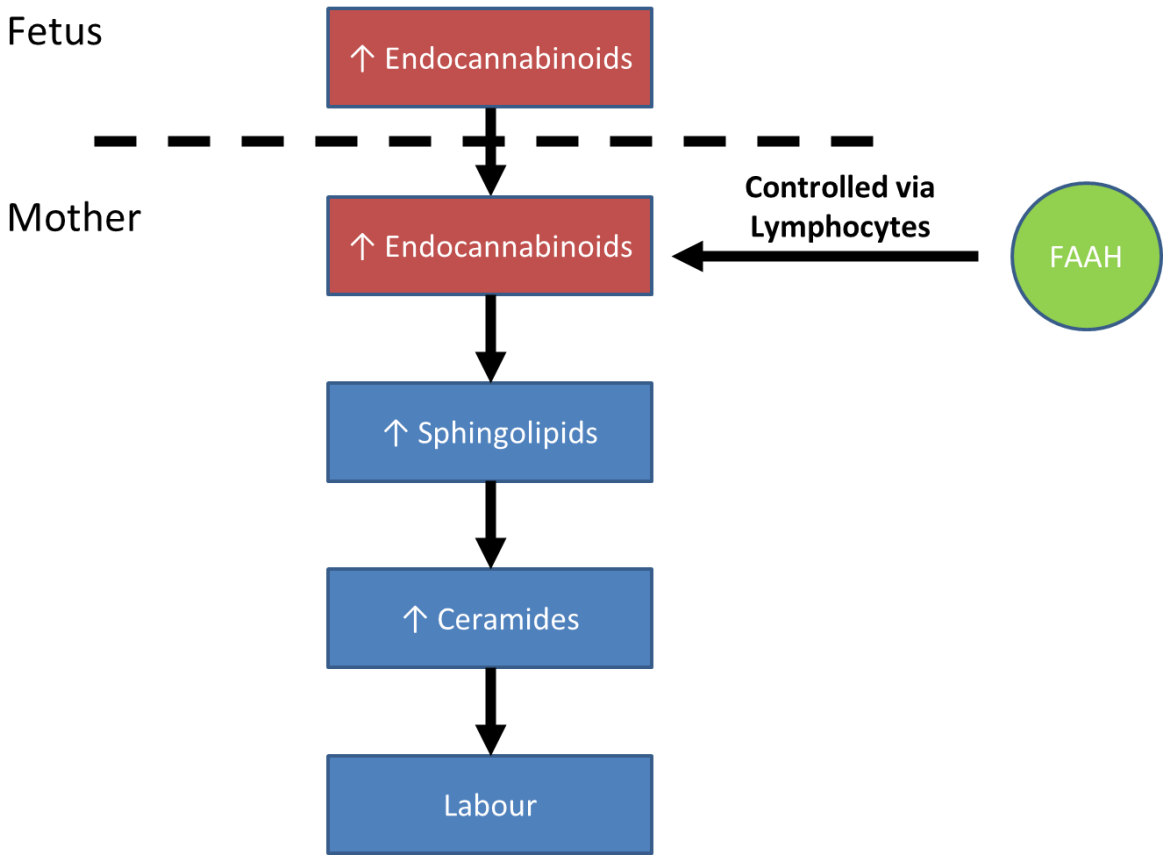

Figure 2.11: Diagram illustrating proposed hypothesis generate from pilot study (FAAH = fatty acid amide hydrolase).

2.12 Limitations

A potential limitation to this study is the relatively low number of participants in each group. However, clear and significant changes in metabolite levels were identified within this pilot group with relatively little noise, demonstrating the reliability of this approach. The platform used is very robust with 26.9% (222/826) of metabolites significantly changing in maternal plasma and 21.1% (174/826) in cord plasma with labour. The method is unlikely to show false positive results: for example metronidazole was given at the time of knife-to-

skin for all women in the elective CS group, but none of the women in the VD group received it; reassuringly, all the maternal and cord plasma of the non-labour group contained metronidazole and none of the labouring group contained it (Tables 2.5 and 2.6). Another limitation is the fact that the study is cross sectional, rather than serial, so it is difficult to know whether the changes observed with labour are part of the mechanism of the initiation of labour or a consequence of the stress and metabolic demands of several hours of active labour. Nevertheless, this data shows that metabolomic techniques are a powerful and reliable approach to identify biochemical changes associated with labour.

The results of this study informed the design of the study presented in the next chapter which investigates maternal metabolomic changes throughout low-risk pregnancies.

CHAPTER 3. SERIAL METABOLOMIC ASSESSMENT OF PREGNANCY AND LABOUR

3.1 Background

This chapter follows on from Chapter 2 where the metabolomic profile of cord and maternal blood at the time of delivery was compared between women who laboured spontaneously and women who did not labour. The hypothesis generated from these results was that the trigger for labour in humans results from an interplay between metabolites in the maternal and fetal circulations, with involvement of endocannabinoid, sphingolipid, ceramide and steroid pathways in the mechanism of active labour[5]. This serial metabolomics study was designed to test and expand this hypothesis in order to better understand any interaction with the suspected functional progesterone withdrawal that is hypothesised to be involved in the onset of spontaneous labour in humans.

As the previous study design was cross sectional, with all samples taken at the time of delivery, it was not possible to definitively conclude that the changes seen were secondary to the physiological stress of labour itself, or as a result of the spontaneous onset of labour. This follow-on study was therefore designed to serially assess the maternal metabolome at different points during low-risk pregnancies, including at the start of labour and at delivery.

The present study had two parts: Part 1 was designed to investigate whether there were differences in the metabolomic profile between the intervillous blood (maternal), cord artery (fetal) blood and cord vein (placental) blood, and whether these were affected by the mode of labour onset and delivery; and whether this could give further insight to the fetal/maternal interaction at labour. Given that the cord vein carries blood from the placenta, the cord artery carries blood from the fetus, and the intervillous blood is predominantly maternal, characterising the metabolome of these separate compartments following different onsets of labour and delivery was hoped to further identify the interactions between the maternal and fetal systems, and whether the trigger for labour may originate in the fetus or mother. Part 2 involved taking serial blood samples from women with low-risk pregnancies at 28 weeks' gestation, 34 weeks' gestation, and latent phase of spontaneous labour. 28- and 34-weeks' gestation were chosen as these are times when women routinely have blood tests with midwives, and therefore would not require the women to attend extra visits or have extra blood tests. This was considered particularly important given this study was exploratory. The metabolome of these samples would be analysed to determine whether there were associations between concentrations of metabolites and gestational age at delivery.

3.2 Aims and objectives

Aim 1 (Part 1 of study): To determine if there are differences in the metabolomic profile of the intervillous (maternal) blood, cord artery (fetal), and cord vein (placental) at the time of delivery (in Chapter 2 cord blood was not separated into arterial and venous).

Aim 2 (Part 1 of study): Determine if any differences in Aim 1 are affected by the mode of labour onset and delivery. The different modes of labour onset and delivery assessed were spontaneous labour (SL), induction of labour (IOL) followed by vaginal delivery, and elective caesarean section (ECS).

Objective 1.1: To answer aims 1 and 2, samples were taken from four women who spontaneously laboured at term (37 to 42 completed weeks' gestation) and delivered vaginally, four women who had an IOL at term and delivered vaginally, and four women who had an ECS at term. As this was an exploratory study, four participants per group were selected, with the intention of informing power calculations for follow-on studies if indicated by the results. The samples taken for each woman were a sample in latent phase for the SL group (early labour, before 4 cm cervical dilatation[30]), or a sample at the start of the induction process for the IOL group, or at the pre-operative clinic (day before ECS) for the ECS group. Further samples were taken from the placenta: intervillous (IV) (as with the pilot study), cord vein (CV) and cord artery (CA). To answer aim 1, the

metabolomic profile of these different samples were compared between the different modes of labour, and to answer aim 2, the metabolomic profile of each of the different vascular compartments were compared between the mode of labour onset and delivery groups.

Aim 3 (Part 2 of study): To determine whether there is an association between the maternal metabolomic profile at 28 weeks' gestation, 34 weeks' gestation, or in latent phase, and gestational age at the onset of spontaneous labour.

Objective 3.1: Maternal blood was sampled at 28 weeks' gestation, 34 weeks' gestation, and in latent phase in women with low-risk pregnancies who went on to spontaneously labour. Samples were sent to Metabolon, Inc., for global MS metabolomics analysis.

3.3 Funding, ethical approval and role of thesis author

Funding for this study (£19 940) was granted by the Above and Beyond charity in July 2018. Ethical approval was granted by from the National Research Ethics Service Committee-South West, Bristol (reference number: E5431).

The thesis author (Katherine Birchenall) came up with the idea for the study, designed the study protocol, wrote the grant for funding, recruited all women, collected, and processed all samples, and analysed all the data. The MS

metabolomics and initial statistical analysis were performed by Metabolon, Inc., as described further below.

3.4 Methodology

3.4.1 Inclusion and exclusion criteria

The inclusion criteria were:

- Low-risk singleton pregnancy
- BMI 18-30
- Age 20-40 years

The exclusion criteria were:

- BMI under 18 or over 30
- Age under 20 or over 40 years
- Multiple pregnancy
- Taking medications likely to alter metabolomics
- Development of chorioamnionitis or other infection
- Development of maternal or fetal complications such as diabetes or pre-eclampsia

ECSs were for non-maternal or fetal disease, such as breech, previous caesarean section, or previous third-degree tear.

IOL were also for non-maternal or fetal disease indications, to include post-term and perceived reduced fetal movements.

3.4.2 Gestational age at spontaneous labour

To answer aim 3, it was important to know the gestational age when the included women spontaneously laboured. Women who had iatrogenic labour, for example IOL or ECS were therefore excluded. However, women who had IOL for post-term (recommended by NICE to be offered around 40 weeks' and 12 days gestation to reduce the risk of stillbirth that increases after 42 weeks' gestation) were included as this would be the latest that women would be expected to labour in England under current protocols[29].

3.4.3 Recruitment

In order to recruit women with low-risk pregnancies to the study to answer the first aim, the thesis author attended two community health centres and met with the community midwives to discuss the study and intentions, and confirm the feasibility of the study design. During booking appointments (in first trimester of pregnancy), the community midwives gave eligible women the study information leaflet (3A1, Appendix) and asked if they would be interested in speaking with the researcher (thesis author) about the study. If they were, the researcher would then meet the women after their booking appointment, explain the study further, answer any questions and talk through the patient

information leaflet. Women who were still interested in the study agreed to be met at the dating scan (performed between 11-14 weeks' gestation). At the dating scan, if the women were still happy to take part, the consent form (3A2, Appendix) was signed, and any further questions answered. Each woman was allocated a study code at the time of recruitment.

The women were subsequently met at their routine appointments with the community midwives at 28 weeks' gestation and 34 weeks' gestation. At these appointments, the researcher took an extra blood draw for the study at the same time as the routine Full Blood Count that women are offered at these gestations.

3.4.4 Collecting samples in early labour and at delivery

3.4.4.1 Spontaneously labouring group

Stickers were placed on the maternity notes with the study name, the researcher and the researcher's contact number. Posters were also placed on Central Delivery Suite and the Midwifery-Led Unit so that the sole researcher (Katherine Birchenall) was alerted when the women attended hospital in early labour (latent phase). A further blood sample was then drawn in latent phase. At delivery, the researcher would be outside the delivery room and the midwife would double-clamp the cord (following routine delayed cord clamping that is routinely performed at St Michael's Hospital provided all is well with both the

mother and baby[5]) and then bring the placenta outside the room to the researcher as soon as the cord was cut (there are always two midwives in the rooms at the time of delivery so this did not take care away from the women). For all women, one sample was taken from the cord vein (CV), one from the cord artery (CA) and one intervillous (IV sample), within 30 minutes of delivery.

3.4.4.2 Induction of labour group

At St Michael's Hospital, Bristol, the IOL protocol involves women attending the IOL suite on the antenatal ward and remaining in hospital for the duration of the induction process. Dinoprostone is a prostaglandin PGE2 analogue[126]. 10 mg of dinoprostone within a pessary (slow-release, trade name Propess) is inserted into the woman's vagina, close to the cervix. It is removed after 24 hours, or earlier if the woman starts labouring before the 24-hour period. A cervical assessment then occurs, and a Bishop's Score calculated, as per protocol at St Michael's Hospital. If the woman's cervix has dilated sufficiently to allow artificial rupture of the membranes, then the woman is transferred to the central delivery suite (CDS) for this. If not, then a further 0.5 – 1 mg of dinoprostone within a gel (faster-release, trade name Prostin) is again inserted into the vagina close to the cervix. A further cervical assessment is performed after 6 hours, and if the cervix remains closed, a further 0.5 mg Prostin is administered. By this stage, an artificial rupture of membranes is usually

possible. For the purpose of this study, as the aim was to compare with latent phase, the blood sample to match with the latent phase sample of the spontaneously labouring women was taken when the women had been transferred to CDS for their ARM. By this point the women in this study had a cervical dilatation that would be defined as latent stage (1-4 cm dilated)[29]. The researcher stayed outside the room and the placenta was brought to her following delivery of the baby as for the SL group.

3.4.4.3 Elective caesarean section group

Regarding ECSs, the initial intention was to recruit these women at the booking appointments and that some of these women would require ECS. If not enough women were recruited by this method, it was intended that women would be recruited from the pre-operative clinic on the day prior to their ECS. For all women with ECS, a peripheral blood sample was taken from the woman at the time of the pre-operative clinic (at the same time as other routine blood tests), and this was to match the blood test taken in latent phase for the SL group. During ECS, again following delayed clamping of the cord for approximately one minute, the surgeon double-clamped the cord and the placenta was brought immediately to the researcher who was in a room adjacent to the theatre. Again, for all women one sample was taken from the CV, one from the CA and one IV sample, within 30 minutes of delivery.

3.4.5 Sample collection and processing

As described, peripheral blood samples were taken from low-risk women at 28 weeks' gestation and 34 weeks' gestation, in latent phase of spontaneous labour/early IOL/pre-operative clinic for the ECS group, and IV blood and CA and CV immediately following delivery. All samples were collected into EDTA tubes and processed following Metabolon, Inc., standard operating procedures as with Chapter 2[5].

As Metabolon, Inc., recommends that all samples are processed within an hour, a motorised scooter was purchased so that the researcher could transport the samples from the community centres to the laboratory within the time frame. In addition, this scooter helped with being able to attend deliveries at all times of the day and night. As with the study in Chapter 2, plasma samples were stored at -80 degrees Celsius until analysis. The metabolites in maternal and fetal blood were measured with the Metabolon, Inc., MS metabolomics platforms (http://www.metabolon.com/), as described in Chapter 2.

3.4.6 Statistical analysis

While the statistical analysis plan was designed by the researcher, Metabolon, Inc. performed the initial statistical analysis as part of their collaboration in the project. As with Chapter 2, metabolites were normalised to sample volume then log transformed. For comparisons between metabolites, each metabolite value

was then rescaled to set the median to be equal to 1, giving a scaled value for each metabolite. Missing values were imputed with the minimum value.

To answer aims 1 and 2, samples from four women who spontaneously laboured (SL group), four women who were induced (IOL group), and four women who had an ECS (ECS group) were taken at the following time points: latent phase of SL/day before ECS/day of commencement of IOL; and at delivery (collected within 30 minutes of delivery of placenta) from the CV, CA and IV (maternal) blood. The following statistical analyses were performed in ArrayStudio and R statistical packages for part 1 of the study:

a. A one-way analysis of variance (ANOVA) test to compare potential differences in mean values of the recorded participant demographic variables and delivery outcomes between the SVD, IOL and ECS groups.
b. Welch's t-test to compare the mean amount of each metabolite measured within the latent phase/day of IOL/pre-operative samples between the SL, IOL and ECS groups, respectively.
c. Welch's t-test to compare the mean amount of each metabolite measured within the CA samples between the SL, IOL and ECS groups
d. Welch's t-test to compare the mean amount of each metabolite measured within the CV samples between the SL, IOL and ECS groups

e. Welch's t-test to compare the mean amount of each metabolite measured within the IV blood between the SL, IOL and ECS group.
f. Paired t-test to compare the mean amount of each metabolite measured between each of the different samples (latent/CV/CA/IV) within the same mode of delivery groups.

To answer aim 3, samples taken at 28 weeks', 34 weeks' and in latent phase of labour had MS metabolomics analysis performed and the gestational age at spontaneous delivery (in days) were also known. The following statistical analyses were performed in ArrayStudio and R statistical packages:

a. Spearman's rank correlation to individually assess whether there were associations between:
    i. Metabolomic profile at 28 weeks and gestational age at spontaneous labour.
    ii. Metabolomic profile at 34 weeks and gestational age at spontaneous labour.
    iii. Metabolomic profile in latent phase and gestational age at spontaneous labour.
b. Paired t-test to determine changes between metabolites at each time point (28 vs 34, 28 vs latent, and 34 vs latent).

To account for multiple testing, a q-value was calculated by Metabolon, Inc., based on the understanding that when 200 compounds are analysed, approximately ten compounds would reach a significance of $p \leq 0.05$ by chance. As described in Chapter 2 (section 2.8.4), a q-value <0.1 indicates high confidence in a result.

3.4.7 Over-representation analysis

As discussed in Chapter 2, given the large amount of data produced by 'omics studies, ORA is a useful tool for interpretation of results[155, 157, 158, 189]. MetaboAnalyst software (www.metaboanalyst.ca) has been developed using the statistical programme R, specifically for analysis of metabolomics studies, and was used for analysis of the metabolomics data generated by this study. MetaboloAnalyst (version 4.0) is a free web-based tool that maps common compound names to a range of database identifiers, including KEGG, HMDB, METLIN, and PubChem[158].

For the MS metabolomics analysis in this thesis, HMDB identifiers were inputted into the MetaboAnalyst programme online (www.metaboanalyst.ca) and enrichment analysis performed. The pathway enrichment analysis selected implements the hypergeometric test to evaluate whether a specific metabolite group is represented more than would be expected by chance within the given compound list. Raw one-tailed p values are calculated, as well as a p-value

calculated following adjustment for multiple testing using the Holm method[157, 158]. For this chapter, those metabolites which had a significant p-value (≤0.05) were entered into the MetaboAnalyst software for each of the experimental comparisons (a full list of these comparisons are presented in Appendices 3A3 to 3A7) .

3.5 Results

3.5.1 Recruitment

61 women with low-risk pregnancies were approached by the researcher at booking appointments with the community midwives and of these: five declined, one had a first-trimester miscarriage, thirteen booked at a different hospital, one booked a home birth, nine were excluded for maternal conditions, and one moved out of the area. 31 women agreed to meet at their dating scan (11-14 weeks' gestation) and signed the consent form. Two women required referral to the fetal medicine unit for fetal concerns and were therefore excluded, and one woman developed pregnancy-induced hypertension and was therefore excluded. Rolling recruitment continued throughout the study in order to ensure that enough women were recruited, and once the intended number of women had complete sample sets, three women were thanked and removed from the

study as no further samples were required (Figure 3.1). The first woman was approached in August 2018 and the final woman recruited in August 2019.

3.5.2 Sample collection

24 women had plasma samples taken at 28 weeks' gestation; and one woman declined having a sample taken at this time point as she had her routine bloods taken early and did not want extra tests (Figure 3.1). One woman spontaneously delivered at 34 weeks' gestation and her delivery was missed, therefore no sample was taken at 34 weeks' gestation. Thus 24 women had samples taken at 34 weeks' gestation. Of these women, one woman had a spontaneously preterm labour at 35 weeks and 3 days and delivered out of area, twelve women spontaneously laboured at term, nine had labour induced at term or post-term, and two booked for an ECS at term (Figure 3.1).

Of the women who went into spontaneous labour at term, eleven had a sample taken in the latent phase of labour. One woman was missed as she spontaneously laboured with a baby in breech position and therefore had an emergency caesarean section (Figure 3.2). Of the eleven women for whom a latent phase sample was taken: four had a normal vaginal delivery, two had a ventouse or kiwi delivery, one had a forceps delivery and four had an emergency caesarean section in labour. CV, CA and IV samples were taken within thirty minutes of delivery of the placenta for all eleven women.

Of the nine women who had an IOL (Figure 3.3): three were induced for post-term, one for obstetric cholestasis, one for maternal request for family reasons, one for suspected reduced fetal growth velocity, one for pregnancy-induced hypertension, and two for reduced fetal movements. Seven of these women had blood taken at the initial stage of induction (insertion of pessary; before artificial rupture of membranes); two were missed: one had an emergency caesarean section for suspected fetal distress following IOL for reduced fetal movements, and one had a normal vaginal delivery following IOL for reduced fetal movements. Of those women for whom a blood sample was taken at the time of induction: two had a normal vaginal delivery, three had a forceps assisted vaginal delivery, one had a ventouse assisted vaginal delivery, and one woman had an emergency caesarean section in labour. Excepting the woman who had the emergency caesarean section, CA, CV and IV samples were taken within thirty minutes of delivery of the placenta for all. Other than the women who were induced for post-dates, all women who were induced were excluded from Part 2 of the study as they did not spontaneously labour, and therefore the samples taken from them at 28 weeks and 34 weeks' gestation were not processed. Samples at the start of IOL and at delivery collected from four of the women who had IOL were included for Part 1 to answer aims 1 and 2. The

indications for IOL at term for the four women who were included were: reduced fetal movements at term, post-term, and maternal request for family reasons.

Two of the women recruited at booking planned an ECS (Figure 3.4). As four women with ECS were required for Part 1, two women with low-risk pregnancies booked to have an ECS at term were recruited from the ECS pre-operative clinic. All four of these women had a sample taken the day before their ECS at the same time as their routine pre-operative blood tests, and CA, CV and IV blood samples were taken within thirty minutes of delivery of the placenta.

Overall, 89 samples were sent to Metabolon, Inc., for global metabolomic analysis. The samples from the placenta and cord were taken immediately following delivery of the placenta, and all samples were processed within 60 minutes of collection, as per Metabolon, Inc., recommendations.

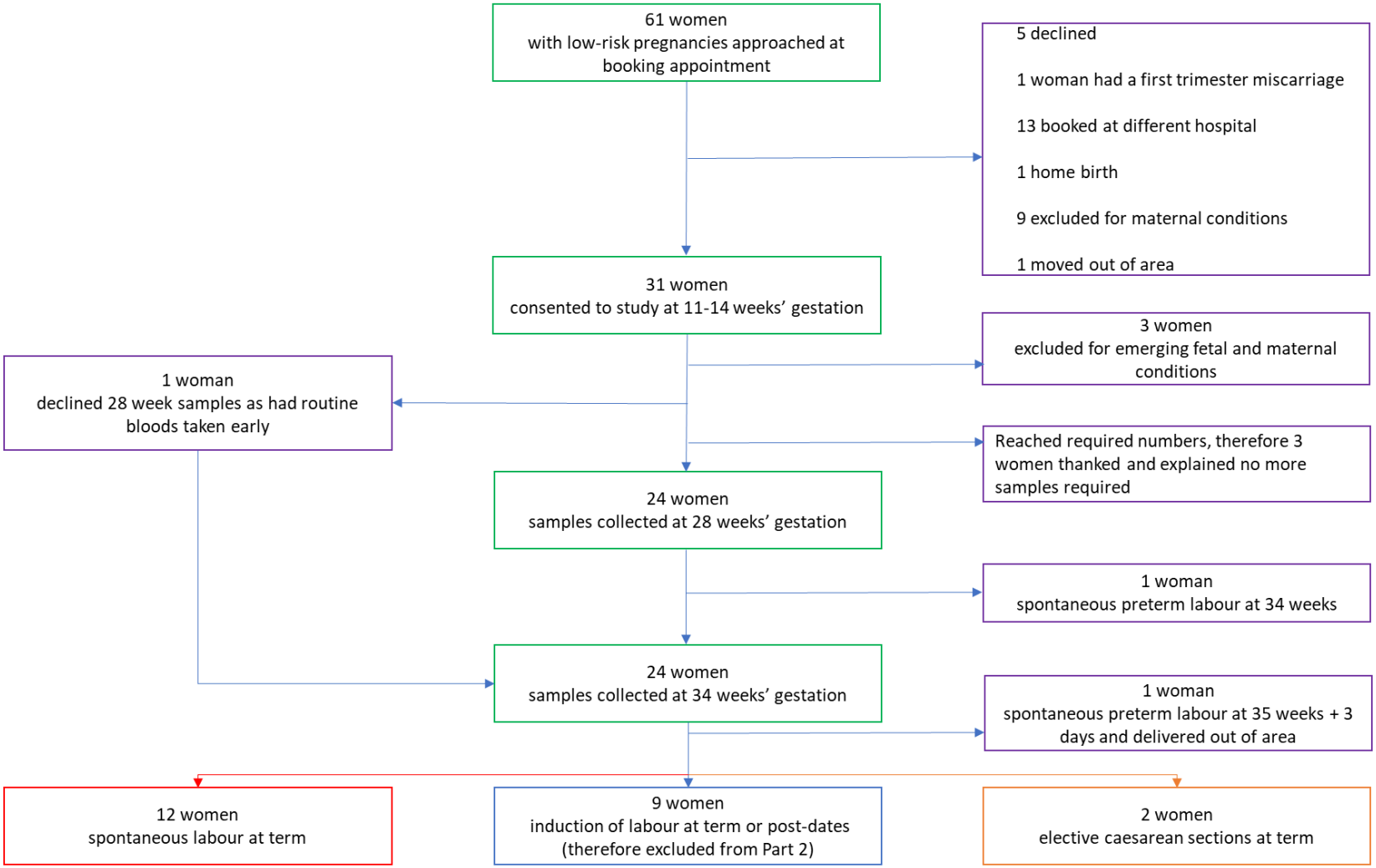

Figure 3.1: Flow diagram showing number of women approached, recruited, and withdrawn from the study, as well as number of women who had 28 week and 34 weeks' gestation blood samples taken.

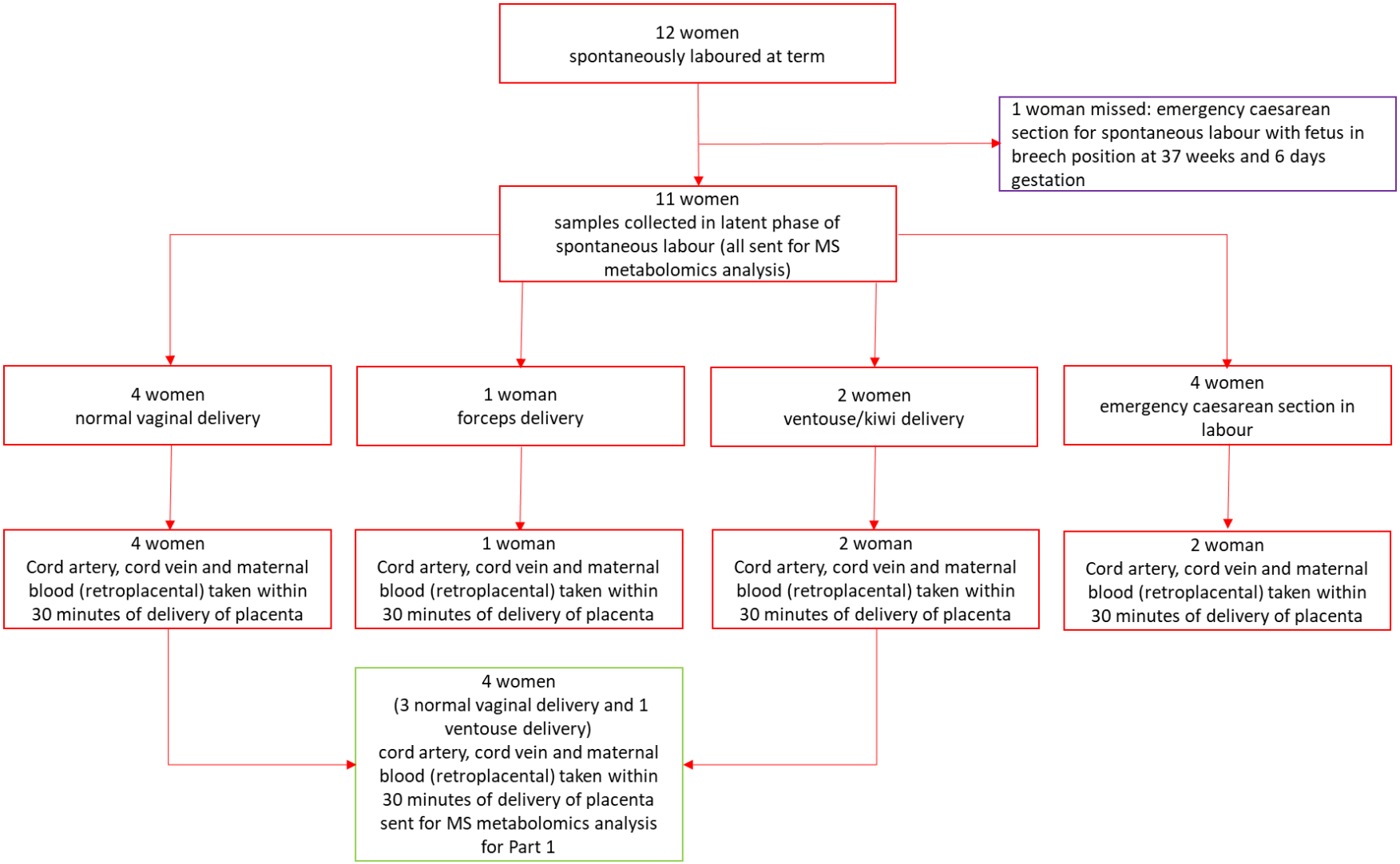

Figure 3.2: Samples taken and delivery outcomes for women who spontaneously laboured at term.

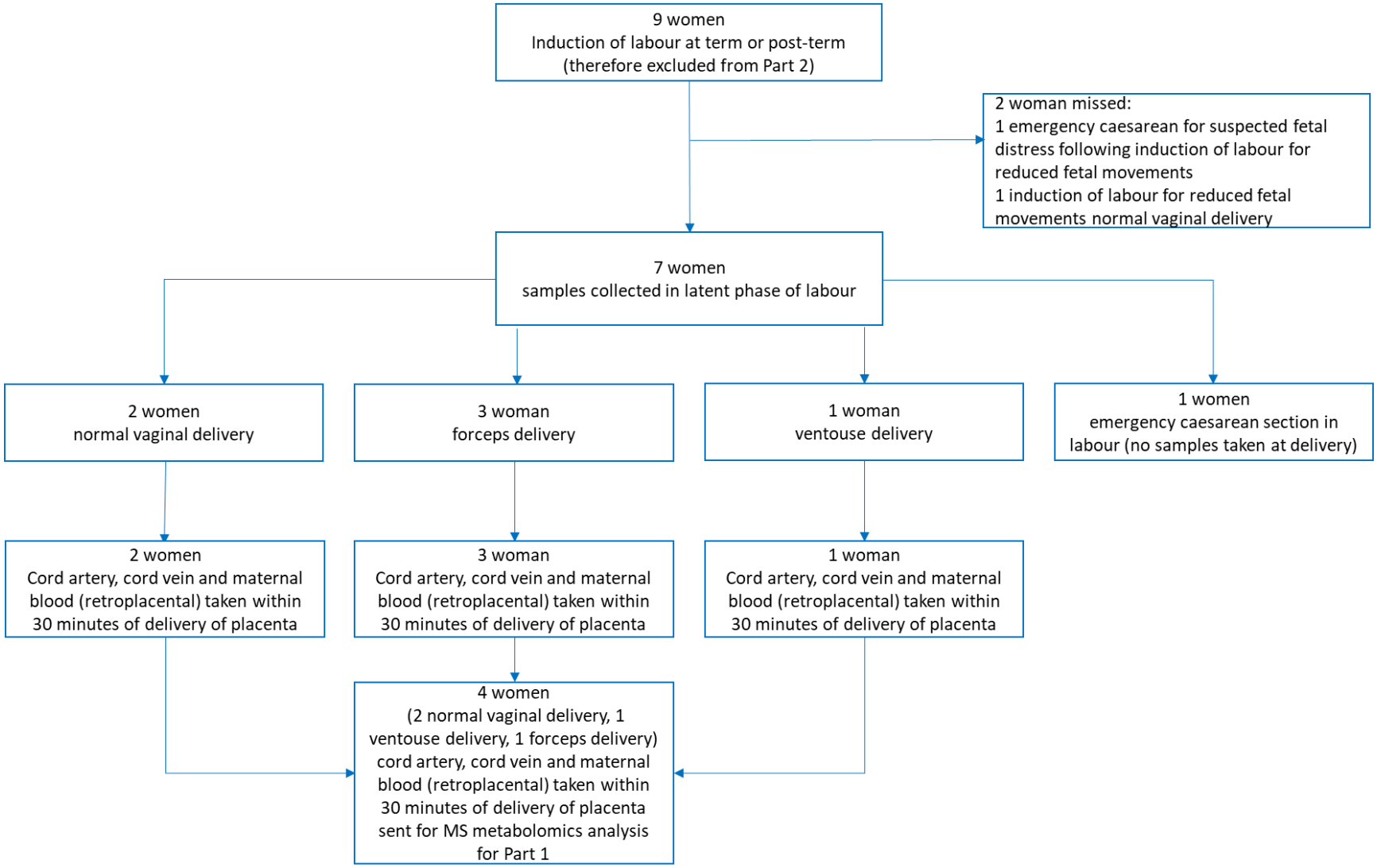

Figure 3.3: Samples taken and delivery outcomes for women who had induction of labour at term or post-term.

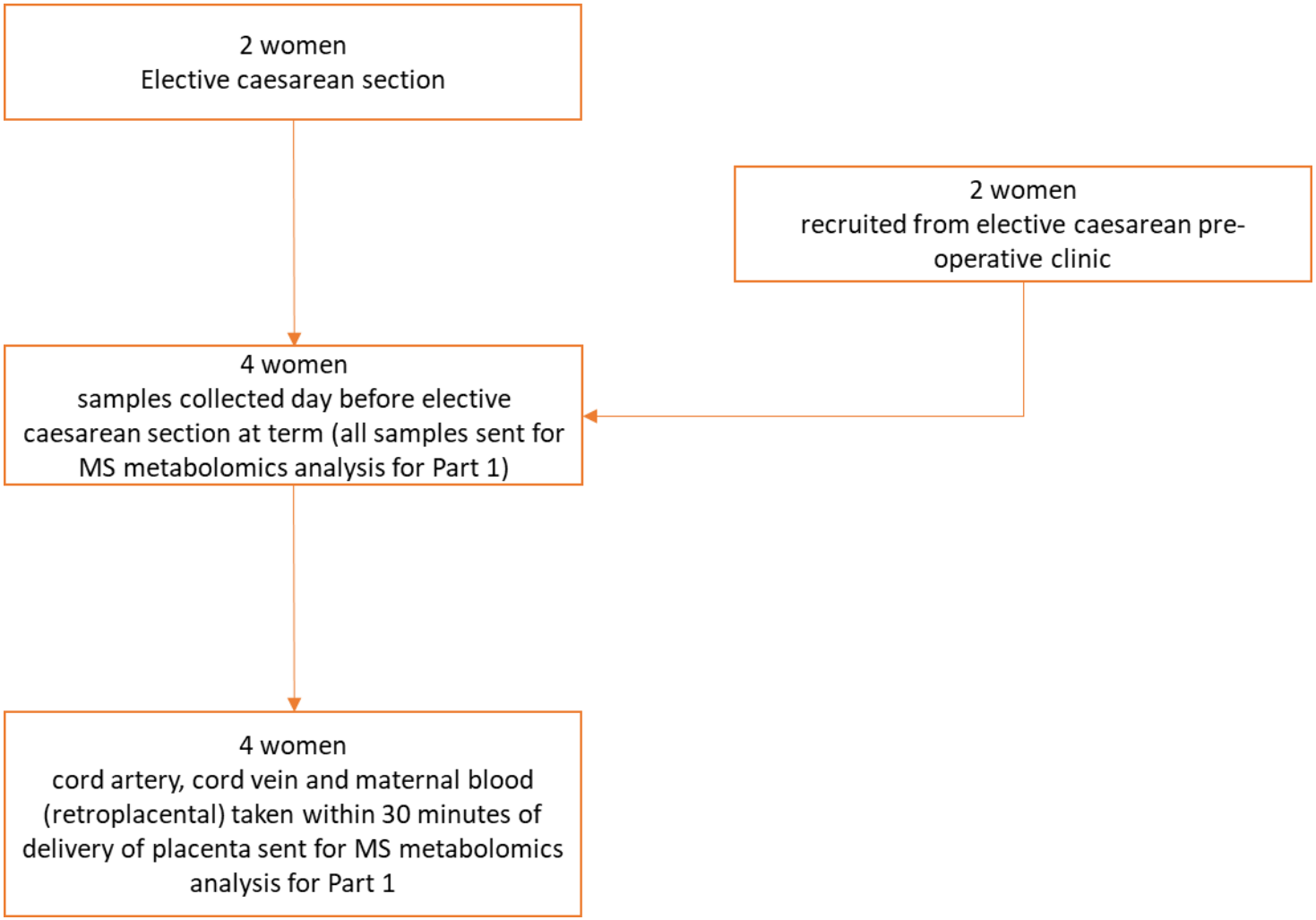

Figure 3.4: Samples taken and delivery outcomes for women who had an elective caesarean section at term.

3.5.3 Part 1 participants

Part 1 compared the metabolomic profile of latent/start of induction/pre-operative samples, CA, CV and IV samples from four women who spontaneous laboured (Figure 3.2), four women who had IOL (Figure 3.3), and four women who had an ECS (Figure 3.4).

Table 3.1 presents the demographic profile, pregnancy course and delivery outcomes for these women. Using a one-way ANOVA analysis, there were no statistical differences between the three groups regarding age at recruitment, booking BMI, Gravida, parity, estimated blood loss at delivery and birthweight. However, there was a significant difference between gestational age at delivery ($p=0.03$). In this study, the women who delivered spontaneously laboured after term, whereas two of those who were induced were induced before 40 completed weeks' gestion, and all ECSs occurred prior to 40 completed weeks' gestation.

Table 3.1: Maternal demographics and delivery outcomes for participants of Part 1 according to mode of delivery.

Demographic	Spontaneous labour (range)	Induction of labour (range)	Elective CS (range)	p-value
Age at recruitment (years)	31.5[a] (24-36)	31.75 [a] (29-34)	34.5 [a] (34-35)	0.421
BMI	23.925 [a] (23.1-25.4)	24.125 [a] (19.4-29.2)	25.325 [a] (22.5-28.7)	0.855
Past medical history	ALL NIL	ALL NIL	3 NIL 1 MILD ASTHMA	
Gravida	1.5[b] (1-3)	1.5 (1-4)	2 [b] (2-3)	0.79
Parity	0 [b] (0-1)	0 (0-2)	1 [b] ALL 1	0.3
Alcohol during pregnancy	ALL NO	ALL NO	ALL NO	
Ethnicity	ALL WE	ALL WE	ALL WE	
Smoking during pregnancy	ALL NO	ALL NO	1 3/day 3 NO	
Indication for El CS	NA	NA	2 PREV CS 1 MAT REQ 1 PREV 3RD	
Indication for IOL	NA	1 PT 2 RFM 1 MAT REQ	NA	
Gestational age at delivery	287.5 [b] (287-290)	276 [b] (269-292)	274.5 [b] (272-278)	0.035
Mode of delivery	3 NVD 1 VEN	2 NVD 1 VEN 1 FORC	4 EL CS	
Intrapartum oxytocin	ALL NO	2 YES 2 NO	NA	
Maternal complications	ALL NO	ALL NO ALL NO	ALL NA	
Estimated blood loss	512.5 [a] (250-1100)	350 [a] (100-800)	600 [a] (500-700)	0.51
Analgesia	3 ENT 1 LA	2 EPI 1 ENT 1 SP	4 sp	

Management third stage	ALL SYM	3 SYM 1 CAR	4 CAR	
Labour duration			NA	
1st stage	192.5 [b] (135-265)	194.5 [b] (45-300)		
2nd stage	80.5 [b] (37-209)	73 [b] (31-221)		
3rd stage	10 [b] (3-16)	5.5 [b] (5-11)		
Baby Apgar score	8,9,10-9,10,10	7,10,10-9,10,10	ALL 9,10,10	
Birthweight	3.80925 [a] (3.515-4.18)	3.39 [a] (2.36-4.164)	3.4275 [a] (3.202-3.588)	0.416
Baby sex	3 F 1 M	3 F 1 M	3 F 1 M	
Collection to freezing interval				

[a]Mean ; [b]Median; BMI=Body Mass Index; EBL=Estimated Blood Loss; NA=Not Applicable; WE=White European; PREV CS=Previous Caesarean Section; MAT REQ=Maternal Request; PREV 3rd=Previous Third Degree Perineal Tear; PT=Post-Term; RFM=Reduced Fetal Movements; NVD=Normal Vaginal Delivery; VEN=Ventouse or Kiwi Assisted Vaginal Delivery; FORC=Forceps Assisted Vaginal Delivery; El CS=Elective Caesaran Section; ENT=Entonox; LA=Local Anaesthetic; EPI=Epidural; SP=Spinal Regional Anaesthetic; SYM=Syntometrine (Oxytocin+Ergometrine); CAR=Carbetocin; F=Female; M=Male

3.5.4 Part 2 participants

The aim of Part 2 was to investigate whether there is an association between the metabolomic profile of maternal blood at 28 completed weeks', 34 completed weeks' gestation, or latent phase, and gestational age at spontaneous labour and delivery. All women who remained low-risk and who spontaneously laboured and who had blood samples taken at these time points were included in Part 2. In addition, women who were induced for post-term were included if they had otherwise remained low risk; as described above, this was because these women would be medically recommended not to continue their pregnancies beyond 42 weeks' gestation due to the increased risk for

stillbirth after this date. Therefore, it would be expected that no woman would be recommended to await spontaneous delivery after 42 weeks' gestation, and it was considered appropriate to include these women and to mark their gestational age at delivery as the gestational age at delivery (40 weeks' + 12-14 days). Therefore, all samples collected at 28 weeks' gestation and 34 weeks' gestation were included in Part 2, and samples taken in latent phase for the 12 women who spontaneously laboured at term and the samples taken at the time of induction for two women who were induced at post-term for not having laboured by these dates.

Table 3.2 presents the maternal demographics for the participants at each sample point. Samples from sixteen women were included for those taken at 28 weeks' and 34 weeks' gestation, and samples for fourteen women were included for those taken in the latent phase. Unsurprisingly, as most of the women had samples taken at each time point, there were no significant differences in the participant demographics between three different time points. 15 of the women included in the 28 weeks' group were included in the 34 weeks' group (one woman who was included at 28 weeks' gestation did not have further samples taken as she spontaneously delivered at 34 weeks' gestation; and one woman did not have a sample taken at 28 weeks' gestation as she had her routine blood tests taken early but did have samples taken at 34 weeks'

gestation and in latent phase). Two women had samples taken at 28 weeks' gestation and 34 weeks' gestation but not in latent phase simply because the researcher was not called into the hospital for their deliveries and they were missed.

At all three time points, the average age at recruitment was 31 years (range 24-40) for all groups, the average BMI was 24 (range 20-29.2), the median parity was 0 (range 0-1), none of the women drank alcohol during pregnancy, 15 women were non-smokers and one woman smoked up to five cigarettes a day. 15 of the women who were included in the 28 weeks' and 34 weeks' gestation samples were white European and one woman was white European/Chinese; and 13 of the women included in the latent phase samples were white European and one white European/Chinese. Thirteen of the women included at 28 weeks' and 34 weeks' gestation spontaneously laboured and three were induced for post-term. For the latent phase samples this reduced to 11 women who spontaneously laboured and 3 women who were induced for post-term. The average gestational age at delivery was 287 days for all three groups (range 240 to 295 for 28 weeks' gestation samples; 252 to 295 at 34 weeks' gestation samples; and 280 to 295 for latent phase samples.

Table 3.2: Maternal demographics for included participants for samples taken at 28- and 34-weeks' gestation and in latent phase: (majority same women in each group).

Demographics	28 weeks' Gestation n=16 (range)	34 weeks' Gestation n=16 (range)	Latent Phase n=14 (range)	p-value
Age at Recruitment (years)	**31.6**[a] (24-40)	**31.3**[a] (24-40)	**31.4**[a] (24-40)	**0.975**
BMI	**24.5**[a] (20-28.8)	**24.8**[a] (20-29.2)	**24.6**[a] (20-29.2)	**0.959**
PMH	**Nil**	**NIL**	**NIL**	
Gravida	**2**[b] (1-4)	**2**[b] (1-4)	**1.5**[b] (1-4)	**0.95**
Parity	**0**[b] (0-1)	**0**[b] (0-2)	**0**[b] (0-2)	**0.878**
Alcohol during pregnancy	**16 NIL**	**16 NIL**	**14 NIL**	
Ethnicity	**15 WE** **1 WE/Chinese**	**15 WE** **1 WE/Chinese**	**13 WE** **1 WE/Chinese**	
Smoking during pregnancy	**1 5/day** **15 NIL**	**1 5/day** **15 NIL**	**1 5/day** **13 NIL**	
Type of labour onset	**13 SP** **3 IOL PT**	**13 SP** **3 IOL PT**	**11 SP** **3 IOL PT**	
Gestational age at delivery	**287**[b] (240-295)	**287**[b] (252-295)	**287**[b] (280-295)	**0.192**
Maternal complications	**NIL**	**NIL**	**NIL**	
Apgar scores	**7,8,9-9,10,10**	**7,10,10-9,10,10**	**7,10,10-9,10,10**	
Birthweight	**3.620**[a] (2.44-4.18)	**3.707**[a] (3.196-4.18)	**3.734**[a] (3.196-4.18)	**0.679**
Baby sex	**6 M** **10 F**	**5 M** **11 F**	**4 M** **10 F**	
Sample collection to freezing time				

3.5.5 Correlations with gestational age at delivery

63 of the 1032 metabolites measured at 28 weeks' gestation were significantly ($p \leq 0.05$) correlated with gestational age at delivery (49 negative association; 14 positive association), and three of these had $q<0.1$ (Table 3.3); 84 of the 1032

metabolites measured at 34 weeks' gestation were significantly ($p \leq 0.05$) correlated with gestational age at delivery (75 negative association; 9 positive association), and two of these had $q < 0.1$ (Table 3.4); and 53 of the 1032 metabolites measured in latent phase (n=11) or at the beginning of IOL for post-term (n=3) were significantly ($p \leq 0.05$) correlated with gestational age at delivery (50 negative association; 3 positive association), and one of these had a $q < 0.1$ (Table 3.5).

Seven metabolites were significantly correlated with gestational age at all three time points; a further 15 were significantly correlated with gestational age at 28 weeks' gestation and 34 weeks' gestation, but not in latent phase; a further four significantly correlated with gestational age at 28 weeks' gestation and latent phase but not at 34 weeks' gestation; and a further ten metabolites were significantly correlated with gestational age at 34 weeks' gestation and in latent phase, but not at 28 weeks' gestation (Table 3.6). The seven metabolites significantly correlated at all time points were: octadecanedioylcarnitine (C18-DC), octadecenedioylcarnitine (C18:1-DC), 5alpha-androstan-3alpha,17alpha-diol disulfate, glycochenodeoxycholate glucuronide (1), tauroursodeoxycholic acid sulfate (1), N4-acetylcytidine and perfluorooctanesulfonate (PFOS) (Table 3.6 and Figure 3.5). At all-time points, all of these were negatively correlated with gestational age at spontaneous labour.

Table 3.3: Metabolites which significantly correlate with gestational age at delivery for those samples taken at 28 weeks' gestation, ordered according to p-value (considered significant if p≤0.05.

Metabolite	Sub pathway	Super pathway	Spearman rank correlation	p-value	q-value
5alpha-pregnan-3beta,20alpha-diol disulfate	Progestin Steroids	Lipid	-0.8149	**0.0001**	0.0806
Glucuronide of piperine metabolite C17H21NO3 (4)	Food Component/Plant	Xenobiotics	0.7971	**0.0002**	0.0806
Indolin-2-one	Food Component/Plant	Xenobiotics	-0.7839	**0.0003**	0.0806
Octadecenedioylcarnitine (C18:1-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	-0.7465	**0.0009**	0.1657
Beta-cryptoxanthin	Vitamin A Metabolism	Cofactors and Vitamins	0.7287	**0.0014**	0.2026
5alpha-pregnan-3beta,20alpha-diol monosulfate (1)	Progestin Steroids	Lipid	-0.7004	**0.0025**	0.3107
3-hydroxyhexanoylcarnitine (1)	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	-0.6883	**0.0032**	0.3388
Tauro-beta-muricholate	Primary Bile Acid Metabolism	Lipid	-0.6751	**0.0041**	0.3388
Octadecanedioylcarnitine (C18-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	-0.6682	**0.0047**	0.3388
2-naphthol sulfate	Chemical	Xenobiotics	-0.6603	**0.0054**	0.3388
Deoxycholic acid glucuronide	Secondary Bile Acid Metabolism	Lipid	-0.6499	**0.0064**	0.3388
Pelargonate (9:0)	Medium Chain Fatty Acid	Lipid	-0.6484	**0.0066**	0.3388
Glyco-beta-muricholate	Primary Bile Acid Metabolism	Lipid	-0.6484	**0.0066**	0.3388
6-hydroxyindole sulfate	Chemical	Xenobiotics	-0.6409	**0.0075**	0.3388
Perfluorooctanesulfonate (PFOS)	Chemical	Xenobiotics	-0.6394	**0.0076**	0.3388
(2 or 3)-decenoate (10:1n7 or n8)	Medium Chain Fatty Acid	Lipid	-0.6380	**0.0078**	0.3388
Taurodeoxycholate	Secondary Bile Acid Metabolism	Lipid	-0.6365	**0.0080**	0.3388
Tridecenedioate (C13:1-DC)	Fatty Acid, Dicarboxylate	Lipid	-0.6350	**0.0082**	0.3388

(2-butoxyethoxy) acetic acid	Chemical	Xenobiotics	0.6276	**0.0092**	0.3392
Androstenediol (3beta,17beta) monosulfate (1)	Androgenic Steroids	Lipid	0.6275	**0.0093**	0.3392
Indolepropionate	Tryptophan Metabolism	Amino acid	0.6171	**0.0109**	0.3392
Tauroursodeoxycholic acid sulfate (1)	Secondary Bile Acid Metabolism	Lipid	-0.6147	**0.0113**	0.3392
N-acetylglucosamine/N-acetylgalactosamine	Aminosugar Metabolism	Carbohydrate	-0.6142	**0.0114**	0.3392
Glycochenodeoxycholate glucuronide (1)	Primary Bile Acid Metabolism	Lipid	-0.6112	**0.0119**	0.3392
Ascorbic acid 3-sulfate	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	0.6112	**0.0119**	0.3392
Glucuronide of piperine metabolite C17H21NO3 (5)	Food Component/Plant	Xenobiotics	0.6101	**0.0121**	0.3392
21-hydroxypregnanolone disulfate	Pregnenolone Steroids	Lipid	-0.6082	**0.0124**	0.3392
Dodecanedioate (C12-DC)	Fatty Acid, Dicarboxylate	Lipid	-0.6038	**0.0133**	0.3392
Taurodeoxycholic acid 3-sulfate	Secondary Bile Acid Metabolism	Lipid	-0.6038	**0.0133**	0.3392
Dodecadienoate (12:2)	Fatty Acid, Dicarboxylate	Lipid	-0.5978	**0.0145**	0.3573
Dehydroepiandrosterone sulfate (DHEA-S)	Androgenic Steroids	Lipid	0.5933	**0.0154**	0.3644
Gamma-glutamylhistidine	Gamma-glutamyl Amino Acid	Peptide	-0.5919	**0.0157**	0.3644
3-hydroxyoleoylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	-0.5802	**0.0185**	0.4148
Chenodeoxycholic acid sulfate (1)	Primary Bile Acid Metabolism	Lipid	-0.5773	**0.0192**	0.4167
1-linoleoylglycerol (18:2)	Monoacylglycerol	Lipid	-0.5755	**0.0197**	0.4167
17alpha-hydroxypregnenolone 3-sulfate	Pregnenolone Steroids	Lipid	0.5695	**0.0213**	0.4384
8-methoxykynurenate	Tryptophan Metabolism	Amino acid	-0.5665	**0.0222**	0.4440
Aconitate [cis or trans]	TCA Cycle	Energy	-0.5577	**0.0248**	0.4840
Gamma-glutamylmethionine	Gamma-glutamyl Amino Acid	Peptide	-0.5532	**0.0262**	0.4954
Gamma-CEHC	Tocopherol Metabolism	Cofactors and Vitamins	-0.5517	**0.0267**	0.4954
3-indoxyl sulfate	Tryptophan Metabolism	Amino acid	-0.5487	**0.0277**	0.5014
Phenol sulfate	Tyrosine Metabolism	Amino acid	-0.5458	**0.0288**	0.5077
Glycodeoxycholate 3-sulfate	Secondary Bile Acid Metabolism	Lipid	-0.5428	**0.0298**	0.5142
Acesulfame	Food Component/Plant	Xenobiotics	0.5398	**0.0309**	0.5212

Oxalate (ethanedioate)	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	0.5339	**0.0332**	0.5275
4-hydroxyphenylacetylglutamine	Acetylated Peptides	Peptide	-0.5309	**0.0343**	0.5275
Laurylcarnitine (C12)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	-0.5309	**0.0344**	0.5275
3-formylindole	Food Component/Plant	Xenobiotics	0.5309	**0.0344**	0.5275
N-stearoyltaurine	Endocannabinoid	Lipid	-0.5283	**0.0354**	0.5275
N-acetylalanine	Alanine and Aspartate Metabolism	Amino acid	-0.5279	**0.0356**	0.5275
10-undecenoate (11:1n1)	Medium Chain Fatty Acid	Lipid	-0.5220	**0.0381**	0.5422
5alpha-androstan-3alpha,17alpha-diol disulfate	Androgenic Steroids	Lipid	-0.5190	**0.0394**	0.5422
N6-succinyladenosine	Purine Metabolism, Adenine containing	Nucleotide	-0.5170	**0.0403**	0.5422
Trans-3,4-methyleneheptanoate	Food Component/Plant	Xenobiotics	-0.5161	**0.0407**	0.5422
Pregnanediol-3-glucuronide	Progestin Steroids	Lipid	-0.5130	**0.0421**	0.5422
9-hydroxystearate	Fatty Acid, Monohydroxy	Lipid	0.5119	**0.0426**	0.5422
Mead acid (20:3n9)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	-0.5086	**0.0443**	0.5422
Glcnac sulfate conjugate of C21H34O2 steroid	Partially Characterized Molecules	Partially Characterised Molecules	-0.5086	**0.0443**	0.5422
N-acetylcitrulline	Urea cycle; Arginine and Proline Metabolism	Amino acid	0.5084	**0.0444**	0.5422
3-hydroxyadipate	Fatty Acid, Dicarboxylate	Lipid	-0.5079	**0.0446**	0.5422
Hydroquinone sulfate	Drug - Topical Agents	Xenobiotics	-0.5079	**0.0446**	0.5422
N4-acetylcytidine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	-0.5041	**0.0465**	0.5557
Myristoleoylcarnitine (C14:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	-0.5011	**0.0480**	0.5648

Table 3.4: Metabolites which significantly correlate with gestational age at delivery for those samples taken at 34 weeks' gestation, ordered according to p-value (considered significant if p≤0.05).

Metabolite	Sub pathway	Super pathway	Spearman Correlation	p-value	q-value
Hydroxy-N6,N6,N6-trimethyllysine	Lysine Metabolism	Amino acid	-0.8111	0.0001	0.0797
N6-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide	-0.8066	0.0002	0.0797
Tauroursodeoxycholic acid sulfate (1)	Secondary Bile Acid Metabolism	Lipid	-0.7584	0.0007	0.1694
Hydroxyasparagine	Alanine and Aspartate Metabolism	Amino acid	-0.7486	0.0008	0.1694
Octadecanedioylcarnitine (C18-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	-0.7501	0.0008	0.1694
Gamma-glutamylthreonine	Gamma-glutamyl Amino Acid	Peptide	-0.7396	0.0011	0.1756
5alpha-androstan-3alpha,17alpha-diol disulfate	Androgenic Steroids	Lipid	-0.7277	0.0014	0.1861
Dehydroepiandrosterone sulfate (DHEA-S)	Androgenic Steroids	Lipid	0.7248	0.0015	0.1861
Androstenediol (3beta,17beta) monosulfate (1)	Androgenic Steroids	Lipid	0.7099	0.0021	0.2192
5-(galactosylhydroxy)-L-lysine	Lysine Metabolism	Amino acid	-0.7069	0.0022	0.2192
N-acetylglucosamine/N-acetylgalactosamine	Aminosugar Metabolism	Carbohydrate	-0.6965	0.0027	0.2467
C-glycosyltryptophan	Tryptophan Metabolism	Amino acid	-0.6875	0.0032	0.2698
Flavin adenine dinucleotide (FAD)	Riboflavin Metabolism	Cofactors and Vitamins	-0.6831	0.0035	0.2714
N-acetylglucosaminylasparagine	Aminosugar Metabolism	Carbohydrate	-0.6771	0.0040	0.2819
Suberoylcarnitine (C8-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	-0.6682	0.0047	0.3101
Oestriol 16-glucuronide	Estrogenic Steroids	Lipid	-0.6578	0.0056	0.3378
Perfluorooctanesulfonate (PFOS)	Chemical	Xenobiotics	-0.6563	0.0058	0.3378
N-stearoyl-sphingosine (d18:1/18:0)	Ceramides	Lipid	-0.6459	0.0069	0.3809
Pro-hydroxy-pro	Urea cycle; Arginine and Proline Metabolism	Amino acid	-0.6399	0.0076	0.3982

Ribonate	Pentose Metabolism	Carbohydrate	-0.6355	0.0082	0.4068
3-hydroxy-2-ethylpropionate	Leucine, Isoleucine and Valine Metabolism	Amino acid	-0.6280	0.0092	0.4164
Nicotinamide adenine dinucleotide (NAD+)	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	-0.6280	0.0092	0.4164
Sulfate	Chemical	Xenobiotics	-0.6250	0.0096	0.4173
1-linoleoyl-GPA (18:2)	Lysophospholipid	Lipid	-0.6146	0.0113	0.4186
Gamma-glutamylglutamate	Gamma-glutamyl Amino Acid	Peptide	-0.6117	0.0118	0.4186
1-stearoyl-2-oleoyl-GPC (18:0/18:1)	Phosphatidylcholine (PC)	Lipid	-0.6102	0.0121	0.4186
Deoxycholic acid glucuronide	Secondary Bile Acid Metabolism	Lipid	-0.6102	0.0121	0.4186
Pseudouridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	-0.6102	0.0121	0.4186
N4-acetylcytidine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	-0.6057	0.0129	0.4186
Succinoyltaurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino acid	-0.6043	0.0132	0.4186
N-palmitoyltaurine	Endocannabinoid	Lipid	-0.6009	0.0138	0.4186
Pregnenediol sulfate (C21H34O5S)	Pregnenolone Steroids	Lipid	0.5997	0.0141	0.4186
5alpha-pregnan-3beta,20alpha-diol disulfate	Progestin Steroids	Lipid	-0.5983	0.0144	0.4186
(N(1) + N(8))-acetylspermidine	Polyamine Metabolism	Amino acid	-0.5953	0.0150	0.4186
Phytanate	Food Component/Plant	Xenobiotics	-0.5953	0.0150	0.4186
3-(3-amino-3-carboxypropyl) uridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	-0.5927	0.0155	0.4186
5alpha-androstan-3beta,17alpha-diol disulfate	Androgenic Steroids	Lipid	-0.5923	0.0156	0.4186
Nonadecanoate (19:0)	Long Chain Saturated Fatty Acid	Lipid	-0.5908	0.0160	0.4186
Dimethylguanidino valeric acid (DMGV)	Urea cycle; Arginine and Proline Metabolism	Amino acid	-0.5849	0.0173	0.4430
5alpha-androstan-3alpha,17beta-diol disulfate	Androgenic Steroids	Lipid	-0.5823	0.0179	0.4445
Stearoyl sphingomyelin (d18:1/18:0)	Sphingomyelins	Lipid	-0.5789	0.0188	0.4445

Hypoxanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	-0.5789	0.0188	0.4445
5,6-dihydrouridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	-0.5774	0.0192	0.4445
N,N,N-trimethyl-alanylproline betaine (TMAP)	Urea cycle; Arginine and Proline Metabolism	Amino acid	-0.5715	0.0207	0.4641
4-hydroxyglutamate	Glutamate Metabolism	Amino acid	-0.5655	0.0224	0.4641
3-hydroxyhexanoylcarnitine (1)	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	-0.5655	0.0224	0.4641
Oestriol 3-sulfate	Estrogenic Steroids	Lipid	-0.5655	0.0224	0.4641
1-stearoyl-GPG (18:0)	Lysophospholipid	Lipid	-0.5645	0.0227	0.4641
Cis-urocanate	Histidine Metabolism	Amino acid	-0.5637	0.0230	0.4641
1-palmitoyl-GPA (16:0)	Lysophospholipid	Lipid	-0.5615	0.0236	0.4641
Sphingomyelin (d18:2/24:1, d18:1/24:2)	Sphingomyelins	Lipid	0.5596	0.0242	0.4641
Pregnanediol-3-glucuronide	Progestin Steroids	Lipid	-0.5581	0.0247	0.4641
Beta-cryptoxanthin	Vitamin A Metabolism	Cofactors and Vitamins	0.5581	0.0247	0.4641
21-hydroxypregnanolone disulfate	Pregnenolone Steroids	Lipid	-0.5548	0.0257	0.4655
3-formylindole	Food Component/Plant	Xenobiotics	0.5536	0.0261	0.4655
Octadecenedioylcarnitine (C18:1-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	-0.5506	0.0271	0.4655
Glycochenodeoxycholate glucuronide (1)	Primary Bile Acid Metabolism	Lipid	-0.5506	0.0271	0.4655
2-naphthol sulfate	Chemical	Xenobiotics	-0.5506	0.0271	0.4655
N,N,N-trimethyl-5-aminovalerate	Lysine Metabolism	Amino acid	-0.5462	0.0286	0.4835
Stearate (18:0)	Long Chain Saturated Fatty Acid	Lipid	-0.5432	0.0297	0.4930
1,5-anhydroglucitol (1,5-AG)	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	-0.5417	0.0302	0.4937
S-adenosylhomocysteine (SAH)	Methionine, Cysteine, SAM and Taurine Metabolism	Amino acid	-0.5395	0.0310	0.4955
Glyco-beta-muricholate	Primary Bile Acid Metabolism	Lipid	-0.5387	0.0313	0.4955

5alpha-androstan-3alpha,17alpha-diol monosulfate	Androgenic Steroids	Lipid	-0.5358	0.0324	0.5053
Succinylcarnitine (C4-DC)	TCA Cycle	Energy	-0.5328	0.0336	0.5137
N-stearoyltaurine	Endocannabinoid	Lipid	-0.5318	0.0340	0.5137
Deoxycholic acid 12-sulfate	Secondary Bile Acid Metabolism	Lipid	-0.5280	0.0355	0.5290
(R)-3-hydroxybutyrylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	-0.5253	0.0366	0.5373
N-palmitoylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	-0.5209	0.0386	0.5493
Sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)	Sphingomyelins	Lipid	0.5209	0.0386	0.5493
Arachidate (20:0)	Long Chain Saturated Fatty Acid	Lipid	-0.5194	0.0392	0.5502
N-acetylhistidine	Histidine Metabolism	Amino acid	-0.5149	0.0412	0.5502
Gamma-glutamylphenylalanine	Gamma-glutamyl Amino Acid	Peptide	-0.5149	0.0412	0.5502
17alpha-hydroxypregnanolone glucuronide	Pregnenolone Steroids	Lipid	-0.5149	0.0412	0.5502
Adipoylcarnitine (C6-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	-0.5134	0.0419	0.5502
Urate	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	-0.5134	0.0419	0.5502
Hexadecanedioate (C16-DC)	Fatty Acid, Dicarboxylate	Lipid	-0.5090	0.0441	0.5561
Stearoylcarnitine (C18)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	-0.5090	0.0441	0.5561
Linoleoyl-linoleoyl-glycerol (18:2/18:2) [2]	Diacylglycerol	Lipid	0.5090	0.0441	0.5561
Hyocholate	Secondary Bile Acid Metabolism	Lipid	-0.5049	0.0461	0.5696
3-aminoisobutyrate	Pyrimidine Metabolism, Thymine containing	Nucleotide	0.5045	0.0463	0.5696
N1-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide	-0.5030	0.0470	0.5718
Tiglylcarnitine (C5:1-DC)	Leucine, Isoleucine and Valine Metabolism	Amino acid	-0.5000	0.0486	0.5764

Pantothenate	Pantothenate and CoA Metabolism	Cofactors and Vitamins	-0.5000	0.0486	0.5764

Table 3.5: Metabolites which significantly correlate with gestational age at delivery for those samples taken in latent phase (n=11)/prior start of IOL (n=3) for post-term, ordered according to p-value (considered significant if p≤0.05).

Metabolite	Sub pathway	Super pathway	Correlation	p-value	q-value
N-acetylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino acid	-0.8860	0.0000	0.0249
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino acid	-0.7697	0.0013	0.5114
Glycochenodeoxycholate glucuronide (1)	Primary Bile Acid Metabolism	Lipid	-0.7563	0.0017	0.5114
N-acetylserine	Glycine, Serine and Threonine Metabolism	Amino acid	-0.7496	0.0020	0.5114
5-(galactosylhydroxy)-L-lysine	Lysine Metabolism	Amino acid	-0.7294	0.0031	0.5736
Hydroxyasparagine	Alanine and Aspartate Metabolism	Amino acid	-0.7227	0.0035	0.5736
Cis-4-decenoylcarnitine (C10:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	-0.7160	0.0040	0.5736
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)	Methionine, Cysteine, SAM and Taurine Metabolism	Amino acid	-0.6936	0.0059	0.7075
5alpha-androstan-3alpha,17alpha-diol disulfate	Androgenic Steroids	Lipid	-0.6847	0.0069	0.7075
Octadecenedioylcarnitine (C18:1-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	-0.6780	0.0077	0.7075
Oestriol 16-glucuronide	Estrogenic Steroids	Lipid	-0.6780	0.0077	0.7075
Arabitol/xylitol	Pentose Metabolism	Carbohydrate	-0.6623	0.0099	0.8299
N-acetylhistidine	Histidine Metabolism	Amino acid	-0.6533	0.0113	0.8414
N-acetylalanine	Alanine and Aspartate Metabolism	Amino acid	-0.6511	0.0117	0.8414
Perfluorooctanesulfonate (PFOS)	Chemical	Xenobiotics	-0.6422	0.0133	0.8415
Oestriol 3-sulfate	Estrogenic Steroids	Lipid	-0.6354	0.0146	0.8415
Hexanoylcarnitine (C6)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	-0.6332	0.0151	0.8415
Phenylacetylglutamate	Acetylated Peptides	Peptide	-0.6206	0.0179	0.8415

Tetradecanedioate (C14-DC)	Fatty Acid, Dicarboxylate	Lipid	-0.6198	0.0181	0.8415
N,N,N-trimethyl-alanylproline betaine (TMAP)	Urea cycle; Arginine and Proline Metabolism	Amino acid	-0.6153	0.0192	0.8415
1-palmitoyl-GPE (16:0)	Lysophospholipid	Lipid	0.6086	0.0209	0.8415
5-hydroxymethyl-2-furoylcarnitine	Food Component/Plant	Xenobiotics	-0.6030	0.0225	0.8415
17alpha-hydroxypregnanolone glucuronide	Pregnenolone Steroids	Lipid	-0.5996	0.0234	0.8415
Gamma-carboxyglutamate	Glutamate Metabolism	Amino acid	-0.5974	0.0241	0.8415
Cortisol 21-sulfate	Corticosteroids	Lipid	-0.5966	0.0243	0.8415
Hydroquinone sulfate	Drug - Topical Agents	Xenobiotics	-0.5911	0.0260	0.8415
1-oleoyl-GPE (18:1)	Lysophospholipid	Lipid	0.5885	0.0268	0.8415
Octadecanedioylcarnitine (C18-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	-0.5862	0.0276	0.8415
N6-carbamoylthreonyladenosine	Purine Metabolism, Adenine containing	Nucleotide	-0.5862	0.0276	0.8415
Octadecanedioate (C18-DC)	Fatty Acid, Dicarboxylate	Lipid	-0.5795	0.0299	0.8415
Chiro-inositol	Inositol Metabolism	Lipid	0.5776	0.0305	0.8415
Linoleoyl-linoleoyl-glycerol (18:2/18:2) [2]	Diacylglycerol	Lipid	-0.5773	0.0306	0.8415
O-sulfo-L-tyrosine	Chemical	Xenobiotics	-0.5773	0.0306	0.8415
5alpha-androstan-3alpha,17beta-diol disulfate	Androgenic Steroids	Lipid	-0.5723	0.0325	0.8415
3-indoxyl sulfate	Tryptophan Metabolism	Amino acid	-0.5706	0.0331	0.8415
Decanoylcarnitine (C10)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	-0.5638	0.0357	0.8415
N-acetyl-cadaverine	Lysine Metabolism	Amino acid	-0.5616	0.0366	0.8415
Phenylacetylglutamine	Acetylated Peptides	Peptide	-0.5616	0.0366	0.8415
Suberate (C8-DC)	Fatty Acid, Dicarboxylate	Lipid	-0.5616	0.0366	0.8415
Tauroursodeoxycholic acid sulfate (1)	Secondary Bile Acid Metabolism	Lipid	-0.5580	0.0381	0.8415
Octanoylcarnitine (C8)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	-0.5571	0.0385	0.8415
4-ethylcatechol sulfate	Benzoate Metabolism	Xenobiotics	-0.5571	0.0385	0.8415
Glycodeoxycholate glucuronide (1)	Secondary Bile Acid Metabolism	Lipid	-0.5543	0.0397	0.8415

N-acetylputrescine	Polyamine Metabolism	Amino acid	-0.5527	0.0404	0.8415
Pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	-0.5527	0.0404	0.8415
N4-acetylcytidine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	-0.5504	0.0414	0.8415
Pro-hydroxy-pro	Urea cycle; Arginine and Proline Metabolism	Amino acid	-0.5482	0.0424	0.8415
Indoleacetylglutamine	Tryptophan Metabolism	Amino acid	-0.5424	0.0451	0.8415
5-hydroxyindoleacetate	Tryptophan Metabolism	Amino acid	-0.5417	0.0454	0.8415
N-acetyl-beta-alanine	Pyrimidine Metabolism, Uracil containing	Nucleotide	-0.5415	0.0455	0.8415
5alpha-pregnan-3beta,20alpha-diol monosulfate (1)	Progestin Steroids	Lipid	-0.5370	0.0477	0.8415
4-acetamidobutanoate	Polyamine Metabolism	Amino acid	-0.5348	0.0488	0.8415
Linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [2]	Diacylglycerol	Lipid	-0.5324	0.0500	0.8415

Table 3.6: Metabolites which significantly correlate with gestational age at delivery for at least two of the sampled time points (p≤0.05).

Metabolite	Sub pathway	Super-pathway	Correlation 28 weeks	p-value 28 weeks	Correlation 34 weeks	p-value 34 weeks	Correlation Latent	p-value Latent
Octadecanedioylcarnitine (C18-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	**-0.6682**	0.0047	**-0.7501**	0.0008	**-0.5862**	0.0276
Octadecenedioylcarnitine (C18:1-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	**-0.7465**	0.0009	**-0.5506**	0.0271	**-0.6780**	0.0077
5alpha-androstan-3alpha,17alpha-diol disulphate	Androgenic Steroids	Lipid	**-0.5190**	0.0394	**-0.7277**	0.0014	**-0.6847**	0.0069
Glycochenodeoxycholate glucuronide (1)	Primary Bile Acid Metabolism	Lipid	**-0.6112**	0.0119	**-0.5506**	0.0271	**-0.7563**	0.0017
Tauroursodeoxycholic acid sulphate (1)	Secondary Bile Acid Metabolism	Lipid	**-0.6147**	0.0113	**-0.7584**	0.0007	**-0.5580**	0.0381
N4-acetylcytidine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**-0.5041**	0.0465	**-0.6057**	0.0129	**-0.5504**	0.0414
Perfluorooctanesulphonate (PFOS)	Chemical	Xenobiotics	**-0.6394**	0.0076	**-0.6563**	0.0058	**-0.6422**	0.0133
N-acetylglucosamine/N-acetylgalactosamine	Aminosugar Metabolism	Carbohydrate	**-0.6142**	0.0114	**-0.6965**	0.0027		

3-hydroxyhexanoylcarnitine (1)	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**-0.6883**	0.0032	**-0.5655**	0.0224		
N-stearoyltaurine	Endocannabinoid	Lipid	**-0.5283**	0.0354	**-0.5318**	0.0340		
21-hydroxypregnanolone disulfate	Pregnenolone Steroids	Lipid	**-0.6082**	0.0124	**-0.5548**	0.0257		
5alpha-pregnan-3beta,20alpha-diol disulfate	Progestin Steroids	Lipid	**-0.8149**	0.0001	**-0.5983**	0.0144		
Pregnanediol-3-glucuronide	Progestin Steroids	Lipid	**-0.5130**	0.0421	**-0.5581**	0.0247		
Dehydroepiandrosterone sulfate (DHEA-S)	Androgenic Steroids	Lipid	**0.5933**	0.0154	**0.7248**	0.0015		
Androstenediol (3beta,17beta) monosulfate (1)	Androgenic Steroids	Lipid	**0.6275**	0.0093	**0.7099**	0.0021		
Glyco-beta-muricholate	Primary Bile Acid Metabolism	Lipid	**-0.6484**	0.0066	**-0.5387**	0.0313		
Deoxycholic acid glucuronide	Secondary Bile Acid Metabolism	Lipid	**-0.6499**	0.0064	**-0.6102**	0.0121		
Beta-cryptoxanthin	Vitamin A Metabolism	Cofactors and Vitamins	**0.7287**	0.0014	**0.5581**	0.0247		
3-formylindole	Food Component/Plant	Xenobiotics	**0.5309**	0.0344	**0.5536**	0.0261		
2-naphthol sulfate	Chemical	Xenobiotics	**-0.6603**	0.0054	**-0.5506**	0.0271		
N-acetylalanine	Alanine and Aspartate Metabolism	Amino acid	**-0.5279**	0.0356			**-0.6511**	0.0117

3-indoxyl sulfate	Tryptophan Metabolism	Amino acid	**-0.5487**	0.0277			**-0.5706**	0.0331
5alpha-pregnan-3beta,20alpha-diol monosulfate (1)	Progestin Steroids	Lipid	**-0.7004**	0.0025			**-0.5370**	0.0477
Hydroquinone sulfate	Drug - Topical Agents	Xenobiotics	**-0.5079**	0.0446			**-0.5911**	0.0260
Hydroxyasparagine	Alanine and Aspartate Metabolism	Amino acid			**-0.7486**	0.0008	**-0.7227**	0.0035
N-acetylhistidine	Histidine Metabolism	Amino acid			**-0.5149**	0.0412	**-0.6533**	0.0113
5-(galactosylhydroxy)-L-lysine	Lysine Metabolism	Amino acid			**-0.7069**	0.0022	**-0.7294**	0.0031
Pro-hydroxy-pro	Urea cycle; Arginine and Proline Metabolism	Amino acid			**-0.6399**	0.0076	**-0.5482**	0.0424
N,N,N-trimethyl-alanylproline betaine (TMAP)	Urea cycle; Arginine and Proline Metabolism	Amino acid			**-0.5715**	0.0207	**-0.6153**	0.0192
Linoleoyl-linoleoyl-glycerol (18:2/18:2) [2]	Diacylglycerol	Lipid			**0.5090**	0.0441	**-0.5773**	0.0306
17alpha-hydroxypregnanolone glucuronide	Pregnenolone Steroids	Lipid			**-0.5149**	0.0412	**-0.5996**	0.0234
5alpha-androstan-3alpha,17beta-diol disulfate	Androgenic Steroids	Lipid			**-0.5823**	0.0179	**-0.5723**	0.0325

Oestriol 3-sulfate	Estrogenic Steroids	Lipid	**-0.5655**	0.0224	**-0.6354**	0.0146
Oestriol 16-glucuronide	Estrogenic Steroids	Lipid	**-0.6578**	0.0056	**-0.6780**	0.0077

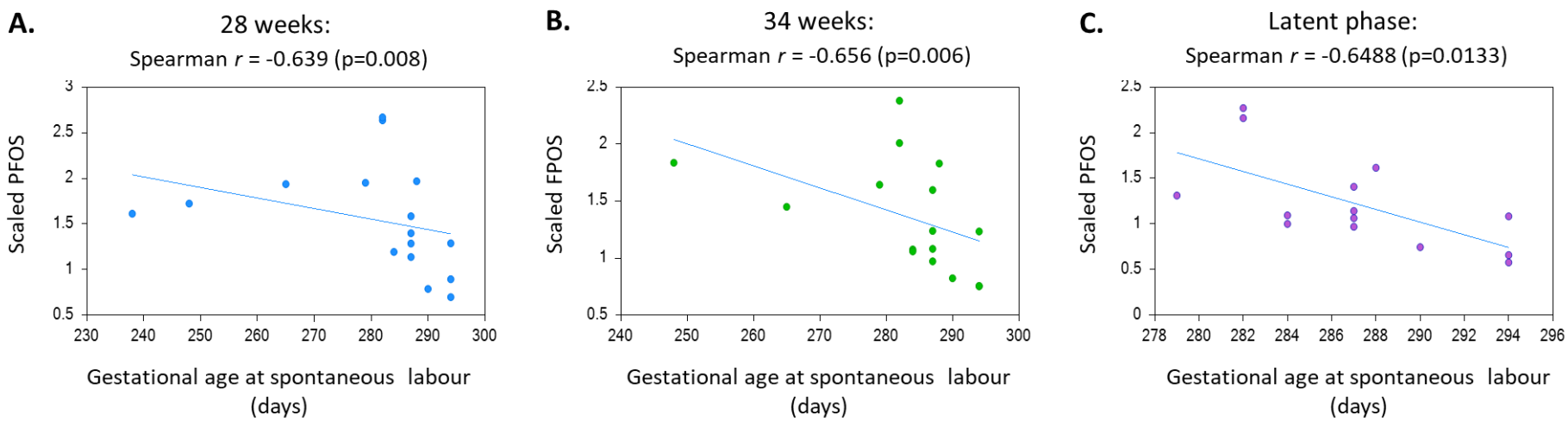

Figure 3.5: Scatter plots showing correlation between metabolite level and gestational age at delivery for perfluorooctanesulfonate (PFOS), the chemical for which the correlation was significant at each of the sample time points with Spearman correlations of: A. -0.639 (p=0.008) at 28 weeks' gestation (n=16); B. -0.656 (p=0.006) at 34 weeks' gestation (n=16); C. -0.6488 (p=0.0133) in latent phase (n=14). Scaled PFOS for each sample determined following normalisation by sample volume, log-transformation, then rescaling to set the median PFOS for all samples to be equal to 1.

3.5.6 Summary of differences in maternal metabolites between sampling points during pregnancy

Using paired t-tests, between 28 and 34 weeks' gestation, 159 metabolites significantly increased ($p \leq 0.05$; 117 of which $q < 0.1$), and 40 metabolites significantly decreased ($p \leq 0.05$; 28 of which $q < 0.1$). Between 28 weeks' gestation and latent phase 364 metabolites significantly increased ($p \leq 0.05$; all of which $q < 0.1$) and 54 metabolites significantly decreased ($p \leq 0.05$; all of which $q < 0.1$). Between 34 weeks' gestation and latent phase 306 metabolites significantly increased ($p \leq 0.05$; all of which $q < 0.1$) and 50 significantly decreased ($p \leq 0.05$; all of which $q < 0.1$) (Table 3A3, Appendices). Figure 3.6 is a Venn diagram designed to illustrate the overlap of the of metabolites with significant differences between paired time points (28 weeks vs 34 weeks; 34 weeks vs latent phase; 28 weeks vs latent phase). This shows that differences between metabolites at the paired time points 28 weeks vs 34 weeks is not simply a subset of 28 weeks vs latent phase, which likewise is not a subset of 34 weeks vs latent phase. This indicates that there are specific changes in certain metabolites which occur at certain gestations of pregnancy, and these could be investigated further in future studies.

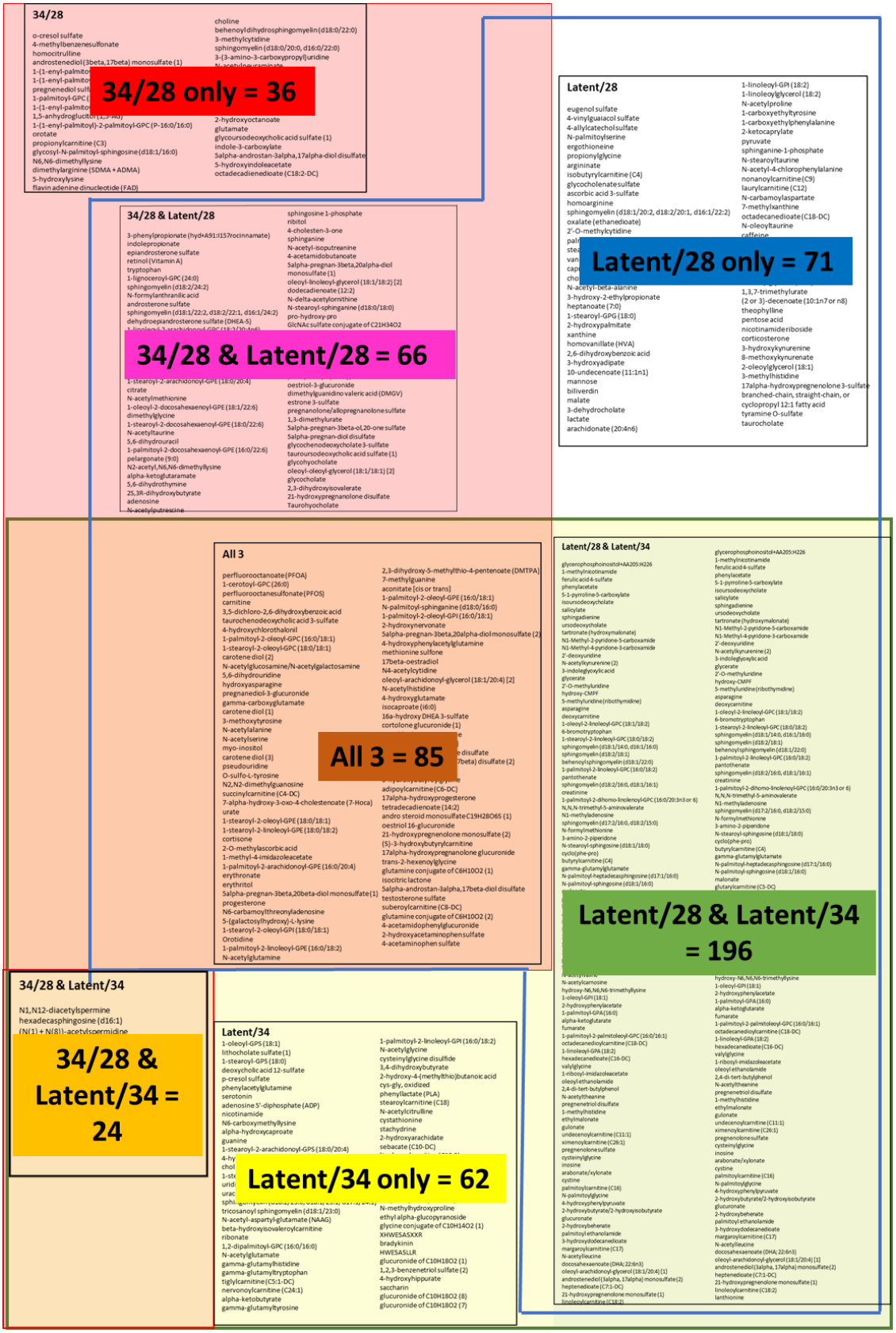

Figure 3.6: Venn diagram illustrating the spread and degree of overlap of metabolites with significant differences between paired time points (28 weeks vs 34 weeks = red; 34 weeks vs latent phase = yellow; 28 weeks vs latent phase = blue).

Table 3.7: Table showing significant changes for the endocannabinoids.

Metabolite	Latent 28	Latent 34	CVCS CACS	CVS IVS	CVCS IVCS	CAIOL IVIOL	CACS IVCS	LCS L	CAIOL CAS	CACS CAS	CVIOL CVS
Oleoyl ethanolamide	**1.41**	**1.33**			**0.43**	**0.40**	**0.43**				
Palmitoyl ethanolamide	**1.52**	**1.42**		**0.68**	**0.72**		**0.69**	**0.56**			
N-oleoyltaurine	**1.48**									**0.52**	
N-stearoyltaurine	**1.40**								**0.65**	**0.64**	**1.56**
N-palmitoyltaurine										**0.63**	
N-linoleoyltaurine			**1.43**								
Linoleoyl ethanolamide	**2.06**	**2.01**		**0.31**	**0.19**	**0.16**	**0.19**	**0.44**			
N-oleoylserine											
N-palmitoylserine	**0.69**				**5.40**		**4.76**	**2.73**			

Significant fold-changes for endocannabinoids (all results shown are significant at p≤0.05); paired t-tests for differences in the mean value between: latent phase samples and samples taken at 28 weeks' gestation (L/28); latent phase samples and samples taken at 34 weeks' gestation (L/34); cord vein samples and cord artery samples of the elective caesarean section group (CVCS/CACS); cord vein samples and intervillous samples of the spontaneously labouring group (CVS/IVS); cord vein samples and intervillous samples of the elective caesarean section group (CVCS/IVCS); cord artery and intervillous samples of the induction of labour group (CAIOL/IVIOL); cord artery and intervillous samples of the elective caesarean section group (CACS/IVCS); and Welch's t-test for differences in the mean value between: samples taken in the pre-operative clinic for the elective caesarean group and samples taken in the latent phase of the spontaneously labouring group (LCS/L); cord artery samples of the induction of labour group and cord artery of the spontaneously labouring group (CAIOL/CAS); cord artery of the elective caesarean section group and cord artery of the spontaneously labouring group (CACS/CAS); and cord vein of the induction of labour group and cord vein of the spontaneously labouring group (CVIOL/CVS). Comparison of metabolites associated with different modes of delivery: (all groups n=4): CVCS= cord vein for elective caesarean section group; CACS = cord artery sample for elective caesarean section group; CVS= cord vein sample for spontaneous labour group; IVS = intervillous (maternal) blood for spontaneous labour group; IVCS = intervillous blood sample for elective caesarean section group; CAIOL = cord artery sample for induction of labour group; IVIOL = intervillous blood sample for induction of labour group; LCS = term not in labour sample taken day prior to elective caesarean section; L = samples taken in latent phase for spontaneous labour group; CVIOL = cord vein sample

for the induction of labour group. Colour code: purple = increase; yellow = decrease. Comparison of metabolites associated with gestational age at delivery at different time points sampled: Latent=samples taken in latent phase spontaneous labour or start of IOL for post-dates (n=14); 28 = samples taken at 28 weeks' gestation (n=16); 34 = samples taken at 34 weeks' gestation (n=16).

3.5.7 Summary of differences in metabolites within vascular compartments at delivery between different modes of labour onset and delivery

Regarding the CA, Welch's two-sample t-tests indicated that: 104/1032 (10%) of the measured metabolites were significantly ($p \leq 0.05$) different between the CA of the IOL group and the CA of the SL group, and 16 of these had a q-value <0.1; 232/1032 (22.4%) were significantly ($p \leq 0.05$) different between the CA of the ECS group and the CA of the SL group, and 230 of these also had a q-value <0.1; and 82/1032 (7.9%) metabolites were significantly ($p \leq 0.05$) different between the CA of the ECS group and CA of the IOL group, and 35 of these also had a q-value <0.1 (Appendix 3A4).

Regarding the CV, Welch's two-sample t-tests indicated that: 113/1032 (10.9%) of the metabolites were significantly ($p \leq 0.05$) different between the CV of the IOL group and the CV of the SL group, and 10 had a $p<0.1$; 130/1032 (12.6%) were significantly ($p \leq 0.05$) different between the CV of the ECS group and the CV of the SL group, and 21 of these had a q-value <0.1 (Appendix 3A5); and 60/1032 (5.8%) of the metabolites were significantly ($p \leq 0.05$) different between the CV of the IOL group and the CV of the ECS group, and 2 of these had a q-value <0.1 (Appendix 3A5).

Regarding the IV blood, Welch's two-sample t-tests indicated that: 30/1032 (2.9%) of the metabolites were significantly ($p \leq 0.05$) different between the IV

blood of the IOL group and the IV blood of the SL group, and none had a significant q value; 111/1032 (10.8%) were significantly ($p \leq 0.05$) different between the IV blood of the ECS group and the IV blood of the SL group, and 10 of these had a q-value <0.1; and 50/1032 (4.9%) were significantly ($p \leq 0.05$) different between the IV blood of the IOL group and the IV blood of the ECS group, and none of these had a q-value <0.1 (Appendix 3A7).

3.5.8 Endocannabinoids

N-palmitoyltaurine was significantly negatively correlated with gestational age at delivery (Figure 3.7) at 34 weeks' gestation. N-palmitoyltaurine was also significantly reduced in the CA of people with ECS when compared with the CA of women who spontaneously laboured (0.63-fold change (-FC), p=0.0032). Whereas the other endocannabinoids measured increased with gestation and were decreased in the CV and CA compared with the concentrations in the IV blood, N-palmitoylserine significantly decreased between 28 weeks' gestation and latent phase (0.69-FC, p=0.0110), and significantly increased in the CV (5.40-fold change, p=0.0197) and the CA (4.76-FC, p=0.0172) compared with the IV blood of the ECS group. It was also significantly increased in the pre-operative sample from the ECS group compared with the latent phase of spontaneous labour (2.73-FC, p=0.0044).

N-stearoyltaurine was negatively correlated with gestational age at delivery in samples taken at 28 weeks' and 34 weeks' gestation (Figure 3.8), it was also significantly reduced in the CA of the IOL group compared with CA of the SL group, and also significantly reduced in the CA of the ECS group compared with the SL group. Again, this would fit with increased levels being associated with earlier gestational age at delivery in spontaneous labour at term compared with no labour, and therefore it may be involved in the trigger for human labour. It was significantly higher in the CV of the IOL group when compared with the SL group (1.56-fold change, p=0.0305), it was also increased in the CV of the ECS group compared with the SL group, but this did not reach significance (1.51-fold change, p=0.0625).

The endocannabinoids oleoyl-ethanolamide (OEA), palmitoyl-ethanolamide (PEA) and linoleoyl-ethanolamide all significantly increased between 28 weeks' gestation and latent phase, and between 34 weeks' gestation and latent phase. OEA increased 1.41-fold (p=0.005) in the latent phase compared with 28 weeks' gestation, and 1.33-fold-change (p=0.0057) between 34 weeks' gestation and latent phase. Further, N-oleoyltaurine and N-stearoyltaurine were both significantly increased in latent phase compared with levels at 28 weeks' gestation, and N-palmitoylserine significantly decreased between 28 weeks' gestation and latent phase (Table 3.7 and Figure 3.9).

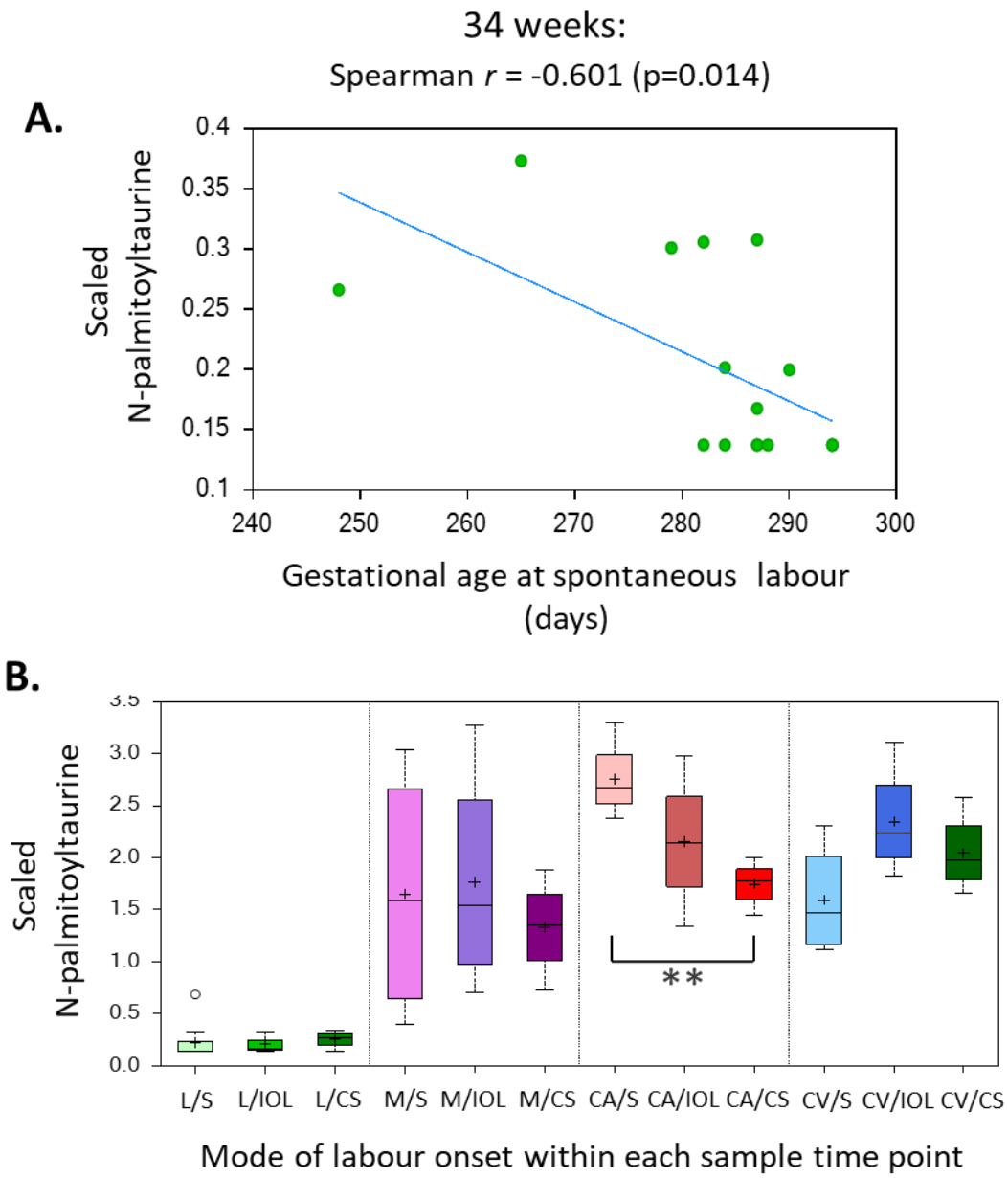

Figure 3.7: Scatter plot showing correlation between N-palmitoyltaurine level and gestational age at delivery at 34 weeks' gestation, and box-plots showing differences between modes of labour onset for latent, intervillous, cord artery and cord vein: A. scatter plot showing correlation between metabolite level and gestational age at delivery for N-palmitoyltaurine at 34 weeks' gestation with Spearman correlation -0.601 (p=0.014) (n=16). B. Box plots for N-palmitoyltaurine for the three modes of labour onset showing median value level (horizontal line within plot) and mean (cross within plot), and interquartile range for each of the different sample points (L=latent/pre-op, intervillous, CA=cord artery, CV=cord vein) for each of the different modes of labour and delivery (S=spontaneous, IOL=induction of labour, CS=elective caesarean section) (each group n=4). ** indicates p≤0.01. Scaled N-palmitoyltaurine for each sample determined following normalisation by sample volume, log-transformation, then rescaling to set the median N-palmitoyltaurine for all samples to be equal to 1.

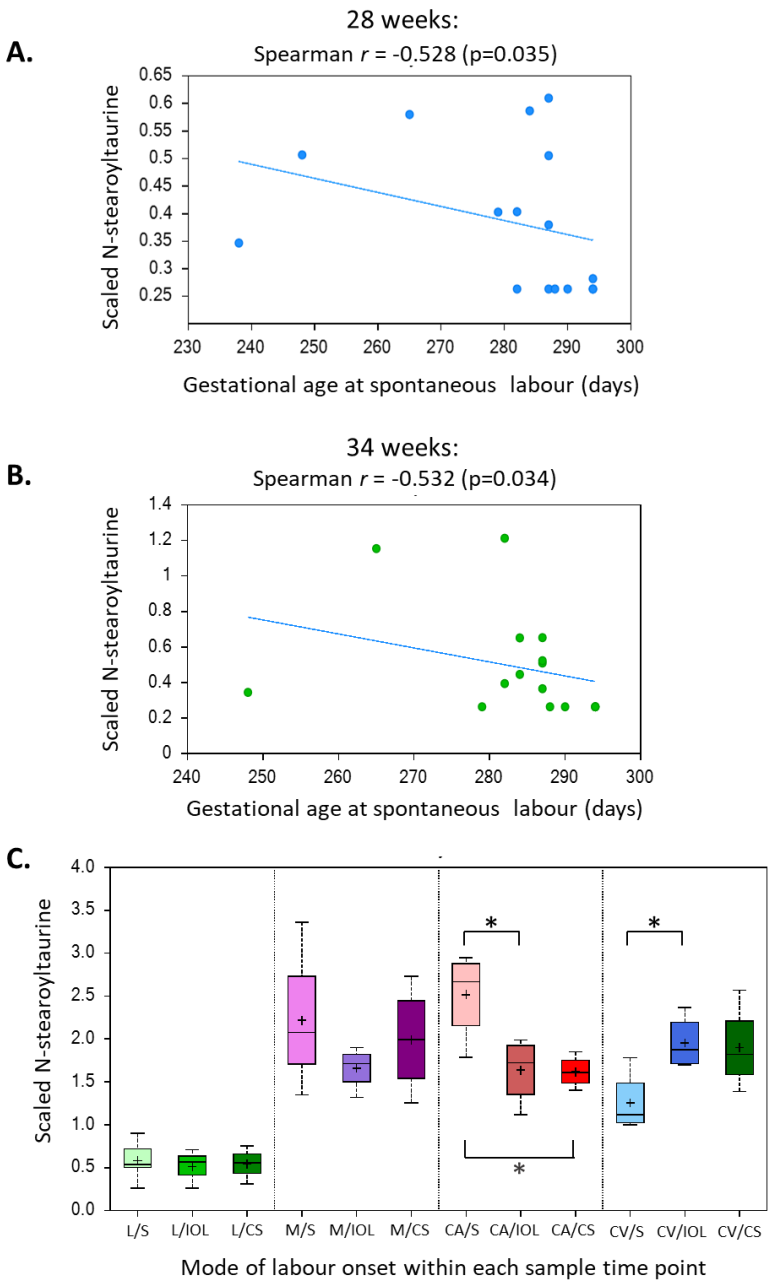

Figure 3.8: Scatter plots showing correlation between N-stearoyltaurine level and gestational age at delivery at 28 weeks' and 34 weeks' gestation, and box-plots showing differences between modes of labour onset for latent, intervillous, cord artery and cord vein. A and B: scatter plots showing correlation between N-stearoyltaurine level and gestational age at delivery at A. 28 weeks' gestation, with Spearman correlation -0.528 (p=0.035) (n=16); and B. 34 weeks' gestation, with Spearman correlation -0.532 (p=0.034) (n=16). C. Box plots for N-stearoyltaurine for the three modes of labour onset types within each of the labour and delivery samples showing median value level (horizontal line within plot) and mean (cross within plot), and interquartile range for each of the different sample points (L=latent/pre-op, intervillous, CA=cord artery, CV=cord vein) for each of the different modes of labour and delivery (S=spontaneous, IOL=induction of labour, CS=elective caesarean section) (each group n=14). * indicates p≤0.05. Scaled N-stearoyltaurine for each sample determined following normalisation by sample volume, log-transformation, then rescaling to set the median N-stearoyltaurine for all samples to be equal to 1.

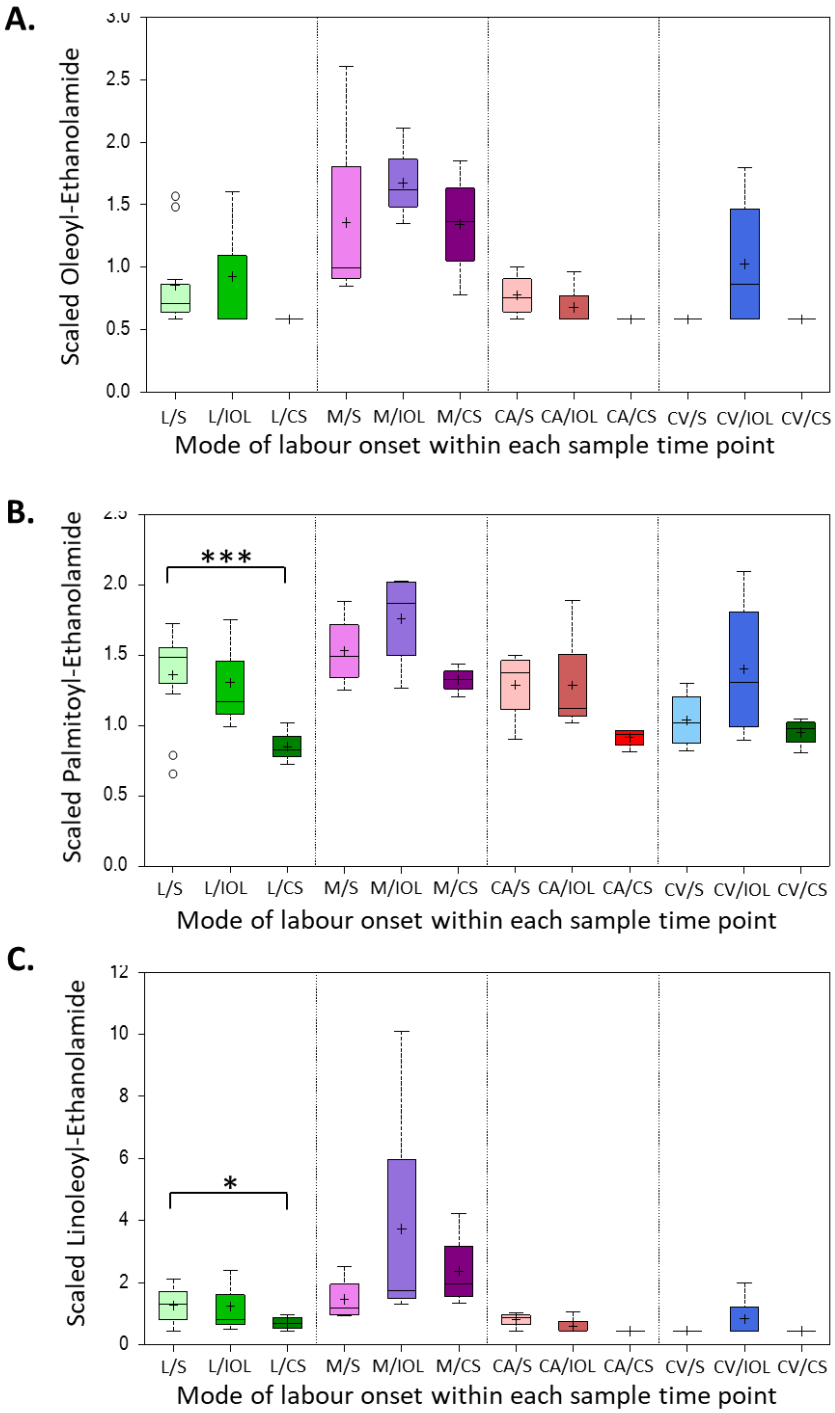

Figure 3.9: Box plots for scaled metabolite for the three labour onset types within each of the labour and delivery samples for oleoyl-ethanolamide, palmitoyl-ethanolamide and linoleoyl-ethanolamide. Box plots for scaled metabolite for the three labour onset types within each of the labour and delivery samples showing median value level (horizontal line within plot) and mean (cross within plot), and interquartile range for each of the different sample points (L=latent/pre-op, intervillous, CA=cord artery, CV=cord vein) for each of the different modes of labour and delivery (S=spontaneous, IOL=induction of labour, CS=elective caesarean section) (each group n=4). *** indicates p≤0.001; * indicates p≤0.05. A. oleoyl-ethanolamide (OEA); B. palmitoyl-ethanolamide (PEA); C. linoleoyl-ethanolamide (LEA). Scaled metabolite for each sample determined following normalisation by sample volume, log-transformation, then rescaling to set the median for all samples to be equal to 1.

PEA was significantly increased in latent phase when compared with both 28 weeks' gestation (1.52-fold change, p=0.0007) and 34 weeks' gestation (1.42-fold change, p=0.002). Levels in the CV were significantly lower than in the IV blood in the SL group (0.68-FC, p=0.023) (see Figure 3.9) and ECS group (0.72-FC, p=0.0179), and significantly reduced in the CA compared with the IV blood in the ECS group (0.69-FC, p=0.0132). PEA was significantly reduced in the pre-operative samples for the ECS than in the latent phase of the group in spontaneous labour (0.56-FC, p=0.0008). PEA was also reduced in the CA of the ECS group when compared with the SL group, although this did not reach significance (0.71-fold change, p=0.0584).

LEA more than doubled between 28 weeks' gestation and latent phase (2.06-FC, p=0.0002) and between 34 weeks' gestation and latent phase (2.01-FC, p=0.0035). It was significantly lower in the CV than the IV blood in the SL group (0.31-FC, p=0.017), and significantly lower in the CV of the ECS group than the IV blood (0.19-fold change, p=0.007). It was significantly reduced in the CA of women in the ECS group compared with the IV blood (0.19-FC, p=0.007). As with PEA, LEA was also significantly reduced in the maternal blood samples taken at term (pre-op) for the ECS group compared with the latent phase of the SL group (0.44-FC, p=0.0239).

Of note, in contrast to the samples analysed in Chapter 2, Anandamide (AEA), one of the main endocannabinoids researched, was not detected in this study. Metabolon, Inc., were asked to investigate, and revisited the spectrometry results and confirmed that there was no quantifiable signal for AEA in any of the samples of this study. It was highlighted that as a non-targeted platform, MS metabolomics is not optimised for the detection of specific compounds. Metabolon, Inc., also looked historically at their database and found that AEA was detected in only approximately 15% of the studies they have conducted in human blood, and concluded that it is likely a labile metabolite that is present in the blood at levels which is close to the detection levels. The samples were treated in exactly the same way for both studies before transfer to the United States and therefore there is no other obvious explanation for this difference.

3.5.9 Sphingolipids

Twenty-eight sphingomyelins (the major member of the sphingolipid family) were measured in this study. In Part 2, the concentration of three of the sphingomyelins in the samples taken at 34 weeks' gestation were significantly correlated with gestational age at delivery. Stearoyl sphingomyelin (d18:1/18:0) was negatively correlated, while sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1), and sphingomyelin (d18:2/24:1, d18:1/24:2) were positively

correlated (-0.5789, p=0.0188 and 0.5596, p=0.0242, q=0.4641, respectively) (Table 3.8).

Nineteen of the 28 sphingomyelins measured were significantly lower (range of-FC 0.17-0.73) in the CV than the IV blood of the SL group, and the same significant changes were observed for 16 of these in the ECS group. Concentrations of eight sphingomyelins were significantly lower in the CA than the IV blood in the SL group, and 19 were significantly lower in the CA of ECS group (Table 3.8). In contrast, two sphingomyelins were significantly higher in the CV when compared with the IV blood in all three modes of labour (Table 3.8).

Most of the sphingomyelins were found in higher concentrations in the IV, CA and CV of the SL than the ECS groups, but the majority of these were not significantly different at the $p \leq 0.05$ level. Concentrations of palmitoyl sphingomyelin (d18:1/16:0), behenoyl sphingomyelin (d18:1/22:0), and sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0) were significantly lower in the CA of the ECS group compared with the SL group (0.87-FC, 0.73-FC, and 0.72-FC, respectively). Further concentrations of sphingomyelin (d18:1/20:1, d18:2/20:0) was significantly lower in the IV of the ECS group than the SL group (0.68-fold-change). There were no significant differences in sphingomyelin concentrations between the SL and IOL groups.

3.5.10 Sphinganine-1-phosphate and sphinganine

Of the metabolites of sphingolipid synthesis, most changes were seen in concentrations of sphinganine-1-phosphate and sphinganine (Table 3.8). Although both sphinganine and sphinganine-1-phosphate increased in the maternal plasma during pregnancy, these metabolites showed differing concentration ratios in the cord and IV blood at delivery. Whereas in the ECS group sphinganine was reduced 0.10-fold (p=0.0013) in the CV compared with the IV, and the CA had 0.17-fold (p=0.0001) the amount present in the IV samples, sphinganine-1-phosphate levels were 2.85-fold (p= 0.0032) greater in the CV than IV samples, and 2.21-fold (p= 0.0191) greater in the CA than the IV blood. In addition, sphinganine-1-phosphate was slightly but significantly increased in the CV compared with the CA (1.29-fold change, p=0.0097) in the ECS group. Similar differences were observed in the SL group where sphinganine concentration was 0.22-fold less (p=0.0008) in the CA compared with the IV samples, while sphinganine-1-phosphate was 6.92-fold (p=0.0007) greater in the CA than the IV sample; and sphinganine-1-phosphate was 8.06-fold (p= 0.0232) greater in the CV than IV sample.

Regarding differences between modes of labour and delivery, concentrations of sphinganine-1-phosphate were significantly lower in the CA of the IOL group (0.49-FC, p=0.0129) and the CA of the ECS group (0.32-fold change, p=0.0017)

than in the CA of the SL group. Sphinganine was significantly lower in the CV of both the IOL group (0.19-FC, p=0.0218) and the ECS group (0.12-FC, p=0.0142) than in the SL group. These results indicate that sphinganine and sphinganine-1-phosphate are differentially expressed by the mother and fetus and between modes of labour and delivery.

In addition, a third lipid involved in sphingolipid metabolism, sphingadienine, decreased during pregnancy between 28 weeks' and latent phase (0.70-fold change, p=0.0048), and between 34 weeks' gestation and latent phase (0.58-fold change, p=0.0064).

Table 3.8: Significant fold-changes for sphingolipids (all results shown are significant at p≤0.05); paired t-tests for differences in the mean value between: cord vein and cord artery of the spontaneously labouring group (CVS/CAS); cord vein and cord artery of the induction of labour group (CVIOL/CAIOL); cord vein and cord artery of the elective caesarean section group (CVCS/CACS); cord vein and intervillous blood of the spontaneously labouring group (CVS/IVS); cord vein and intervillous samples of the elective caesarean section group (CVCS/IVCS); cord artery and intervillous samples of the spontaneously labouring group (CAS/IVS); cord artery and intervillous blood of the elective caesarean section group (CACS/IVCS); and Welch's t-test for differences in the mean value between: pre-operative sample of the elective caesarean section group and the latent phase sample of the spontaneously labouring group (LCS/L); cord artery of the induction of labour group and the cord artery of the spontaneously labouring group (CAIOL/CAS); cord artery of the elective caesarean section group and the cord artery of the spontaneously labouring group (CSCS/CAS); cord vein of the induction of labour group and the cord vein of the spontaneously labouring group (CVIOL/CVS); cord vein of the elective caesarean section group and the cord vein of the spontaneously labouring group (CVCS/CVS); intervillous sample of the elective caesarean section group and the intervillous blood of the spontaneously labouring sample (IVCS/IVS); and the intervillous sample of the induction of labour group and the intervillous sample of the elective caesarean section group (IVIOL/IVCS).

Metabolite	CVS / CAS	CVIOL / CAIOL	CVCS / CACS	CVS / IVS	CVCS / IVCS	CAS / IVS	CACS / IVCS	LCS / LS	CAIOL / CAS	CACS / CAS	CVIOL / CVS	CVCS / CVS	IVCS / IVS	IVIOL / IVCS
Palmitoyl sphingomyelin (d18:1/16:0)							**0.87**			**0.87**				
Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH))		**0.85**					**0.73**							
Stearoyl sphingomyelin (d18:1/18:0)		**0.90**					**1.27**							
Behenoyl sphingomyelin (d18:1/22:0)			**1.14**	**0.47**	**0.45**	**0.60**	**0.40**			**0.73**				
Tricosanoyl sphingomyelin (d18:1/23:0)			**1.14**	**0.18**	**0.17**	**0.24**	**0.15**							

Lignoceroyl sphingomyelin (d18:1/24:0)			**1.10**		**0.59**		**0.54**
Sphingomyelin (d18:2/18:1)			**1.11**	**0.46**	**0.60**		**0.54**
Sphingomyelin (d18:2/23:1)			**1.12**	**0.48**	**0.61**		**0.55**
Sphingomyelin (d18:2/24:2)					**2.10**		**1.95**
Sphingomyelin (d17:1/14:0, d16:1/15:0)	**0.84**			**0.26**	**0.30**	**0.31**	**0.26**
Sphingomyelin (d18:1/14:0, d16:1/16:0)			**1.09**	**0.46**	**0.44**		**0.40**
Sphingomyelin (d18:2/14:0, d18:1/14:1)		**1.11**		**0.45**	**0.60**		**0.52**
Sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)			**1.11**	**0.37**	**0.44**	**0.43**	**0.39**
Sphingomyelin (d17:2/16:0, d18:2/15:0)				**0.32**	**0.46**		**0.41**
Sphingomyelin (d18:2/16:0, d18:1/16:1)			**1.10**	**0.67**			**0.76**
Sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)				**0.55**	**0.56**	**0.57**	**0.52**
Sphingomyelin (d18:1/18:1, d18:2/18:0)				**1.66**	**2.28**	**1.58**	**2.18**
Sphingomyelin (d18:1/19:0, d19:1/18:0)		**1.14**	**1.17**	**0.34**	**0.39**		**0.33**

Sphingomyelin (d18:1/20:0, d16:1/22:0)			**0.51**	**0.51**		**0.48**							**0.79**
Sphingomyelin (d18:1/20:1, d18:2/20:0)	**0.90**		**1.41**	**1.91**	**1.40**	**1.87**						**0.68**	
Sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2)	**1.25**	**1.16**				**1.07**							
Sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)		**1.18**	**0.17**	**0.18**	**0.23**	**0.16**		**0.72**					
Sphingomyelin (d18:2/21:0, d16:2/23:0)		**1.12**	**0.42**	**0.46**		**0.41**							
Sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)			**0.55**	**0.56**		**0.52**							
Sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)						**0.98**							
Sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)		**1.15**	**0.24**	**0.27**		**0.24**							
Sphingomyelin (d18:1/24:1, d18:2/24:0)		**1.08**	**0.65**	**0.59**		**0.55**							
Sphingomyelin (d18:2/24:1, d18:1/24:2)		**1.11**	**0.73**		**0.75**	**0.74**							
Sphinganine				**0.10**	**0.22**	**0.17**			**0.19**	**0.12**			
Sphinganine-1-phosphate		**1.29**	**8.06**	**2.85**	**6.92**	**2.21**	**0.49**	**0.32**					

Sphingadienine			**0.65**	
Myristoyl dihydrosphingomyelin (d18:0/14:0)			**1.08**	
Palmitoyl dihydrosphingomyelin (d18:0/16:0)			**1.12**	
Behenoyl dihydrosphingomyelin (d18:0/22:0)	**1.14**	**1.81**	**1.59**	**1.69**
Sphingomyelin (d18:0/18:0, d19:0/17:0)	**1.12**	**2.18**	**1.94**	
Sphingomyelin (d18:0/20:0, d16:0/22:0)	**1.15**	**1.90**	**1.66**	

All groups n=4: CVCS= cord vein for elective caesarean section group; CACS = cord artery sample for elective caesarean section group; CVS= cord vein sample for spontaneous labour group; IVS = intervillous (maternal) blood for spontaneous labour group; IVCS = intervillous blood sample for elective caesarean section group; CAIOL = cord artery sample for induction of labour group; IVIOL = intervillous blood sample for induction of labour group; LCS = term not in labour sample taken day prior to elective caesarean section; L = samples taken in latent phase for spontaneous labour group; CVIOL = cord vein sample for the induction of labour group. Colour code: purple = increase; yellow = decrease.

Table 3.9: Significant changes for Sphingosines (all results shown are significant at p≤0.05): paired t-tests for cord vein and cord artery of the elective caesarean section group (CVCS/CACS); cord vein and intervillous samples of the elective caesarean section group (CVCS/IVCS); cord vein and intervillous samples of the elective caesarean section group (CVCS/IVCS); cord artery and intervillous samples of the spontaneously labouring group (CAS/IVS); cord artery and intervillous blood of the elective caesarean section group (CACS/IVCS); Welch's t-test for cord artery of the elective caesarean section group and the cord artery of the spontaneously labouring group (CACS/CAS); cord artery of the spontaneously labouring group and the cord artery of the induction of labour group (CACS/CAIOL); cord vein of the induction of labour group and the cord vein of the spontaneously labouring group (CVIOL/CVS); cord vein of elective caesarean section group and cord vein of spontaneously labouring group (CVCS/CVS); and intervillous sample of the elective caesarean section group and intervillous blood of the spontaneously labouring group (IVCS/IVS).

Metabolite	Sub pathway	Super pathway	CVCS CACS	CVS IVS	CVCS IVCS	CAS IVS	CACS IVCS	CACS CAS	CACS CAIOL	CVIOL CVS	CVCS CVS	IVCS IVS
Sphingosine	Sphingosines	Lipid			0.17	0.27	0.20	0.49	0.42	0.28	0.22	
Sphingosine 1-phosphate	Sphingosines	Lipid	1.49	3.69	2.78	4.38		0.43	0.61			
Hexadecasphingosine (d16:1)	Sphingosines	Lipid										0.50

All groups n=4: CVCS= cord vein for elective caesarean section group; CACS = cord artery sample for elective caesarean section group; CVS= cord vein sample for spontaneous labour group; IVS = intervillous (maternal) blood for spontaneous labour group; CVCS = cord vein sample for elective caesarean section group; IVCS = intervillous blood sample for elective caesarean section group; CAS = cord artery sample for spontaneous labour group; CAIOL = cord artery sample for induction of labour group; IVIOL = intervillous blood sample for induction of labour group; LCS = term not in labour sample taken day prior to elective caesarean section; L = samples taken in latent phase for spontaneous labour group; CVIOL = cord vein sample for the induction of labour group.

3.5.11 Sphingosine and sphingosine-1-phosphate

Concentrations of sphingosine did not change during pregnancy. However, concentrations were different at the time of labour between the different compartments and the different modes of labour onset and delivery. Concentrations of sphingosine were significantly lower in the CV (0.17-FC, p=0.003) and CA (0.20-FC, p=0.002) than in the IV samples in the ECS group. It was also lower in the CA than the IV samples (0.27-FC, p=0.007) in the SL group. There were also differences according to type of labour onset: concentrations of sphingosine were significantly lower in the CA of the ECS group than the CA of the SL group (0.49-FC, p=0.016), and lower in the CV in the ECS group than the CV in the SL group (0.22-FC, p=0.034). Further, concentration of sphingosine in the CA of the ECS group was also significantly lower than that in the CA of the IOL group (0.42-FC, p=0.017). And the CV of the IOL group had significantly lower sphingosine concentration than the CV of the SL group (0.28-FC, p=0.049) (Table 3.9).

Differences in sphingosine 1-phosphate between vascular compartments were generally in the opposite direction to those for sphingosine. Levels of sphingosine 1-phosphate were 1.49-fold greater in the CV than CA of the ECS group (p=0.004), and 2.78-fold greater in the CV than IV (p=0.010). In the SL group, levels of sphingosine 1-phosphate were 3.69-fold higher in the CV than

IV (p=0.016), and 4.38-fold higher in the CA than IV (p=0.011). Differences between the different modes of labour onset and delivery were in the same direction as for sphingosine: levels in the CA of the ECS group were significantly lower than both the CA in the SL group (0.43-FC, p=0.001) and IOL group (0.61-FC, p=0.043) (Table 3.9).

3.5.12 Ceramides

Overall, the ceramide metabolites tended to increase slightly with gestation throughout pregnancy (Table 3.10). N-stearoyl-sphingosine (d18:1/18:0) was negatively correlated with gestational age at spontaneous labour (-0.6459, p=0.007), and this was the only ceramide that was associated with gestational age at delivery. Just over half of the measured ceramides were lower in the CV and CA than the IV blood, potentially indicating that the source of these is likely maternal (Table 3.10). There were few differences between the concentrations of ceramide metabolites in the different vascular compartments and the mode of labour onset and delivery, other than a significantly greater level in the CV of women who spontaneously laboured compared with the CV of those who were induced of the Hexosylceramides (HCER) glycosyl-N-stearoyl-sphingosine (d18:1/18:0), glycosyl-N-behenoyl-sphingadienine (d18:2/22:0) and glycosyl ceramide (d18:1/20:0, d16:1/22:0), which had fold changes of 1.54 (p=0.005), 1.98 (p=0.010) and 1.66 (p=0.008), respectively.

Table 3.10: Significant changes for ceramide family (including ceramides, dihydroceramides, hexosylceramides, lactosylceramides and ceramide PE); all results shown are significant at p≤0.05. Paired t-tests for differences in the mean value between: latent phase samples and 28 week's gestation samples of the spontaneously labouring group (L/28); latent phase samples and 34 week's gestation samples of the spontaneously labouring group (L/34); cord vein and cord artery of the spontaneously labouring group (CVS/CAS); cord vein and intervillous blood of the spontaneously labouring group (CVS/IVS);); cord artery and intervillous blood of the elective caesarean section group (CACS/IVCS); cord artery and intervillous blood of the induction of labour group (CAIOL/IVIOL); cord artery and intervillous samples of the elective caesarean section group (CACS/MCS); and Welch's t-test for differences in the mean value between: pre-operative sample of the elective caesarean section group and the latent phase sample of the spontaneously labouring group (LCS/L); cord artery of the induction of labour group and the cord artery of the spontaneously labouring group (CAIOL/CAS); cord artery of the elective caesarean section group and the cord artery of the spontaneously labouring group (CACS/CAS); cord vein of the induction of labour group and the cord vein of the spontaneously labouring group (CVIOL/CVS).

Metabolite	CVS CAS	CVIOL CAIOL	CVCS CACS	CVS IVS	CVIOL IVIOL	CVCS IVCS	CAS IVS	CAIOL IVIOL	CACS IVCS	LIOL L	LCS L	CVIOL CVS	IVCS IVS	IVIOL IVCS
N-palmitoyl-sphinganine (d18:0/16:0)							**0.59**		**0.48**					
N-stearoyl-sphinganine (d18:0/18:0)														**1.66**
N-palmitoyl-sphingosine (d18:1/16:0)						**0.27**	**0.36**		**0.28**					
N-stearoyl-sphingosine (d18:1/18:0)						**0.36**	**0.55**		**0.35**					
N-palmitoyl-sphingadienine (d18:2/16:0)							**0.48**				**0.35**			
N-stearoyl-sphingadienine (d18:2/18:0)														

N-palmitoyl-heptadecasphingosine (d17:1/16:0)				**0.18**			**0.14**	**0.20**	**0.26**	**0.13**	**0.61**	**0.63**		
ceramide (d18:1/14:0, d16:1/16:0)				**0.33**			**0.33**	**0.36**		**0.31**		**0.50**		
glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)							**0.45**			**0.45**				
glycosyl-N-stearoyl-sphingosine (d18:1/18:0)			**1.17**	**0.34**									**1.54**	
glycosyl-N-behenoyl-sphingadienine (d18:2/22:0)			**1.30**	**0.23**						**0.41**			**1.98**	
glycosyl-N-tricosanoyl-sphingadienine (d18:2/23:0)				**0.16**				**0.16**						
glycosyl ceramide (d18:1/20:0, d16:1/22:0)			**1.19**	**0.21**			**0.37**			**0.31**			**1.66**	
glycosyl ceramide (d18:1/23:1, d17:1/24:1)				**0.07**			**0.33**	**0.07**		**0.19**				
glycosyl ceramide (d18:2/24:1, d18:1/24:2)	**0.46**			**0.17**										
lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)		**0.79**			**0.55**		**0.53**			**0.60**				
palmitoyl-sphingosine-phosphoethanolamine (d18:1/16:0)									**1.08**					**0.75**

All groups n=4: CVCS= cord vein for elective caesarean section group; CACS = cord artery sample for elective caesarean section group; CVS= cord vein sample for spontaneous labour group; IVS = intervillous (maternal) blood for spontaneous labour group; IVCS = intervillous blood sample for elective caesarean section group; CAIOL = cord artery sample for induction of labour group; IVIOL = intervillous blood sample for induction of labour group; LCS =

term not in labour sample taken day prior to elective caesarean section; L = samples taken in latent phase for spontaneous labour group; CVIOL = cord vein sample for the induction of labour group. Colour code: purple = increase; yellow = decrease.

3.5.13 Adenosine 3',5'-cyclic monophosphate

Adenosine 3',5'-cyclic monophosphate (cAMP) was significantly reduced in the CV of both the IOL (0.22-FC, p=0.0336) and the ECS (0.11-FC, p=0.0091) groups when compared with the SL group, and also between the ECS pre-operative samples when compared with the latent phase of SL (0.69-FC, p=0.0302). cAMP was also lower in the CA than CV in the IOL (0.56-fc, p=0.0497) and ECS group (0.36-fc, p=0.0397). There was an opposite effect in the SL group with a 2.56-fold increased concentration in the CA than the CV in the SL group, however this was not significant (p=0.081). Further, the concentration in the IV sample was significantly higher than that in the CV sample for the IOl group (0.47-FC, p=0.0284) and ECS group (0.11-FC, p=0.006), whereas there was no change in the SL group (CVS/MS = 1.21-FC, p=0.831).

3.5.14 Steroids correlated with gestational age at delivery

Regarding those steroid metabolites which were significantly correlated with gestational age at delivery at more than one time point: 5alpha-androstan-3alpha,17alpha-diol disulfate was correlated at all time points (Figure 3.10); 21-hydroxypregnanolone disulfate, 5alpha-pregnan-3beta,20alpha-diol disulfate, pregnanediol-3-glucuronide, dehydroepiandrosterone sulfate (DHEA-S) and androstenediol (3beta,17beta) monosulfate (1) were correlated with gestational age at spontaneous labour at 28 weeks' and 34 weeks' gestation

(Table 3.11 and Figure 3.11); 5alpha-pregnan-3beta,20alpha-diol monosulfate (1) was correlated with gestational at delivery at 28 weeks' gestation and in latent phase (Figure 3.12); and 17alpha-hydroxypregnanolone glucuronide, 5alpha-androstan-3alpha,17beta-diol disulfate, oestriol 3-sulfate and oestriol 16-glucuronide were significantly correlated at 38 weeks' gestation and in latent phase (Figure 3.12). All of these were negatively correlated with gestational age at delivery except for DHEA-S and androstenediol (3beta, 17 beta) monosulfate (1) which were positively correlated.

Table 3.11: Mean normalised and scaled values for 17alpha-hydroxyprogesterone in each of the vascular compartments for each of the modes of labour onset and delivery (n = 4 for each group). 95%CI = 95% confidence interval.

Dehydroepiandrosterone sulfate	**Mean normalised and scaled value (95%CI)**		
Mode of labour onset	**Cord vein**	**Cord artery**	**Intervillous blood**
Spontaneous	2.6 (1.4-3.7)	2.6 (1.8-3.5)	1.8 (0.9-2.6)
Induction of labour	1.6 (1.0-2.1)	1.7 (1.2-2.1)	1.1 (0.7-1.6)
Elective caesarean section	1.9 (1.4-2.4)	2.1 (1.5-2.8)	0.9 (0.5-1.2)

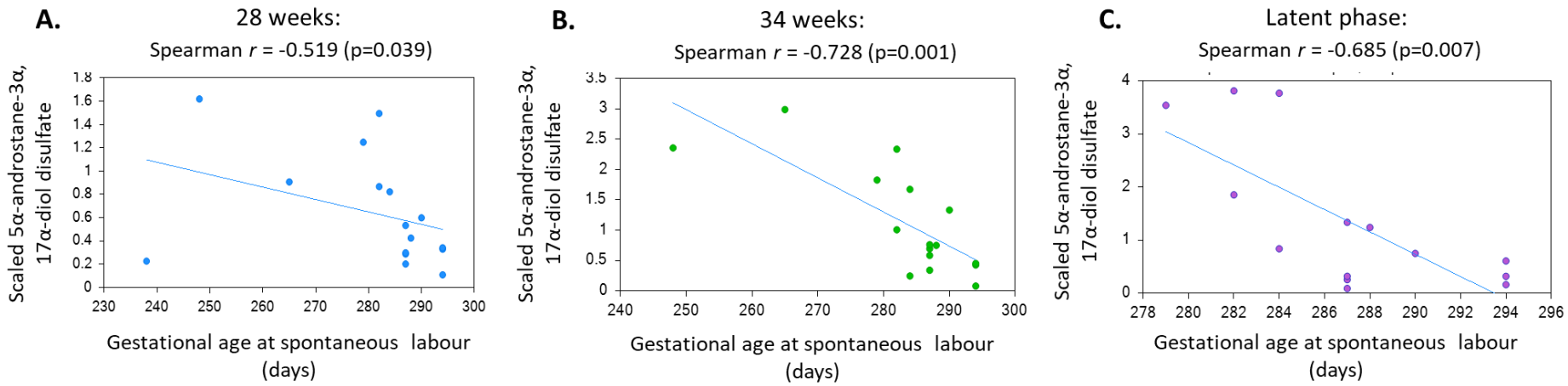

Figure 3.10: Scatter plots showing correlation between 5α-androstan-3α, 17α-diol disulfate level and gestational age at delivery at: A. 28 weeks' gestation, with Spearman correlation -0.519 (p=0.039) (n=16); B. 34 weeks' gestation, with Spearman correlation -0.728 (p=0.001) (n=16); C. Latent phase with Spearman correlation -0.685 (p=0.007) (n=14). Scaled 5α-androstan-3α, 17α-diol disulfate for each sample determined following normalisation by sample volume, log-transformation, then rescaling to set the median 5α-androstan-3α, 17α-diol disulfate for all samples to be equal to 1.

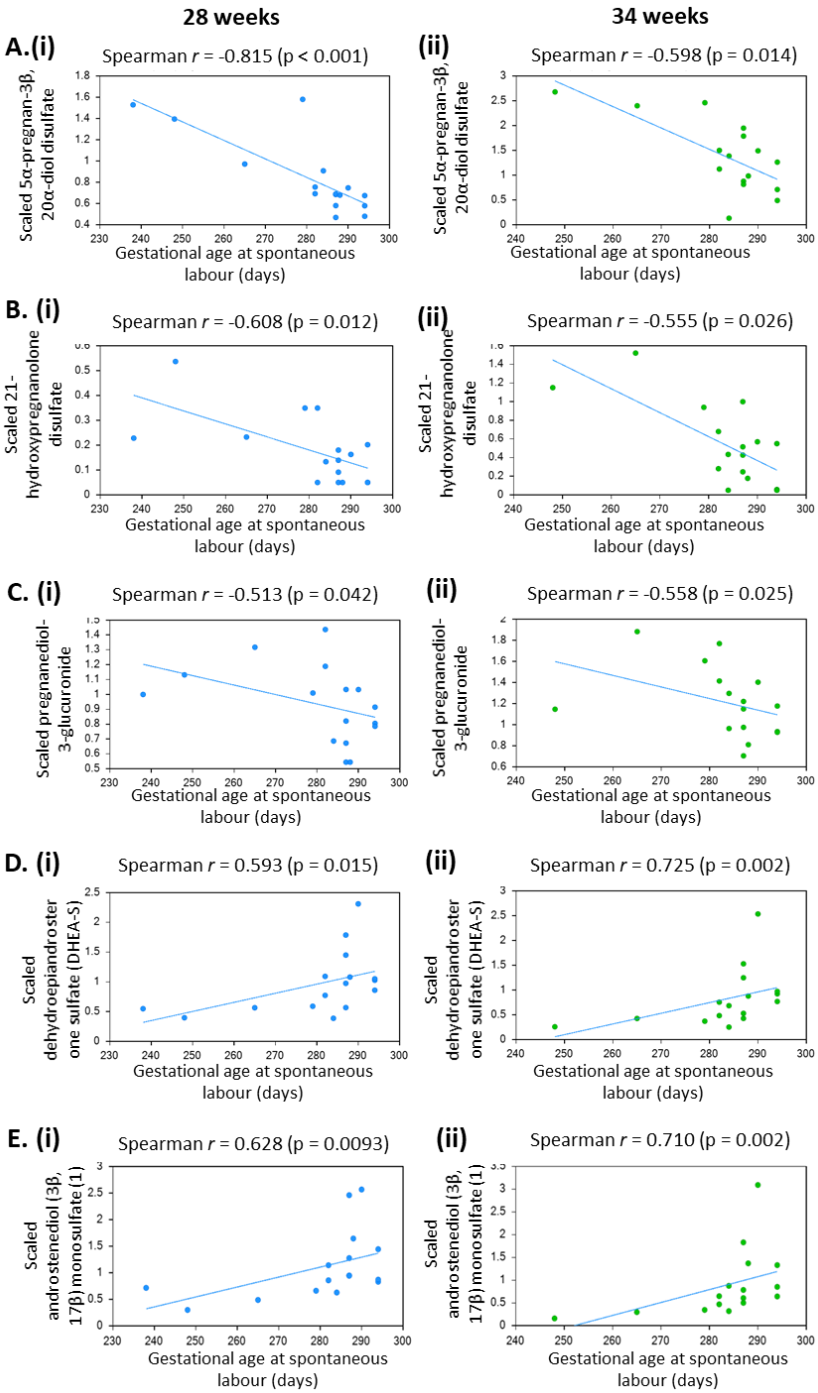

Figure 3.11: Correlations between metabolite and gestational age at delivery for 5α-pregnan-3β, 20α-diol disulfate, 21-hydroxypregnanolone disulfate, pregnanediol-3-glucuronide, dehydroepiandrosterone sulfate and androstenediol (3β, 17β) monosulfate. Scatter plots showing correlation between scaled metabolite and gestational age at delivery at 28 weeks' and 34 weeks' gestation, for: A. 5α-pregnan-3β, 20α-diol disulfate at (i) 28 and (ii) 34 weeks' gestation; B. 21-hydroxypregnanolone disulfate at (i) 28 and (ii) 34 weeks' gestation; C. pregnanediol-3-glucuronide at (i) 28 and (ii) 34 weeks' gestation; D. dehydroepiandrosterone sulfate (DHEA-S) at (i) 28 and (ii) 34 weeks' gestation; and E. androstenediol (3β, 17β) monosulfate (1) at (i) 28 and (ii) 34 weeks' gestation. Spearman correlation with p-value are shown for each comparison. Scaled metabolite for each sample determined following normalisation by sample volume, log-transformation, then rescaling to set the median for all samples to be equal to 1.

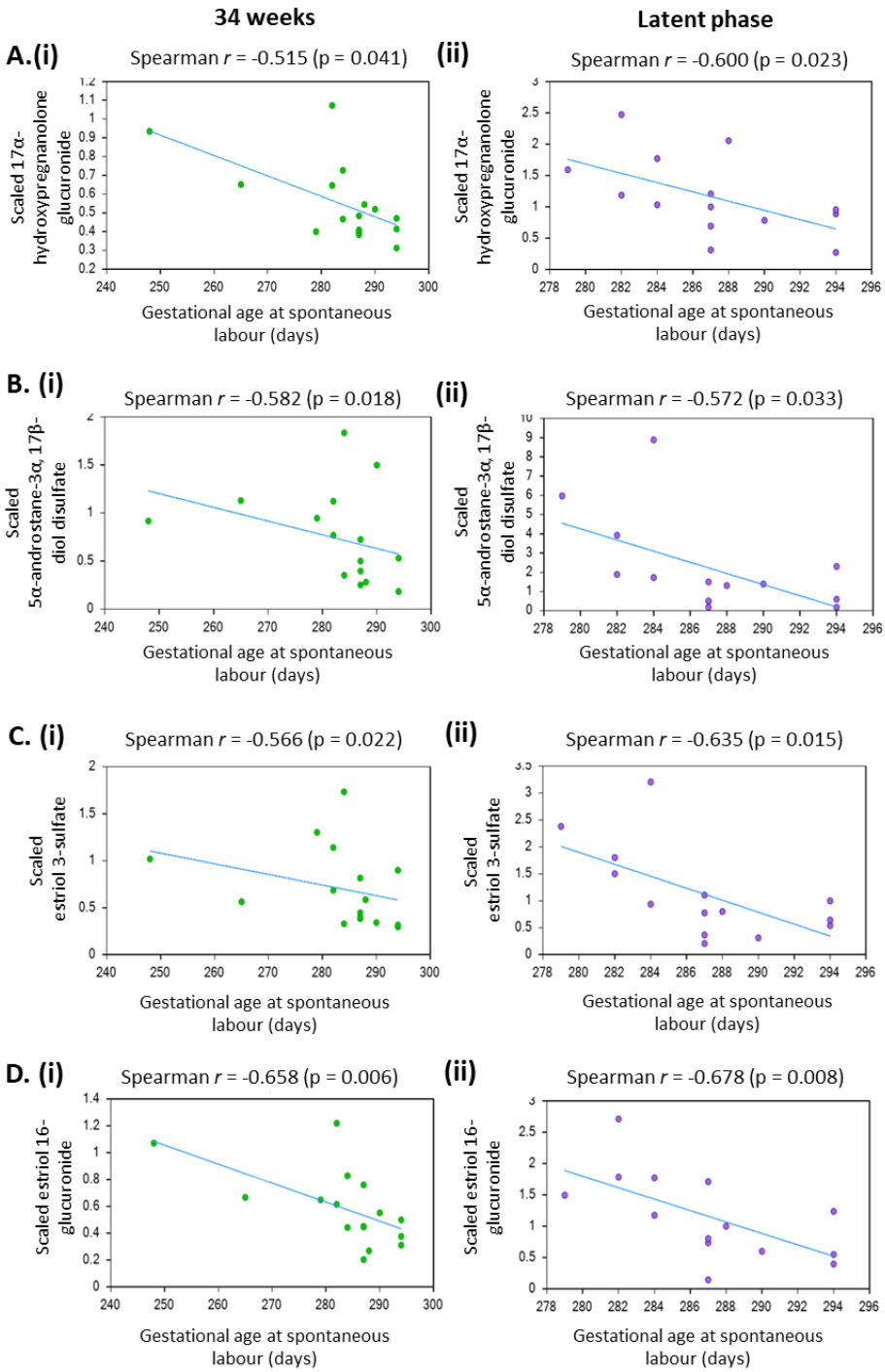

Figure 3.12: Correlations between metabolite and gestational age at delivery for 17α-hydroxypregnanolone glucuronide, 5α-androstan-3α, 17β-diol disulfate, estriol 3-sulfate and estriol 16-glucuronide. Scatter plots showing correlation between scaled metabolite and gestational age at delivery at 34 weeks' gestation and latent phase, for: A. 17α-hydroxypregnanolone glucuronide at (i) 34 weeks' gestation and (ii) latent phase; B. 5α-androstan-3α, 17β-diol disulfate at (i) 28 weeks' gestation and (ii) latent phase; C. estriol 3-sulfate at (i) 28 weeks' gestation and (ii) latent phase; D. estriol 16-glucuronide at (i) 28 weeks' gestation and (ii) latent phase. Spearman correlation with p-value are shown for each comparison. Scaled metabolite for each sample determined following normalisation by sample volume, log-transformation, then rescaling to set the median for all samples to be equal to 1.

3.5.15 Progestin steroids

Progesterone was significantly lower in the CV than CA in the SL group (0.29-FC, p=0.008), but conversely was significantly higher in the CV than CA of the IOL group (2.20-fold change, p=0.021). The concentration of progesterone was also twice as high in the CV sample than the CA sample in the ECS group, however this was not significant. In all three groups, the progesterone concentration in the CV was significantly lower than that of the IV sample (SL: 0.12-fold change, p=0.005; IOL: 0.51-FC, p=0.046; ECS: 0.36-fold change, p=0.015), and likewise the concentration in the CA was significantly lower than in the IV samples (SL: 0.39-fold change, p=0.005; IOL: 0.23-FC, p=0.005; ECS: 0.17-fold change, p=0.010) (Table 3.12 and 3.13).

Importantly there were changes between the labouring groups. Progesterone concentration was significantly lower in the CA of the ECS group compared with the SL group (0.26-FC, p=0.008), and was also lower in the CA of the ECS group compared with the IOL group (0.45-FC, p=0.073), but this was not significant. Progesterone was significantly higher in the CV of the IOL group compared with both the CV of the ECS group (2.28-FC, p=0.043) and SL group (4.27-fold, p=0.011) (Table 3.12 and 3.13).

3.5.16 17alpha hydroxyprogesterone

17alpha hydroxyprogesterone increased between 28 weeks' gestation and 34 weeks' gestation (1.27-FC, p=0.013) and again between 28 weeks' gestation and latent phase (1.67-FC, p=0.001) in the women who spontaneously laboured, but there was no correlation with gestational age at spontaneous delivery. As with progesterone, in the SL group, 17α-hydroxyprogesterone was significantly greater in the CA than the CV at delivery (0.39-FC, p=0.020). And, again as with progesterone, this was reversed in the IOL and ECS group, where the concentration of 17α-hydroxyprogesterone was greater in the CV than the CA at delivery (CVIOL/CAIOL: 1.90-FC, p=0.007; CVCS/CACS: 1.76-FC, p=0.003). Also, as with progesterone, in all modes of labour and delivery, the concentration of 17α-hydroxyprogesterone in the IV samples was consistently higher than in the CA and CV (Table 3.10). The concentration of 17α- hydroxyprogesterone in the CA of the SL group was significantly higher than in the CA of the ECS group (0.34-FC, p=0.021), and non-significantly higher than the CA of the IOL group (0.47-FC, p=0.433); whereas the concentration of 17α-hydroxyprogesterone in the CV of the IOL group was significantly higher than in the CV of the SL group (3.50-FC, p=0.036). The CV of the ECS group had a greater concentration of 17α-hydroxyprogesterone than the CV of the SL group, but this was non-significant,

and the CV of the IOL group had a greater 17α- hydroxyprogesterone concentration than the CV of the ECS group (Table 3.12 and 3.13).

Table 3.12: Mean normalised and scaled values for progesterone in each of the vascular compartments for each of the modes of labour onset and delivery. The values for the metabolites were normalised and scaled by Metabolon, Inc., so that levels of different metabolites could be compared more directly (n=4 for each group). 95%CI = 95% confidence interval.

Progesterone	**Mean normalised and scaled values (95%CI)**		
Mode of labour onset	**Cord vein**	**Cord artery**	**Intervillous blood**
Spontaneous	1.5 (0.7-2.5)	5.3 (4.1-6.5)	13.6 (9.6-17.6)
Induction of labour	6.7 (5.4-8.0)	3 (1.5-4.6)	13.1 (8.6-17.7)
Elective caesarean section	2.9 (1.8-4.1)	1.3 (0.7-2.1)	8.1 (6.3-10.0)

Table 3.13: Significant changes for progestin and oestrogen steroids (all results shown are significant at p≤0.05).

	Ratio	Progesterone	17alpha -hydroxy-progesterone	Oestrone -3-sulfate	Oestriol	Oestriol -16-glucuronide	Oestriol -3-sulfate	Oestriol -3-glucuronide
Mode labour onset	**(paired t-test)**							
Spontaneous	**CV/CA**	**0.29**	**0.39**		**0.19**	**1.04**		
	CV/M	**0.12**	**0.22**		**0.13**			
	CA/M	**0.39**	**0.56**					
IOL	**CV/CA**	**2.20**	**1.90**					
	CV/M	**0.51**	**0.82**		**0.77**			
	CA/M	**0.23**	**0.43**					
ECS	**CV/CA**		**1.76**					
	CV/M	**0.36**	**0.47**	**0.18**		**0.74**	**5.32**	
	CA/M	**0.17**	**0.26**	**0.19**	**0.10**	**0.72**	**5.46**	
Vascular compartment	**(Welch's t-test)**							
Cord artery	**ECS/SL**	**0.26**	**0.34**	**0.42**	**0.18**			
	CS/IOL		**0.47**	**0.35**				
Cord vein	**IOL/SL**	**4.27**	**3.50**		**6.45**			
	CS/SL			**0.34**				
	IOL/CS	**2.28**	**2.29**	**2.22**				
Intervillous	**CS/SL**							**0.21**
GA association	**(Spearman correlation)**							
	34 weeks					**-0.6578**	**-0.6354**	
	Latent					**-0.6780**	**0.0146**	

Latent=samples taken in latent phase spontaneous labour or start of IOL for post-dates (n=14); 34 weeks = samples taken at 34 weeks' gestation (n=16); SL = spontaneous labour group (n=4); CS = elective caesarean section group (n=4); IOL = induction of labour group (n=4); CV = cord vein sample; CA = cord artery sample; M = intervillous (maternal) sample. Colour code: purple = increase; yellow = decrease.

3.5.17 Oestrogen steroids

Oestriol did not change significantly during pregnancy but was 6.45-fold greater in the CV of the IOL group than the CV of the SL group (p=0.0352) (Table 3.10). However, oestriol 16-glucuronide and oestriol 3-sulfate were both correlated with gestational age at delivery in the samples taken at 34 weeks' gestation and in latent phase (Table 3.14). In addition, oestriol 16-glucuronide was reduced in the CA (0.72-FC, p=0.043) and CV (0.74-FC, p=0.029) of the ECS group compared with the IV blood. Oestriol 3-sulfate was the only oestrogenic steroid to be greater in the CA (5.46-fc, p=0.005) and CV (5.32-fc, p=0.006) than the IV blood in the ECS group. Oestriol-3-glucuronide was significantly less in the IV blood of the ECS group than the IV blood of the SL group (0.21-FC, p=0.038) (Table 3.13 and 3.14).

Table 3.14: Mean normalised and scaled values for 17alpha-hydroxyprogesterone in each of the vascular compartments for each of the modes of labour onset and delivery. The values for the metabolites were normalised and scaled by Metabolon, Inc., so that levels of different metabolites could be compared more directly. (n=4 for each group). 95%CI = 95% confidence interval.

Oestriol	Normalised and scaled value (95%CI)		
Mode of labour onset	**Cord vein**	**Cord artery**	**Intervillous blood**
Spontaneous	**0.3 (0.1-0.5)**	**1.3 (0.4-2.1)**	**2.0 (0.7-3.2)**
Induction of labour	**1.6 (1.3-1.9)**	**0.7 (0.1-1.2)**	**2.1 (1.6-2.5)**
Elective caesarean section	**1.1 (0.4-1.8)**	**0.2 (0.1-0.4)**	**2.2 (1.4-3.0)**

Oestrone 3-sulfate was significantly lower in the CV (0.18-FC, p=0.007) and CA (0.19-FC, p=0.010) than IV blood of the ECS group. There were also differences in metabolite levels between different modes of labour and delivery. Oestrone 3-sulfate was significantly lower in the CA of the ECS group than the CA of both the spontaneously labouring (0.37-FC, p=0.030) and IOL groups (0.35-FC, p=0.013). Further, it was significantly lower in the CV of the ECS group compared with the CV of the SL group (0.34-fC, p=0.013), and significantly higher in the CV of the IOL group than the ECS group (2.22-FC, p=0.024) (Table 3.13).

3.5.18 Pregnenolone steroids

Levels of two of the pregnenolone steroids differed in the latent/pre-operative samples between the three modes of labour: 21-hydroxypregnenolone monosulfate (1) was significantly reduced in both the latent phase of the IOL group (0.30-FC, p=0.006) and the ECS group (0.45-FC, p=0.032) compared with the SL group. 21-hydroxypregnenolone monosulfate (2) was also reduced in the latent phase of the IOL group compared with that of the SL group (0.42-FC, p=0.026); it was also reduced in the ECS group, but this was not significant.

3.5.19 Comparisons between spontaneous labour and induction of labour

10.1% (104/1032) of the metabolites measured were significantly ($p \leq 0.05$) different between the CA of the SL and IOL groups (Table 3A4, Appendices), and 11.0% (113/1032) were significantly different between the CV samples (Table

3A5, Appendices). This is in comparison with only 3.1% (32/1032) significantly different between the latent phase samples (Table 3A6, Appendices), and 2.9% (30/1032) significantly different in the IV samples (Table 3A7, Appendices). The greatest difference in all four groups was higher mean concentrations of Ranitidine in the IOL group. This is likely because two of the women in the IOL group had an epidural, and women with epidurals were routinely offered Ranitidine every six hours at St Michael's Hospital to reduce the risk of aspiration in case of the need for a general anaesthetic.

Of those metabolites that were significantly different between the cord arteries, 88 were higher, and 16 lower, in the CA of the SL group compared with the IOL group. Those metabolites which were significantly lower by at least half in the CA of the IOL group compared with the SL group included 1-stearoyl-GPS (18:0) (0.1-FC, p=0.002), inosine 5'-monohosphate (0.18-FC, p=0.002), 1-stearoyl-2-oleoyl-GPS (18:0/19:1) (0.21-FC, p=0.002), androsterone sulphate (0.35-FC, p-0.013) and sphinganine-1-phosphate (0.49-FC, p=0.013). Five fibrinogen peptide metabolites were increased in the CA of the IOL group compared with the SL group: Fibrinopeptide B (142.41-FC, p=0.00), Fibrinopeptide B (1-13) (101.32-FC, p = 0.001), Fibrinopeptide A (76.31-FC, p=0.0003) as well as adenosine 5'-monophosphate (AMP) (3.44-FC, p=0.021).

Of those metabolites that were significantly different between the CV samples, 42 were higher, and 71 lower, in the SL group compared with the IOL group. Those with the greatest fold-change in the CV included oestriol, which was 6.45-fold higher in the CV of the IOL group compared with the SL group (p = 0.035), and progesterone, which was 4.27-fold higher in the CV of the IOL group compared with the ECS group (p = 0.043). Of note, oestriol was 0.52-FC lower in the CA of the IOL group compared with the SL group, but this was not significant (p=0.29), and progesterone was 0.57-fold lower in the CA of the IOL group compared with the SL group, however again this did not reach significance. Although not significant, oestriol was 4.63-fold higher in the CV of the ECS group (non-significant) than the CV of the SL group, and 0.35-fold lower in the CV of the ECS group than the CV of the IOL group (non-significant) (see Figure 13).

Further, 17alpha-hydroxyprogesterone was 3.50-fold higher in the CV of the IOL group compared with the SL group (p=0.036) and arachidonate (20:4n6) was 2.46-fold higher (p=0.005). Those that were significantly lower in the CV of the IOL group compared with the SL group included the corticosteroids corticosterone and cortisol (0.48-FC, p=0.022 and 0.41-FC, p=0.028, respectively), sphingosine (0.28-FC, p=0.049), sphinganine (0.19-FC, p=0.022), adenosine 3',5'-cyclic monophosphate (cAMP) (0.22-FC, p=0.034), and cytidine 5'-monoposphate (5'-CMP) (0.13-FC, p=0.009) (see Figure 3.13).

A.

Metabolite	SL		IOL		ECS	
	Latent	IV	Latent	IV	Latent	IV
Progesterone	0.9	13.6	1.4	13.1	0.9	8.1
Oestriol	0.1	2	0	2.1	0.1	2.2
17α-hydroxyprogesterone	0.6	3.2	0.5	3	0.5	2.3
DHEA-S	0.9	1.8	0.6	1.1	0.8	0.9
Sphingosine	0.9	7.6	1.1	6	1.1	5
Sphingosine 1-phosphate	1.2	0.4	1.6	0.9	1.3	0.4

B.

Metabolite	SL		IOL		ECS	
	CV	CA	CV	CA	CV	CA
Progesterone	1.5	5.3	6.7	3	2.9	1.3
Oestriol	0.3	1.3	1.6	0.7	1.1	0.2
17α-hydroxyprogesterone	0.7	1.8	2.5	1.3	1.1	1.3
DHEA-S	2.6	2.6	1.6	1.7	1.9	2.1
Sphingosine	3.8	2.1	1.1	2.4	0.9	1
Sphingosine 1-phosphate	1.5	1.8	1.4	1.3	1.2	0.8

Figure 3.13: Heat maps comparing scaled normalised values of progesterone, oestriol, 17α-hydroxyprogesterone, DHEA-S, sphingosine and sphingosine 1-phosphate between the spontaneously labouring group, the induction of labour group and the elective caesarean section group for: A. the latent phase and intervillous (IV) samples; and B. the cord vein (CV) and cord artery (CA) samples. N=4 in each group. Heat map coloured according to scale from blue (lowest) to white (middle) to red (highest). SL = spontaneously labouring; IOL = induction of labour; ECS = elective caesarean section.

3.5.20 Over-representation analysis for correlation analysis

Table 3.15 and Figure 3.14 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 63 metabolites which were significantly associated ($p \leq 0.05$) at 28 weeks' gestation with gestational age at spontaneous delivery. Glycerol phosphate shuttle, riboflavin metabolism and glycerolipid metabolism pathways were the most over-represented, although none were significantly over-represented.

Table 3.15: Results of the over-representation enrichment analysis for metabolites sampled at 28 weeks' gestation which were significantly (p≤0.05) correlated with gestational age at spontaneous delivery, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathway	Total	Expected	Hits	Raw p	Holm p
Glycerol Phosphate Shuttle	11	0.269	1	0.239	1
Riboflavin Metabolism	20	0.488	1	0.393	1
Glycerolipid Metabolism	25	0.61	1	0.465	1
Androgen and Estrogen Metabolism	33	0.806	1	0.563	1
Bile Acid Biosynthesis	65	1.59	1	0.81	1

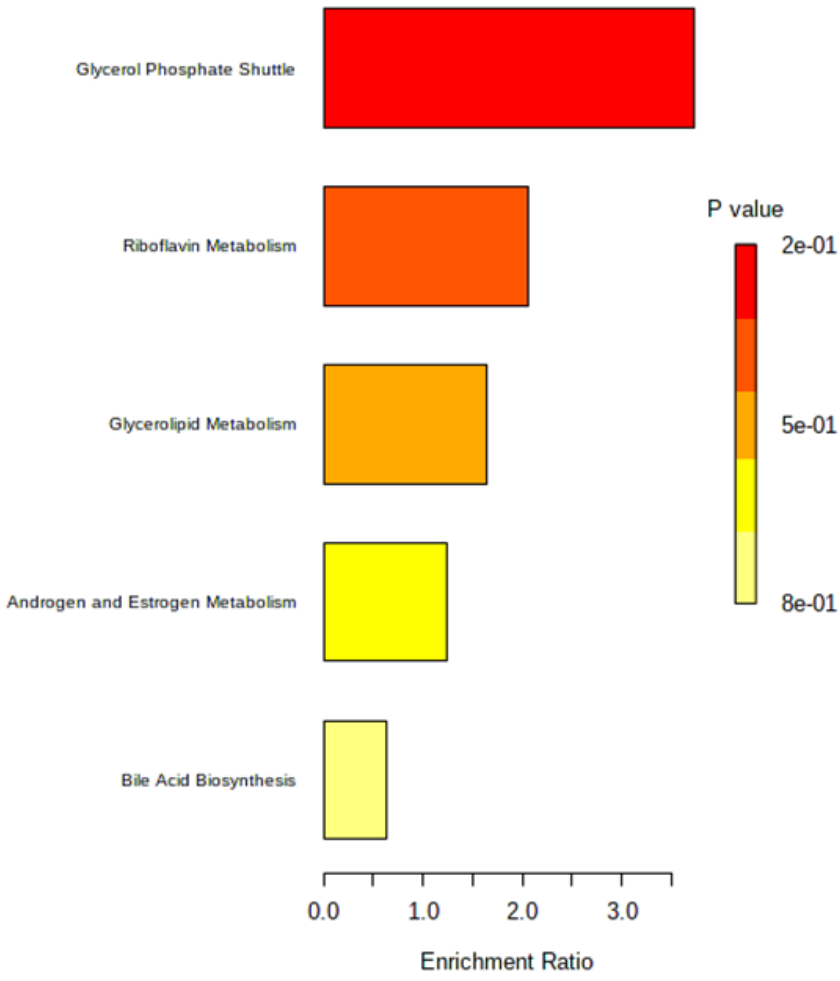

Figure 3.14: Overview of the results of the over-representation analysis for metabolites sampled at 28 weeks' gestation which were significantly (p≤0.05) correlated with gestational age at spontaneous delivery, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

Table 3.16 and Figure 3.15 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 84 metabolites which were significantly associated (p≤0.05) at 34 weeks' gestation with gestational age at spontaneous delivery. Mitochondrial beta-oxidation of long-chain fatty acids, betaine metabolism and Glycerol Phosphate Shuttle were the most over-represented pathways, although none were significantly over-represented.

Table 3.16: Results of the over-representation enrichment analysis for metabolites sampled at 34 weeks' gestation which were significantly (p≤0.05) correlated with gestational age at spontaneous delivery, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathway	Total	Expected	Hits	Raw p	Holm p
Mitochondrial Beta-Oxidation of Long Chain Saturated Fatty Acids	28	1.2	4	0.0286	1
Betaine Metabolism	21	0.902	3	0.0573	1
Glycerol Phosphate Shuttle	11	0.473	2	0.0776	1
Estrone Metabolism	24	1.03	3	0.0798	1
Plasmalogen Synthesis	26	1.12	3	0.0966	1
Methylhistidine Metabolism	4	0.172	1	0.161	1
Androgen and Estrogen Metabolism	33	1.42	3	0.165	1
Beta-Alanine Metabolism	34	1.46	3	0.176	1
Butyrate Metabolism	19	0.816	2	0.195	1
Mitochondrial Electron Transport Chain	19	0.816	2	0.195	1
Nicotinate and Nicotinamide Metabolism	37	1.59	3	0.209	1
Threonine and 2-Oxobutanoate Degradation	20	0.859	2	0.211	1
Carnitine Synthesis	22	0.945	2	0.243	1
Sphingolipid Metabolism	40	1.72	3	0.244	1
Caffeine Metabolism	24	1.03	2	0.276	1
Methionine Metabolism	43	1.85	3	0.28	1
Histidine Metabolism	43	1.85	3	0.28	1
Glycerolipid Metabolism	25	1.07	2	0.292	1
Phytanic Acid Peroxisomal Oxidation	26	1.12	2	0.308	1
Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	27	1.16	2	0.325	1

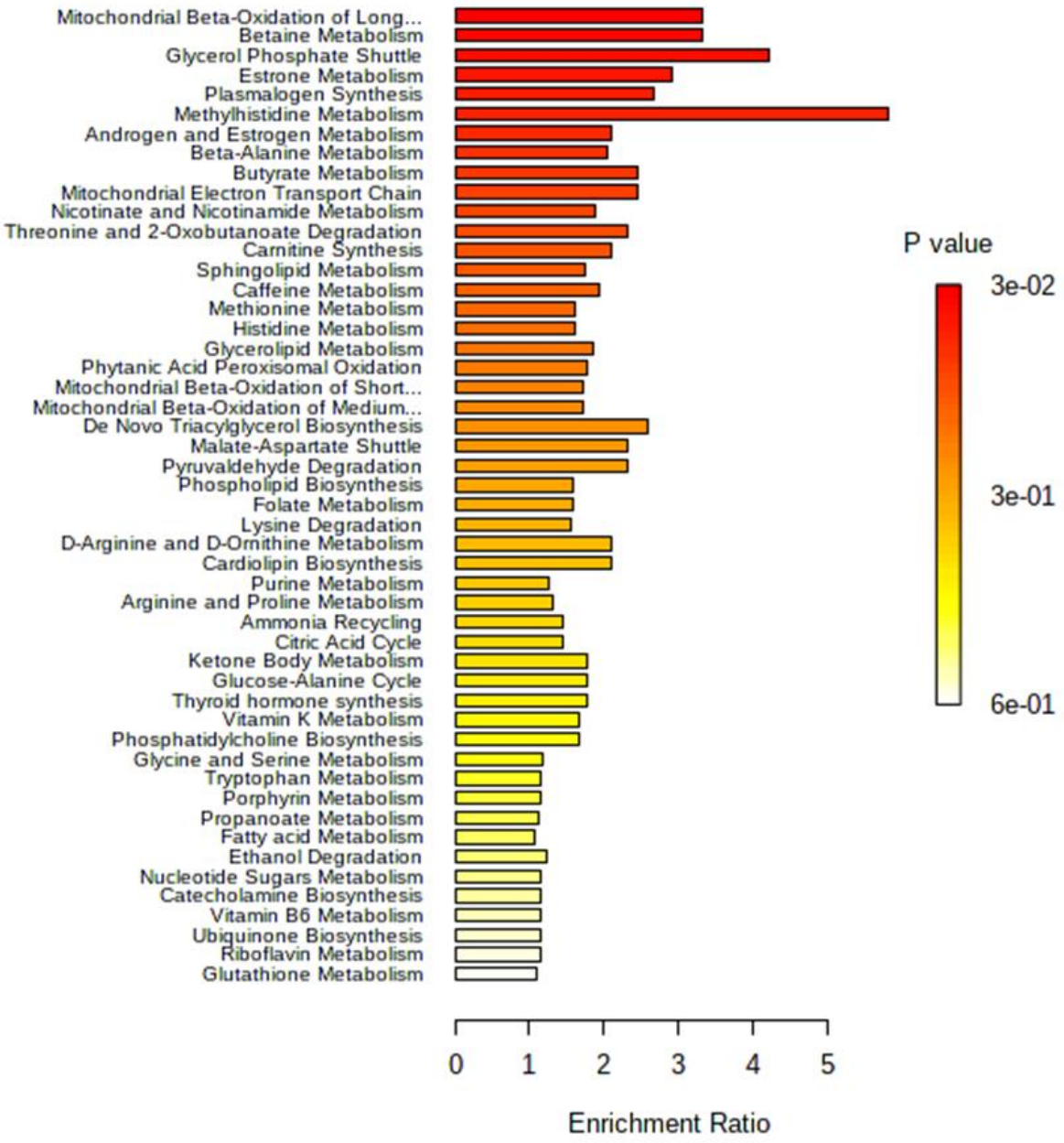

Figure 3.15: Overview of the results of the over-representation analysis for for metabolites sampled at 34 weeks' gestation which were significantly (p≤0.05) correlated with gestational age at spontaneous delivery, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

Table 3.17 and Figure 3.16 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 53 metabolites which were significantly associated (p≤0.05) in latent phase with gestational age at spontaneous delivery. Phenylacetate metabolism, glycerol phosphate shuttle and riboflavin metabolism were the most over-represented pathways, although none were significantly over-represented.

Table 3.17: Results of the over-representation enrichment analysis for metabolites in latent phase which were significantly (p≤0.05) correlated with gestational age at spontaneous delivery, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathway	Total	Expected	Hits	Raw p	Holm p
Phenylacetate Metabolism	**9**	**0.237**	**1**	**0.215**	**1**
Glycerol Phosphate Shuttle	**11**	**0.29**	**1**	**0.256**	**1**
Riboflavin Metabolism	**20**	**0.527**	**1**	**0.417**	**1**
Estrone Metabolism	**24**	**0.633**	**1**	**0.477**	**1**
Glycerolipid Metabolism	**25**	**0.659**	**1**	**0.491**	**1**
Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	**27**	**0.712**	**1**	**0.519**	**1**
Phospholipid Biosynthesis	**29**	**0.765**	**1**	**0.544**	**1**
Tryptophan Metabolism	**60**	**1.58**	**1**	**0.808**	**1**

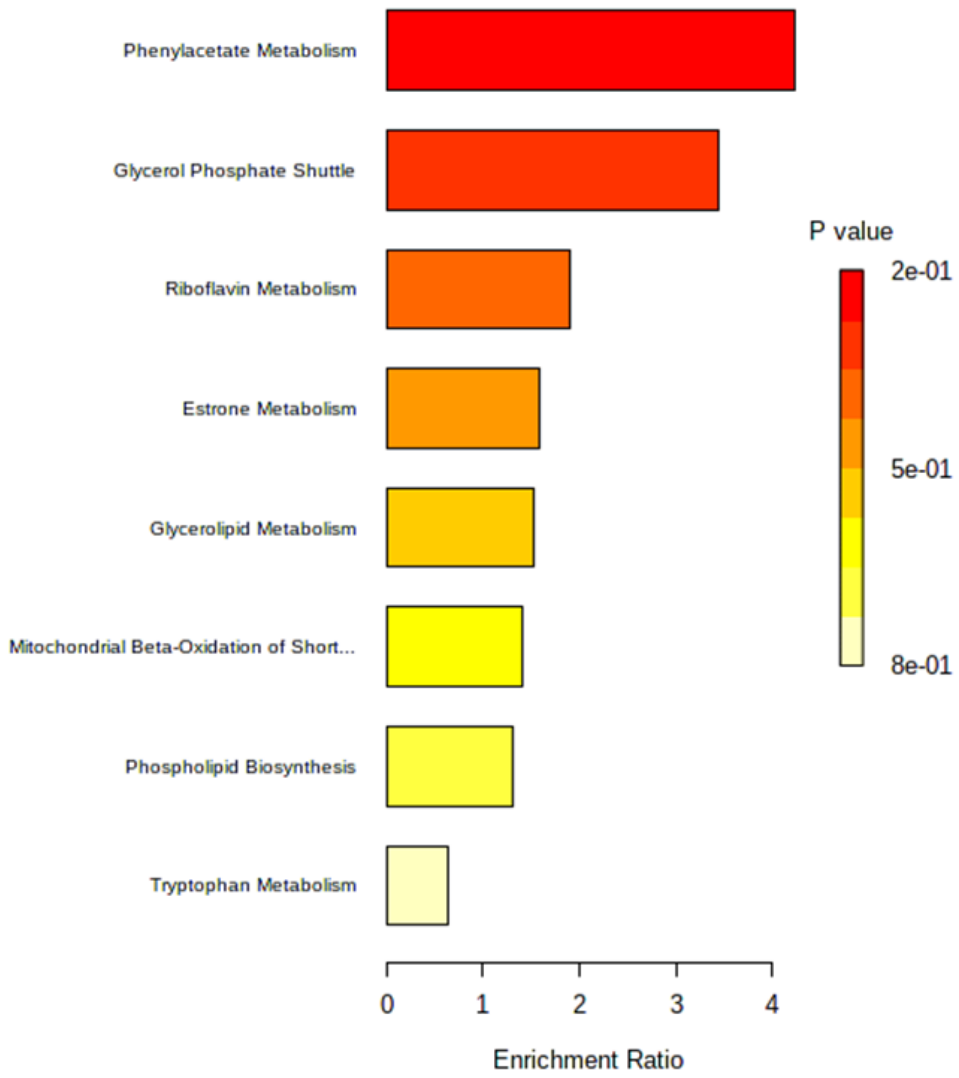

Figure 3.16: Overview of the results of the over-representation analysis for for metabolites sampled in latent phase which were significantly (p≤0.05) correlated with gestational age at spontaneous delivery, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

3.5.21 Over-representation analysis for comparisons of the different vascular compartments at delivery

3.5.21.1 Over-representation analysis for cord artery comparisons

Table 3.18 and Figure 3.17 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 104 metabolites which were significantly different (p≤0.05) between the CA of the IOL and SL groups.

Table 3.18: Results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord artery of the IOL group and cord artery of the SL group, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathways	Total	Expected	Hits	Raw p	Holm p
Alpha Linolenic Acid and Linoleic Acid Metabolism	19	1.43	6	0.00183	0.18
Aspartate Metabolism	35	2.63	7	0.0125	1
Fatty Acid Biosynthesis	35	2.63	6	0.0415	1
Beta Oxidation of Very Long Chain Fatty Acids	17	1.28	3	0.13	1
Butyrate Metabolism	19	1.43	3	0.166	1
Glutathione Metabolism	21	1.58	3	0.205	1
Vitamin K Metabolism	14	1.05	2	0.284	1
Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	27	2.03	3	0.332	1
Mitochondrial Beta-Oxidation of Medium Chain Saturated Fatty Acids	27	2.03	3	0.332	1
Arginine and Proline Metabolism	53	3.99	5	0.368	1
Alanine Metabolism	17	1.28	2	0.37	1
Propanoate Metabolism	42	3.16	4	0.39	1
Methionine Metabolism	43	3.23	4	0.408	1
Histidine Metabolism	43	3.23	4	0.408	1
Ammonia Recycling	32	2.41	3	0.437	1
Riboflavin Metabolism	20	1.5	2	0.452	1
Glycine and Serine Metabolism	59	4.44	5	0.461	1
Biotin Metabolism	8	0.602	1	0.466	1
Thiamine Metabolism	9	0.677	1	0.507	1
Phenylacetate Metabolism	9	0.677	1	0.507	1

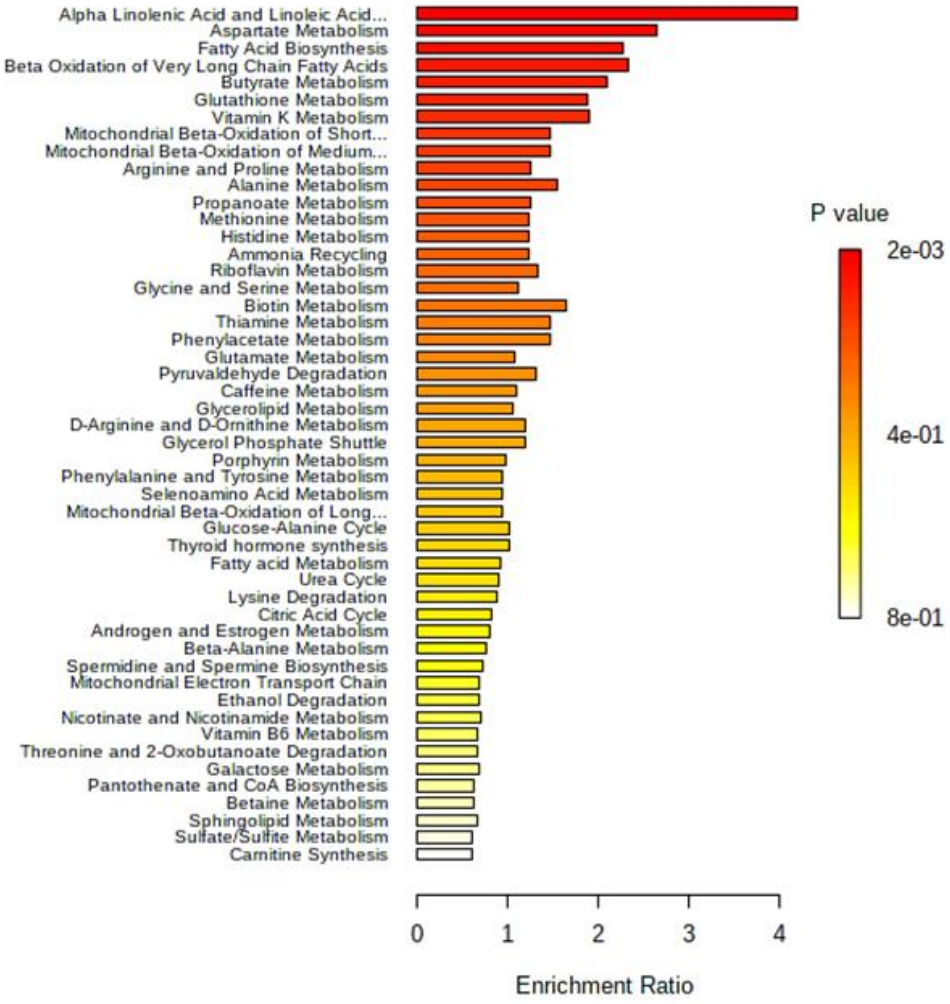

Figure 3.17: Overview of the results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord artery of the IOL group and cord artery of the SL group, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

Table 3.19 and Figure 3.18 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 232 metabolites which were significantly different (p≤0.05) between the CA of the ECS and SL groups.

Table 3.19: Results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord artery of the ECS group and cord artery of the SL group , where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathways	Total	Expected	Hits	Raw p	Holm p
Alpha Linolenic Acid and Linoleic Acid Metabolism	19	2.78	9	0.000615	0.0602
Beta Oxidation of Very Long Chain Fatty Acids	17	2.49	5	0.0893	1
Fatty Acid Biosynthesis	35	5.13	8	0.126	1
Glucose-Alanine Cycle	13	1.9	3	0.294	1
Glutathione Metabolism	21	3.08	4	0.371	1
Transfer of Acetyl Groups into Mitochondria	22	3.22	4	0.406	1
Pyruvaldehyde Degradation	10	1.46	2	0.443	1
Galactose Metabolism	38	5.57	6	0.491	1
Citric Acid Cycle	32	4.69	5	0.515	1
Mitochondrial Electron Transport Chain	19	2.78	3	0.543	1
Taurine and Hypotaurine Metabolism	12	1.76	2	0.544	1
Androgen and Estrogen Metabolism	33	4.83	5	0.544	1
Thyroid hormone synthesis	13	1.9	2	0.589	1
Aspartate Metabolism	35	5.13	5	0.6	1
Steroidogenesis	43	6.3	6	0.621	1
Urea Cycle	29	4.25	4	0.635	1
Sulfate/Sulfite Metabolism	22	3.22	3	0.647	1
Caffeine Metabolism	24	3.52	3	0.707	1
Estrone Metabolism	24	3.52	3	0.707	1
Sphingolipid Metabolism	40	5.86	5	0.721	1

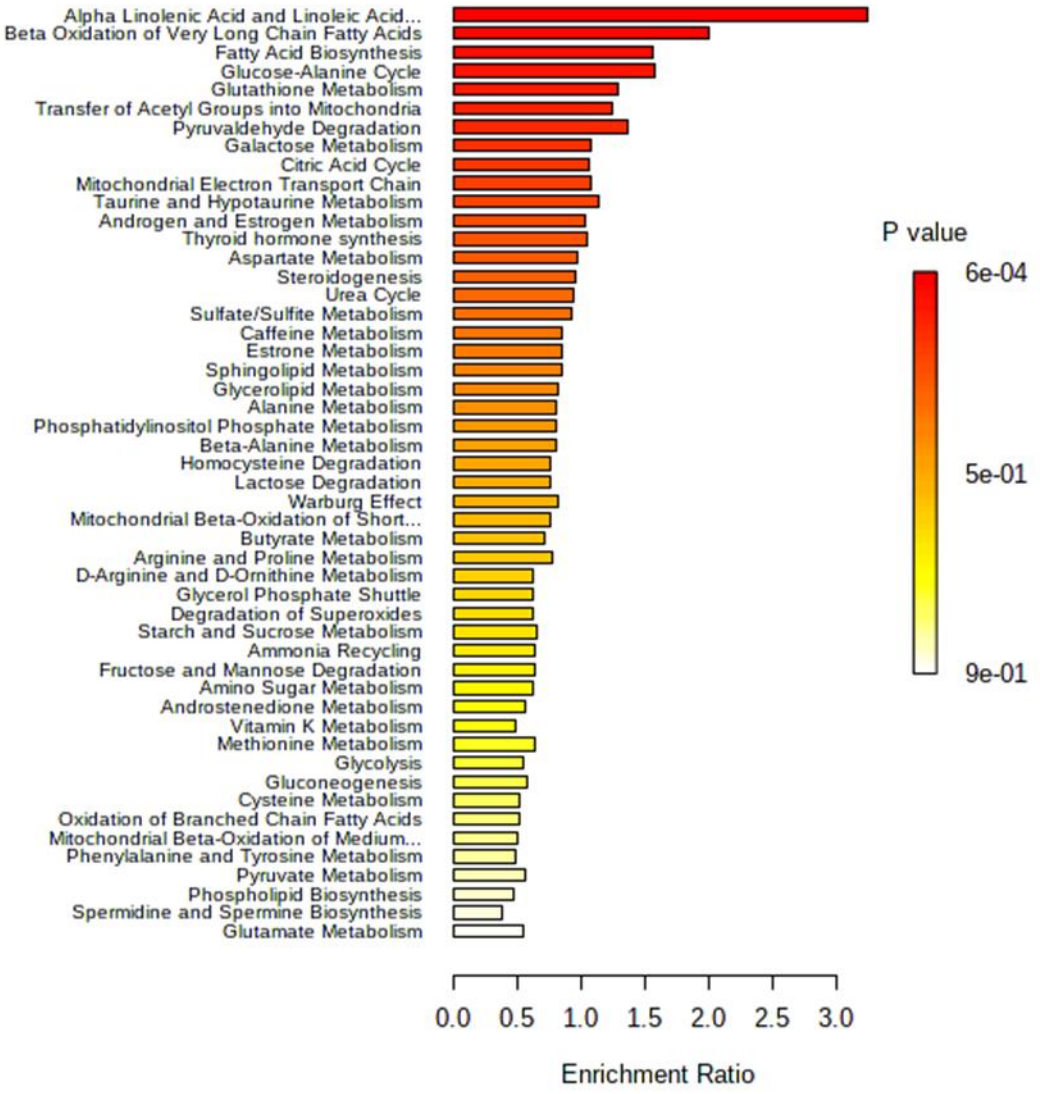

Figure 3.18: Overview of the results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord artery of the ECS group and cord artery of the SL group, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

Table 3.20 and Figure 3.19 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 82 metabolites which were significantly different (p≤0.05) between the CA of the ECS and IOL groups.

Table 3.20: Results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord artery of the ECS group and cord artery of the IOL group, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathways	Total	Expected	Hits	Raw p	Holm p
Citric Acid Cycle	32	1.62	5	0.0197	1
Warburg Effect	58	2.95	6	0.0675	1
Transfer of Acetyl Groups into Mitochondria	22	1.12	3	0.0962	1
Glucose-Alanine Cycle	13	0.66	2	0.138	1
Urea Cycle	29	1.47	3	0.179	1
Alanine Metabolism	17	0.863	2	0.212	1
Mitochondrial Electron Transport Chain	19	0.965	2	0.251	1
Glutathione Metabolism	21	1.07	2	0.289	1
Steroidogenesis	43	2.18	3	0.375	1
Pyruvaldehyde Degradation	10	0.508	1	0.408	1
Pyruvate Metabolism	48	2.44	3	0.445	1
Glutamate Metabolism	49	2.49	3	0.459	1
Taurine and Hypotaurine Metabolism	12	0.609	1	0.467	1
Starch and Sucrose Metabolism	31	1.57	2	0.474	1
Ketone Body Metabolism	13	0.66	1	0.494	1
Amino Sugar Metabolism	33	1.68	2	0.508	1
Androgen and Estrogen Metabolism	33	1.68	2	0.508	1
Beta-Alanine Metabolism	34	1.73	2	0.524	1
Purine Metabolism	74	3.76	4	0.528	1
Aspartate Metabolism	35	1.78	2	0.54	1

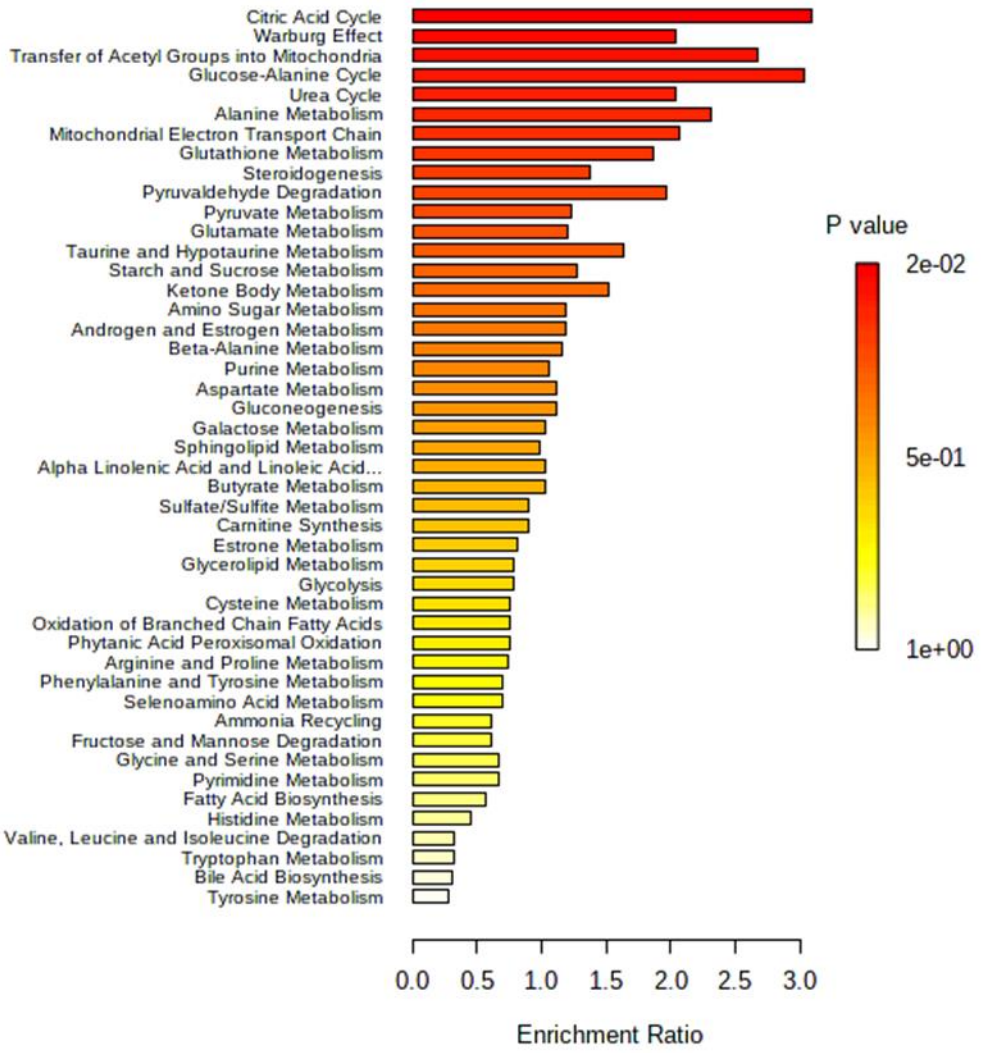

Figure 3.19: Overview of the results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord artery of the ECS group and cord artery of the IOL group, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

3.5.21.2 Over-representation analysis for cord vein comparisons

Table 3.21 and Figure 3.20 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 113 metabolites which were significantly different (p≤0.05) between the CV of the IOL and SL groups.

Table 3.21: Results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord vein of the IOL group and cord vein of the SL group, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathways	Total	Expected	Hits	Raw p	Holm p
Alpha Linolenic Acid and Linoleic Acid Metabolism	**19**	**1.48**	**11**	**$1.53x10^{-8}$**	**$1.50x10^{-6}$**
Beta Oxidation of Very Long Chain Fatty Acids	**17**	**1.33**	**3**	**0.141**	**1**
Glutathione Metabolism	**21**	**1.64**	**3**	**0.222**	**1**
Steroidogenesis	**43**	**3.36**	**5**	**0.24**	**1**
Methylhistidine Metabolism	**4**	**0.312**	**1**	**0.278**	**1**
Fatty Acid Biosynthesis	**35**	**2.73**	**4**	**0.29**	**1**
Phosphatidylcholine Biosynthesis	**14**	**1.09**	**2**	**0.3**	**1**
Galactose Metabolism	**38**	**2.97**	**4**	**0.345**	**1**
Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	**27**	**2.11**	**3**	**0.354**	**1**
Methionine Metabolism	**43**	**3.36**	**4**	**0.437**	**1**
Fructose and Mannose Degradation	**32**	**2.5**	**3**	**0.463**	**1**
Carnitine Synthesis	**22**	**1.72**	**2**	**0.524**	**1**
Malate-Aspartate Shuttle	**10**	**0.781**	**1**	**0.558**	**1**
Nicotinate and Nicotinamide Metabolism	**37**	**2.89**	**3**	**0.564**	**1**
Estrone Metabolism	**24**	**1.88**	**2**	**0.572**	**1**
Cardiolipin Biosynthesis	**11**	**0.859**	**1**	**0.593**	**1**
Phosphatidylethanolamine Biosynthesis	**12**	**0.938**	**1**	**0.625**	**1**
Glucose-Alanine Cycle	**13**	**1.02**	**1**	**0.655**	**1**
Starch and Sucrose Metabolism	**31**	**2.42**	**2**	**0.713**	**1**
Amino Sugar Metabolism	**33**	**2.58**	**2**	**0.746**	**1**

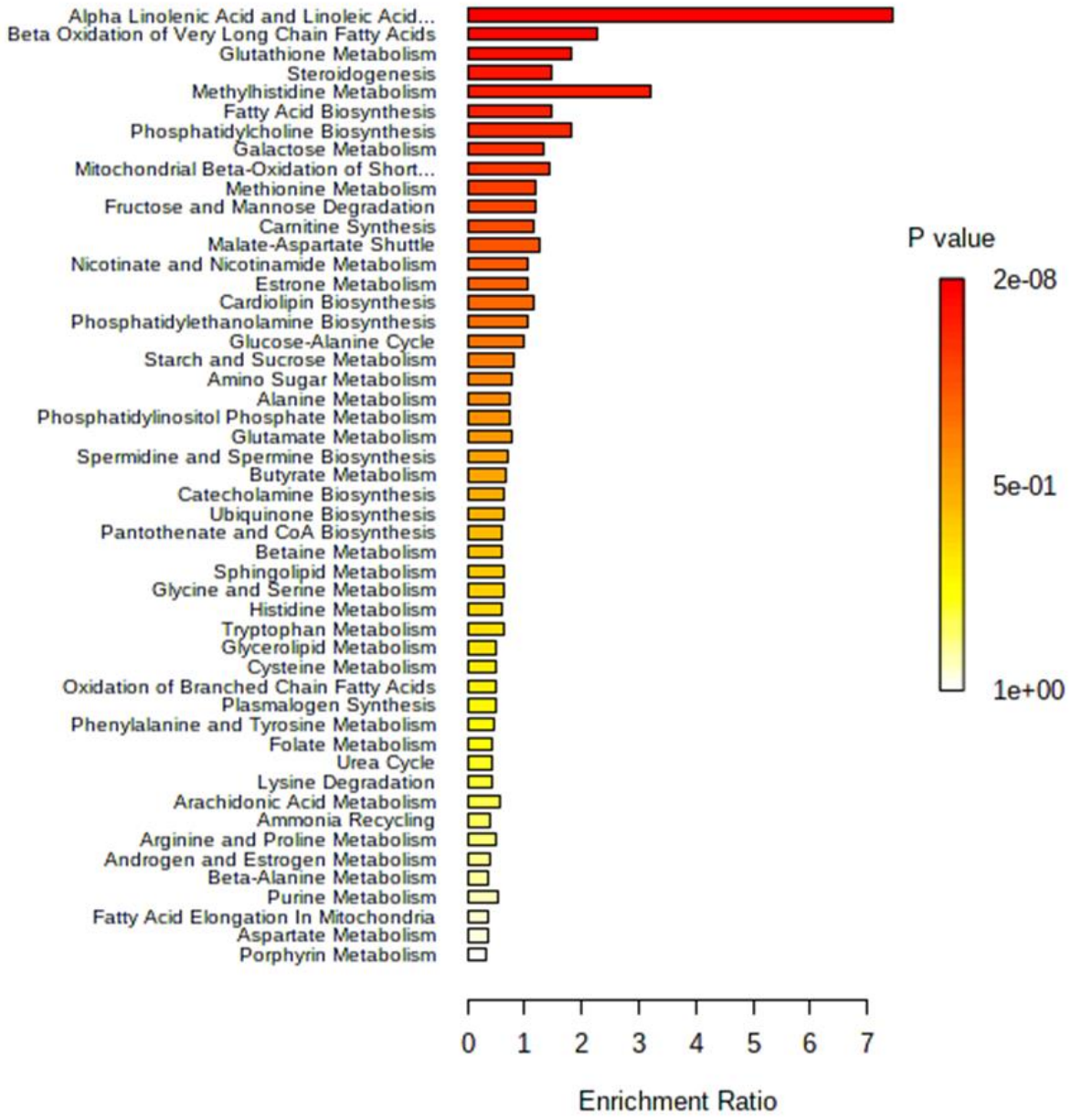

Figure 3.20: Overview of the results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord vein of the IOL group and cord vein of the SL group, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

Table 3.22 and Figure 3.21 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 130 metabolites which were significantly different (p≤0.05) between the CV of the ECS and SL groups.

Table 3.22: Results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord vein of the ECS group and cord vein of the SL group, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathways	Total	Expected	Hits	Raw p	Holm p
Alpha Linolenic Acid and Linoleic Acid Metabolism	19	1.65	4	0.0752	1
Beta Oxidation of Very Long Chain Fatty Acids	17	1.48	3	0.178	1
Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	27	2.35	3	0.421	1
Phosphatidylinositol Phosphate Metabolism	17	1.48	2	0.443	1
Butyrate Metabolism	19	1.65	2	0.503	1
Methionine Metabolism	43	3.74	4	0.524	1
Glutathione Metabolism	21	1.83	2	0.558	1
Pyruvaldehyde Degradation	10	0.869	1	0.599	1
Fatty Acid Biosynthesis	35	3.04	3	0.6	1
Caffeine Metabolism	24	2.09	2	0.633	1
D-Arginine and D-Ornithine Metabolism	11	0.956	1	0.634	1
Glycerol Phosphate Shuttle	11	0.956	1	0.634	1
Cardiolipin Biosynthesis	11	0.956	1	0.634	1
Purine Metabolism	74	6.43	6	0.638	1
Nicotinate and Nicotinamide Metabolism	37	3.22	3	0.639	1
Glycerolipid Metabolism	25	2.17	2	0.655	1
Galactose Metabolism	38	3.3	3	0.658	1
Taurine and Hypotaurine Metabolism	12	1.04	1	0.666	1
Phosphatidylethanolamine Biosynthesis	12	1.04	1	0.666	1
Oxidation of Branched Chain Fatty Acids	26	2.26	2	0.677	1

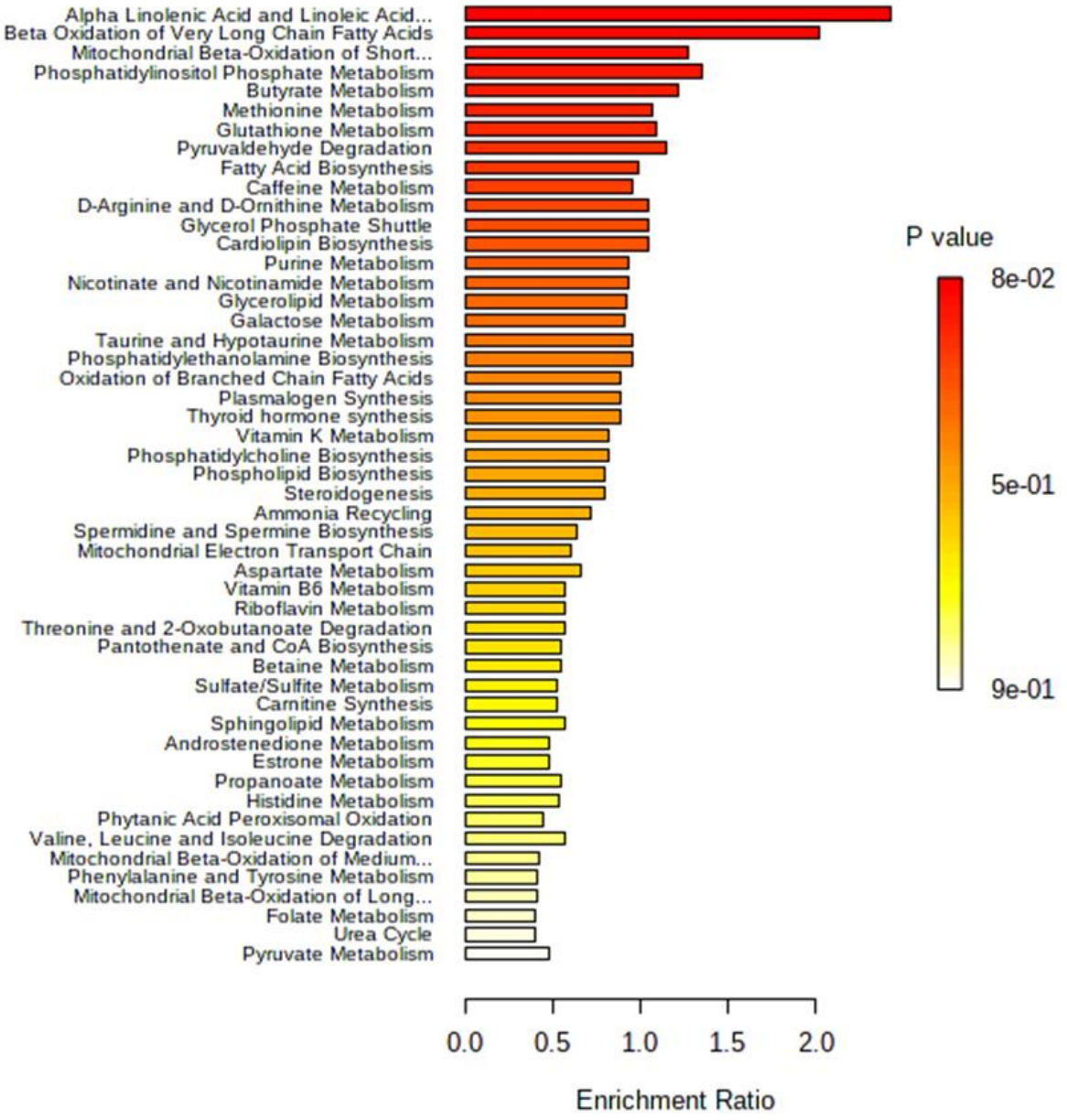

Figure 3.21: Overview of the results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord vein of the ECS group and cord vein of the SL group, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

Table 3.23 and Figure 3.22 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 60 metabolites which were significantly different (p≤0.05) between the CV of the ECS and IOL groups.

Table 3.23: Results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord vein of the ECS group and cord vein of the IOL group, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathways	Total	Expected	Hits	Raw p	Holm p
Steroidogenesis	**43**	**1.55**	**5**	**0.0165**	**1**
Glucose-Alanine Cycle	**13**	**0.47**	**2**	**0.0772**	**1**
Beta-Alanine Metabolism	**34**	**1.23**	**3**	**0.121**	**1**
Methylhistidine Metabolism	**4**	**0.145**	**1**	**0.137**	**1**
Galactose Metabolism	**38**	**1.37**	**3**	**0.154**	**1**
Glutathione Metabolism	**21**	**0.759**	**2**	**0.174**	**1**
Transfer of Acetyl Groups into Mitochondria	**22**	**0.795**	**2**	**0.187**	**1**
Homocysteine Degradation	**9**	**0.325**	**1**	**0.283**	**1**
Lactose Degradation	**9**	**0.325**	**1**	**0.283**	**1**
Androgen and Estrogen Metabolism	**33**	**1.19**	**2**	**0.337**	**1**
Warburg Effect	**58**	**2.1**	**3**	**0.35**	**1**
Taurine and Hypotaurine Metabolism	**12**	**0.434**	**1**	**0.359**	**1**
Gluconeogenesis	**35**	**1.26**	**2**	**0.364**	**1**
Histidine Metabolism	**43**	**1.55**	**2**	**0.467**	**1**
Alanine Metabolism	**17**	**0.614**	**1**	**0.468**	**1**
Lactose Synthesis	**20**	**0.723**	**1**	**0.524**	**1**
Glutamate Metabolism	**49**	**1.77**	**2**	**0.538**	**1**
Pantothenate and CoA Biosynthesis	**21**	**0.759**	**1**	**0.542**	**1**
Sulfate/Sulfite Metabolism	**22**	**0.795**	**1**	**0.559**	**1**
Estrone Metabolism	**24**	**0.867**	**1**	**0.591**	**1**

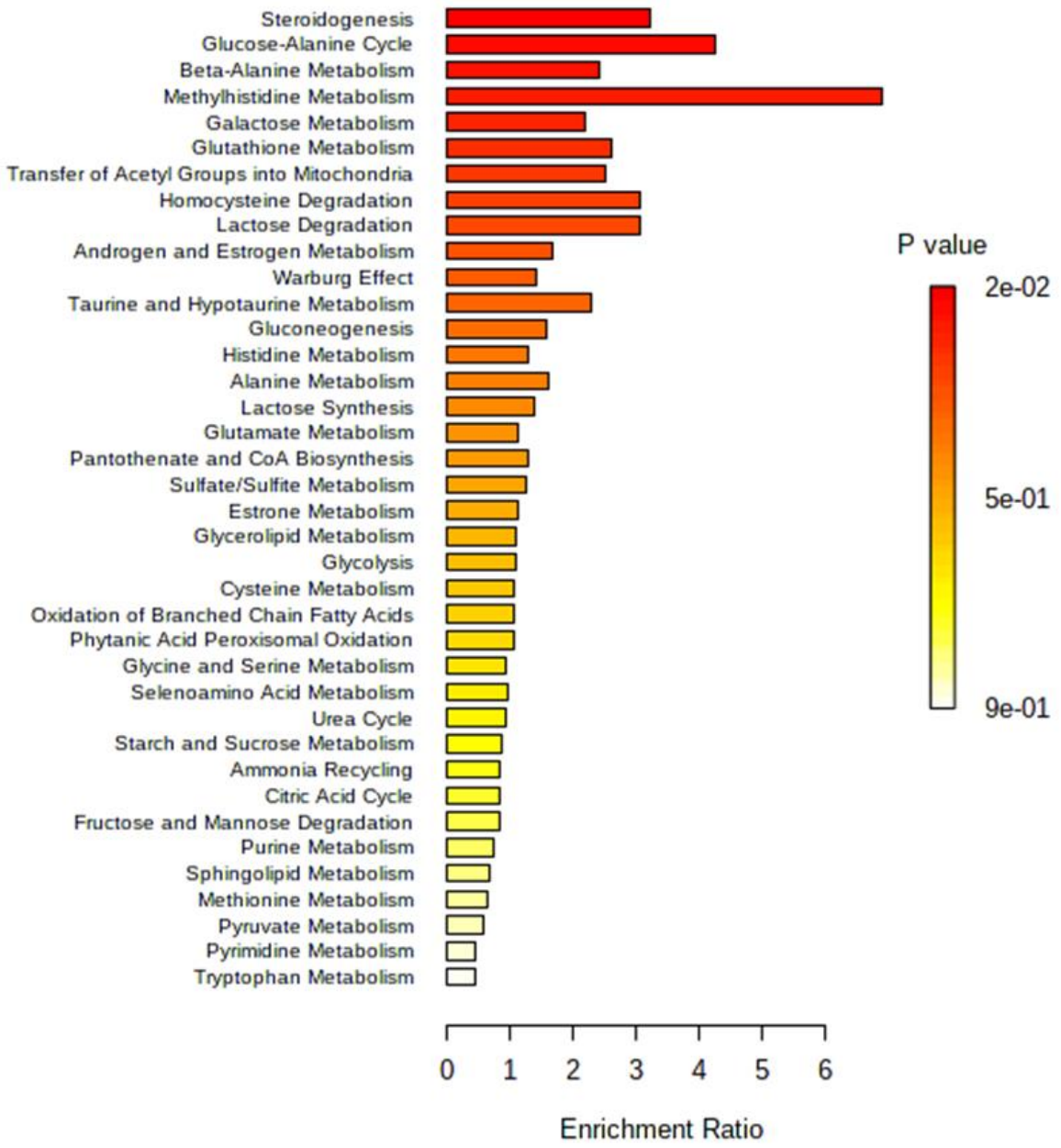

Figure 3.22: Overview of the results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the cord vein of the ECS group and cord vein of the IOL group, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

3.5.21.3 Over-representation analysis for intervillous blood comparisons

Table 3.24 and Figure 3.23 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 30 metabolites which were significantly different (p≤0.05) between the IV blood of the IOL and SL groups.

Table 3.24: Results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the intervillous blood of the IOL group and intervillous blood of the SL group, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathway	Total	Expected	Hits	Raw p	Holm p
Tryptophan Metabolism	**60**	**0.996**	**2**	**0.262**	**1**
Caffeine Metabolism	**24**	**0.398**	**1**	**0.334**	**1**
Glycerolipid Metabolism	**25**	**0.415**	**1**	**0.345**	**1**
Phospholipid Biosynthesis	**29**	**0.481**	**1**	**0.389**	**1**
Nicotinate and Nicotinamide Metabolism	**37**	**0.614**	**1**	**0.468**	**1**
Retinol Metabolism	**37**	**0.614**	**1**	**0.468**	**1**
Galactose Metabolism	**38**	**0.631**	**1**	**0.477**	**1**
Histidine Metabolism	**43**	**0.714**	**1**	**0.521**	**1**

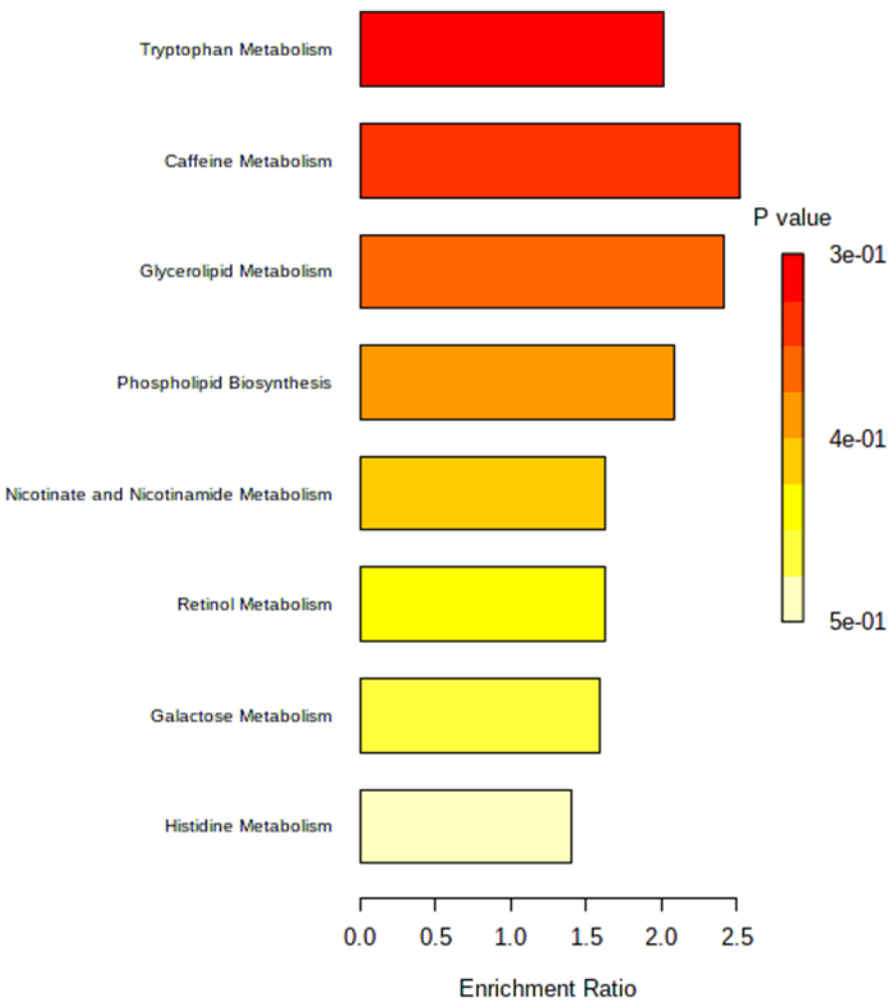

Figure 3.23: Overview of the results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the intervillous blood of the IOL group and intervillous blood of the SL group, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

Table 3.25 and Figure 3.24 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 111 metabolites which were significantly different (p≤0.05) between the IV of the ECS and SL groups.

Table 3.25: Results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the intervillous blood of the ECS group and intervillous blood of the SL group, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

	Total	Expected	Hits	Raw p	Holm p
Glucose-Alanine Cycle	**13**	**0.686**	**3**	**0.0272**	**1**
Homocysteine Degradation	**9**	**0.475**	**2**	**0.0775**	**1**
Transfer of Acetyl Groups into Mitochondria	**22**	**1.16**	**3**	**0.105**	**1**
Alanine Metabolism	**17**	**0.896**	**2**	**0.225**	**1**
Gluconeogenesis	**35**	**1.85**	**3**	**0.279**	**1**
Glutathione Metabolism	**21**	**1.11**	**2**	**0.305**	**1**
Warburg Effect	**58**	**3.06**	**4**	**0.366**	**1**
Glycine and Serine Metabolism	**59**	**3.11**	**4**	**0.379**	**1**
Glycolysis	**25**	**1.32**	**2**	**0.384**	**1**
Lactose Degradation	**9**	**0.475**	**1**	**0.387**	**1**
Cysteine Metabolism	**26**	**1.37**	**2**	**0.403**	**1**
Pyruvaldehyde Degradation	**10**	**0.527**	**1**	**0.42**	**1**
Selenoamino Acid Metabolism	**28**	**1.48**	**2**	**0.441**	**1**
Urea Cycle	**29**	**1.53**	**2**	**0.459**	**1**
Taurine and Hypotaurine Metabolism	**12**	**0.633**	**1**	**0.48**	**1**
Glutamate Metabolism	**49**	**2.58**	**3**	**0.485**	**1**
Citric Acid Cycle	**32**	**1.69**	**2**	**0.512**	**1**
Galactose Metabolism	**38**	**2**	**2**	**0.607**	**1**
Pyrimidine Metabolism	**59**	**3.11**	**3**	**0.615**	**1**
Lactose Synthesis	**20**	**1.05**	**1**	**0.665**	**1**

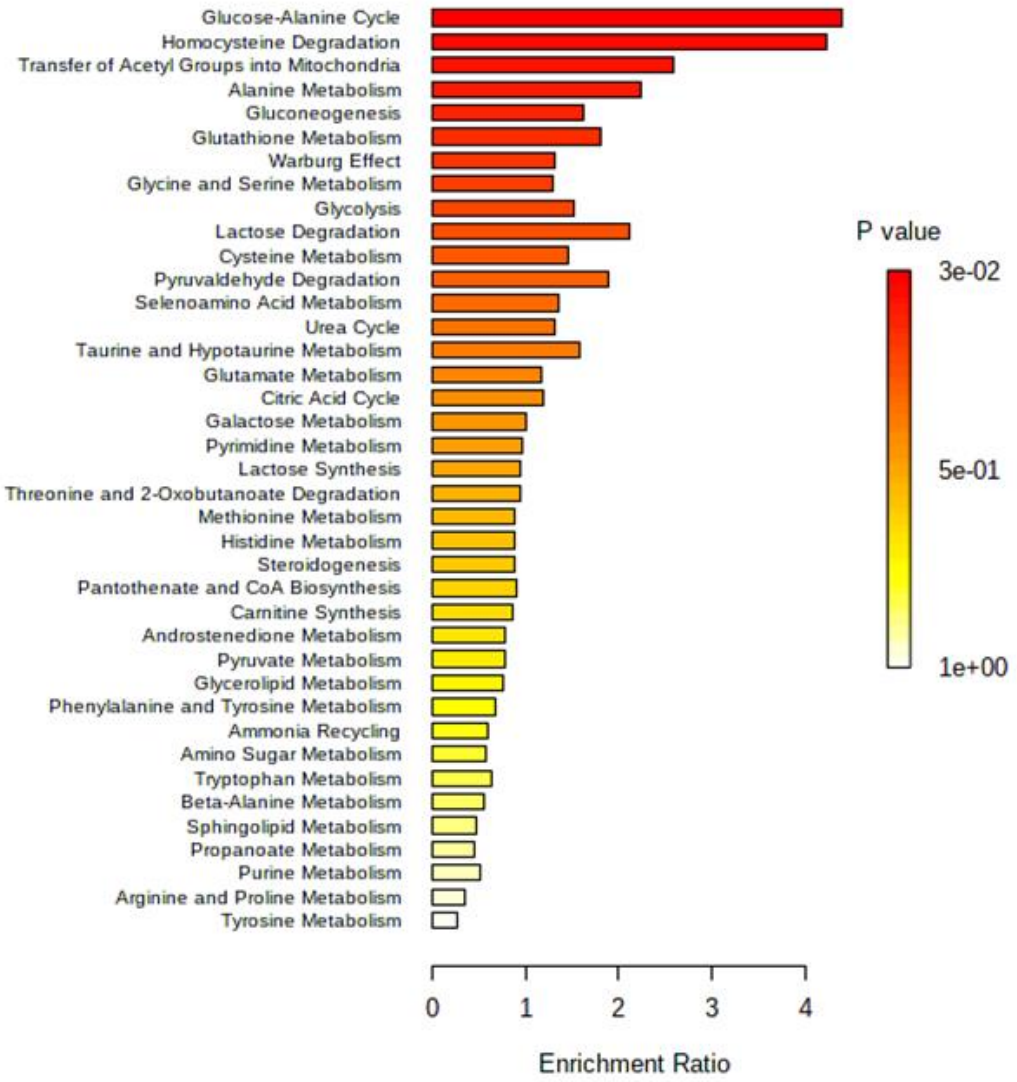

Figure 3.24: Overview of the results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the intervillous blood of the ECS group and intervillous blood of the SL group, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

Table 3.26 and Figure 3.25 show the results of the ORA enrichment analysis performed using the MetaboloAnalyst software for the 50 metabolites which were significantly different (p≤0.05) between the IV of the ECS and IOL groups.

Table 3.26: Results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the intervillous blood of the ECS group and intervillous blood of the IOL group, where total is the total number of metabolites in the pathway according to the MetaboAnalyst database, expected is the number of metabolites that would be expected by chance, hit is the number of metabolites observed in the current study, raw p is the original p value calculated from the pathway analysis, and Holm p is the p value adjusted for multiple testing using the Holm-Bonferroni method.

Metabolite pathways	Total	Expected	Hits	Raw p	Holm p
Urea Cycle	29	1.08	3	0.0886	1
Glutamate Metabolism	49	1.82	4	0.103	1
Arginine and Proline Metabolism	53	1.97	4	0.128	1
Homocysteine Degradation	9	0.334	1	0.289	1
Malate-Aspartate Shuttle	10	0.371	1	0.316	1
Pyruvaldehyde Degradation	10	0.371	1	0.316	1
Ammonia Recycling	32	1.19	2	0.335	1
Amino Sugar Metabolism	33	1.22	2	0.349	1
Aspartate Metabolism	35	1.3	2	0.376	1
Gluconeogenesis	35	1.3	2	0.376	1
Glucose-Alanine Cycle	13	0.482	1	0.39	1
Alanine Metabolism	17	0.631	1	0.477	1
Spermidine and Spermine Biosynthesis	18	0.668	1	0.497	1
Alpha Linolenic Acid and Linoleic Acid Metabolism	19	0.705	1	0.516	1
Threonine and 2-Oxobutanoate Degradation	20	0.742	1	0.534	1
Pyruvate Metabolism	48	1.78	2	0.541	1
Transfer of Acetyl Groups into Mitochondria	22	0.816	1	0.569	1
Glycolysis	25	0.928	1	0.616	1
Cysteine Metabolism	26	0.965	1	0.631	1
Oxidation of Branched Chain Fatty Acids	26	0.965	1	0.631	1

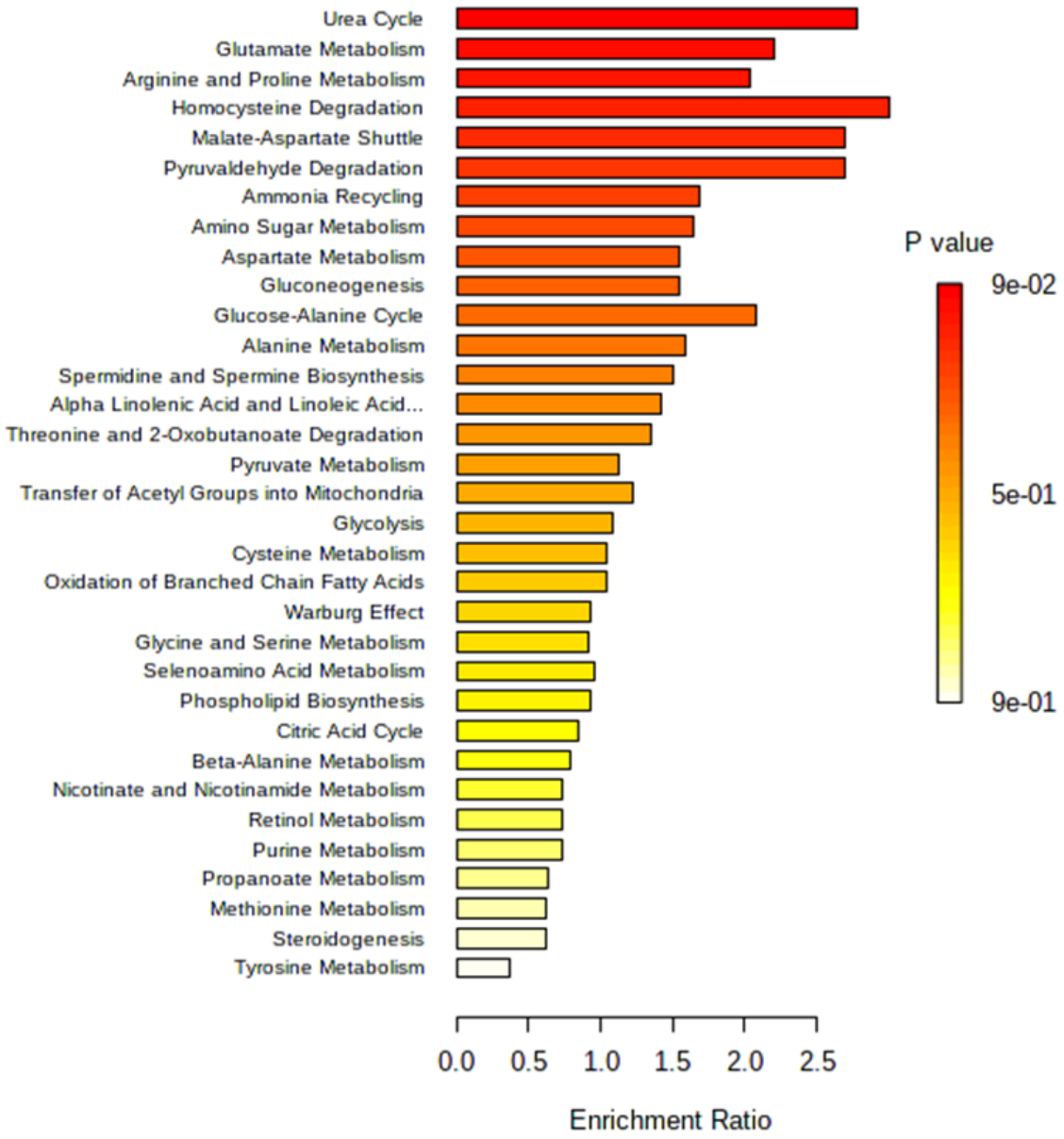

Figure 3.25: Overview of the results of the over-representation enrichment analysis for metabolites significantly different (p≤0.05) between the intervillous blood of the ECS group and intervillous blood of the IOL group, with the metabolite pathways on the y-axis, and the enrichment ratio (calculated by number of metabolites in the pathway identified by this study divided by the number of metabolites expected to be identified by chance in that pathway); the colour of the bar represents the unadjusted p value, as shown by the key.

3.5.22 Linoleic acid and arachidonate

Therefore, the ORA performed identified only one pathway as being significantly over-represented when adjusted for multiple testing, the alpha linolenic acid

and linoleic acid metabolism pathway, which was significantly over-represented in those metabolites significantly different between the CV of the IOL group and CV of the SL group (Table 3.21). Likewise, the raw-p indicated that this pathway was also over-represented between the CA of the ECS and CA of the SL group, and between the CA of the IOL group and the CA of the SL group, although these did not reach significance when adjusted for multiple testing. Linoleate is the conjugate base of linoleic acid. Figure 3.26 is a heat map illustrating the different concentrations of linoleate (18:2n6) found in the CA, CV and IV samples of the SL, IOL and ECS groups. Figure 3.26 also presents those concentrations for the metabolite arachidonate (20:4n6), which was significantly 2.46-fold lower in the CV of the SL group than the CV of the IOL group, and 2.42-fold greater in the CV of the elective CS group than the CV of the SL group, although this was not significant.

	Arachidonate (20:4n6)			Linoleate (18:2n6)		
Sample	**SL**	**IOL**	**ECS**	**SL**	**IOL**	**ECS**
Latent/preoperative	1.2	1.5	0.8	2.2	2	1
Intervillous	6.6	4.4	6.6	2.3	2.3	2.4
Cord Vein	0.6	1.6	1.6	0.2	1	0.5
Cord Artery	1.8	1.1	0.8	1.1	0.4	0.2

Figure 3.26: Heat maps comparing scaled normalised values of arachidonate (20:4n6) and linoleate (18:2n6) concentrations in the latent/preoperative, intervillous, cord artery, and cord vein samples between the SL, IOL, and ECS groups: n=4 in each group; heat map coloured according to scale from blue (lowest) to white (middle) to red (highest); SL = spontaneously labouring; IOL = induction of labour; ECS = elective caesarean section.

3.6 Discussion

In this timely and original study, several important groups of metabolites were found to be correlated with gestational age at delivery; and there were also relationships between the different vascular compartments at delivery, and between different modes of labour and delivery. Given that relatively few detailed clinical studies of this type have been performed in humans, this global analysis of metabolites at the multiple time-points sampled provide valuable information and insights into the trigger for labour in humans. These can be taken forward into future clinical studies. It is acknowledged that the sample sizes in this metabolomic study are small, especially compared with the numbers of women in the Born in Bradford cohort of Chapter 4. In particular, increased confidence in the correlations calculated for the samples taken at 28 weeks' gestation would be gained from inclusion of more women who delivered before 34 weeks' gestation. However, given the limitations of cost and resources for recruitment of women, this study is important as a hypothesis-free global assessment of metabolites during pregnancy, at delivery, and between the different modes of labour; taking advantage of the increased number of metabolites measured with the MS metabolomics platform compared with NMR metabolomics. For example, the separate sampling of the CA and CV of the SL, IOL and ECS groups has shown the pattern of concentrations of progesterone

and oestriol of the IOL group are closer to the ECS concentrations than the SL group, despite the physical event of labour. This could point to inherent differences between spontaneous and induced labour. In summary, this study has successfully built upon the study presented in Chapter 2 and has identified metabolites that can be taken forward in further studies, providing a rationale for the design of clinical protocols in future research. The major findings will now be discussed. Results from part 1 and part 2 will be discussed together for groups of metabolites to gain a fuller understanding as to the findings for individual and groups of metabolites both during pregnancy and at delivery.

3.6.1 Endocannabinoids

The previous study presented in Chapter 2 found that the endocannabinoids OEA, PEA, LEA and AEA significantly increased with labour in cord blood, and that PEA significantly increased and N-palmitoylserine decreased in the IV blood of labouring women. The subsequent hypothesis generated was that the trigger for human labour may involve an increase in endocannabinoid concentrations that stimulates production of ceramides and sphingolipids, which in turn stimulates production of prostaglandins and labour. It was suggested that these endocannabinoids may originate in the fetal circulation and associated with a decrease in lymphocyte production of the enzyme that catabolises endocannabinoids, fatty acid amide hydrolase (FAAH). The present study was in

part designed to test this hypothesis, and the findings related to these metabolites will be discussed first.

3.6.1.1 N-palmitoyltaurine and N-stearoyltaurine

While concentrations of most of the endocannabinoids measured tended to rise during pregnancy, N-stearoyltaurine and N-palmitoyltaurine were negatively correlated with gestational age at delivery. N-palmitoyltaurine was also significantly higher in the CA of the SL group when compared with ECS group. N-stearoyltaurine was significantly higher in the CA of the SL group than both the IOL and ECS groups. For both metabolites, these findings would fit with the hypothesis of increased endocannabinoid levels being associated with earlier gestational age at spontaneous labour, and increased levels produced by the fetus at delivery following spontaneous labour at term compared with no labour (ECS) or induced labour. As with other metabolites that will be discussed in this section, it is interesting that there are differences in metabolite levels in the CA between the SL and IOL group, given that both groups underwent the physiological stress of labour. These changes could point towards the inherent changes that occur with the trigger for spontaneous labour, and in particular fetal-derived metabolites.

N-palmitoyltaurine and N-stearoyltaurine are N-acyl taurines. N-acyl taurines are composed of fatty acids conjugated to amino acids and are regulated by the

enzyme FAAH[190]. At present relatively little is known about the physiological role of these molecules in human physiology. Members of the N-acyl taurine family have been shown to activate transient receptor potential vanilloid type 1 (TRPV1), which is a non-selective ligand-gated cation channel. Activation of TRPV1 by N-acyl taurines results in calcium ion influx and membrane depolarisation and secretion of insulin[191, 192]. In pancreatic β-cell lines *in vitro* such activation stimulates insulin secretion[192], and accumulation of N-palmitoyltaurine was found to be associated with dysfunction of islet of Langerhans cells[193]. AEA has also been found to be an agonist at TRPV1 in Human Embryonic Kidney 293 cells (HEK293 cell line) and *Xenopus* oocytes *in vitro*[194, 195], although this effect was not replicated in the human myometrial smooth cell-line ULTR *in vitro* [196]. While it is relatively early days in the investigation of the N-acyl taurines in human pregnancy, both metabolites may be involved in the pathway for spontaneous labour, and may be produced by the fetus, and warrant further investigation in a larger study. This could include investigation of a potential role in calcium ion regulation with contraction, including with myometrial organ baths.

3.6.1.2 Anandamide

While AEA was detected in the samples of Chapter 2, AEA was not detected in any of the samples in the present study and it is unclear why given that the

samples were processed in the same manner as previously. AEA is broken down by FAAH into arachidonate and ethanolamine[197] which results in AEA having a short half-life. Of note, arachidonate was significantly increased between 28 weeks' gestation and latent phase and was also significantly lower in the maternal blood taken in pre-operative clinic for the ECS group compared with the samples taken in latent phase for the SL group. Arachidonate was also significantly higher in the CV than CA of women who had IOL, and significantly lower in the CV and CA than IV samples in all three modes of labour onset. Arachidonate was significantly higher in the CV of the IOL group than the SL group. While this certainly does not prove that AEA is involved, it is possible that these changes in arachidonate were in part a result of rapid AEA breakdown by FAAH.

3.6.1.3 OEA, PEA and LEA

OEA was significantly increased in the cord blood of labouring women compared with no-labour in the pilot study. In the present study, OEA concentrations increased significantly between 28- and 34-weeks' gestation, pregnancy, but there was no correlation found between OEA levels and gestational age at spontaneous labour. There were no differences in the levels of OEA between the three labour types, which suggests that it is not directly involved in the pathway for labour but rather it generally increased at term and is found at greater levels

in the maternal circulation (IV) than in the fetal/placental circulation (CV and CA). Thus, OEA increases generally in pregnancy, but it is unlikely to be directly involved in the trigger for human labour.

In Chapter 2, PEA was significantly increased in the IV and cord blood with labour compared with no labour. In the current study, PEA had a similar patter to OEA, and was significantly increased in latent phase when compared with both 28 weeks' gestation and 34 weeks' gestation. Levels in the IV were consistently higher than that of the CV samples for all three labour onset groups, although only significant with SL and ECS. However, unlike OEA, PEA was significantly higher in the latent phase samples of the SL group when compared with the pre-operative samples for the ECS group, and it was also higher in the CA of the SL group compared with the ECS group, although this was not significant. Accordingly, PEA is potentially involved in the trigger for human labour. The higher concentrations measured in the IV samples compared with CV for all three groups indicate that any increase originates in the mother rather than the placenta as had been suggested by the pilot study. The differences with the findings of the pilot study may be because the cord blood was not separated into venous and arterial.

OEA and PEA are fatty acid ethanolamides, classified as endocannabinoids by Metabolon, Inc., and are hydrolysed by FAAH. OEA and PEA are agonists for the

peroxisome proliferator-activated receptor-α (PPAR- α) nuclear receptor. OEA does not bind to the cannabinoid receptors, and it has been shown that many of the physiological responses of OEA, such as lipolysis and anti-inflammatory effects are mediated via PPAR-α[198, 199]. PEA has similar anti-inflammatory actions via activation of PPAR-α[199-201]. Cells such as macrophages express phospholipase D (PLD) which promotes the release of OEA and PEA from N-acyl-phosphatidylethanolamine, a cell membrane phospholipid[202]. PPARs are a nuclear receptor family with α, β and γ isoforms, and ligand activation promotes target gene transcription. These heterodimerise with retinoid X receptor and bind to sequences in the DNA called PPAR response elements. Transactivation of PPARs is believed to be modulated by recruitment of regulator proteins which bind to a further site on the PPAR molecules. PPARs transcribe genes related to metabolism, inflammation and cell differentiation, and PPARα has been investigated in inflammatory pathways and fatty acid catabolism regulation [200]. Of note, peroxisomal proliferator-activated receptor A interacting complex 285 (PPAR-α interacting complex 285) at least halved between the pre-contraction and relaxed contracting state of myometrium (REL/PRE) in the upcoming Chapter 5, PPAR-α interacting complex 285 functions as a coactivator of PPAR-α[203]. Given the finding of increased PEA in the spontaneous labour latent phase compared with the no labour group, and the change in the PPAR-α interacting

complex 285 in the proteomics study, PEA may be involved in the spontaneous onset of labour but is likely to have a different impact to AEA.

In Chapter 2, concentrations of LEA, another endocannabinoid, were similar between the IV and cord blood. In this study LEA was found to be in higher concentrations as pregnancy progressed; and was higher in the latent phase samples of the SL group than the pre-operative samples of the ECS group. Therefore, as with PEA, it is possible that LEA is associated with the trigger for labour and this warrants further investigation.

3.6.2 Sphingolipids and ceramides

In the present study, a number of sphingomyelins were significantly lower in the CA and CV than the IV samples in both the SL and ECS group. This indicates that higher concentrations of sphingomyelins are found in the maternal circulation rather than the fetal or placental circulations, and this fits with the original hypothesis where an increase in endocannabinoids, potentially derived from the fetus, resulted in increased sphingolipids and ceramides in the maternal blood.

Sphingolipids are a lipid family of molecules which are made up of a long-chain aminediol base N-linked to a fatty acid via an amide bond. The most common base is sphingosine, which is a bioactive lipid in-its-own right. Family members of the sphingolipid group include ceramides, sphingomyelins, cerebrosides and glycosphingolipids[204]. Sphingolipids exist within the plasma membrane and have

both structural and lipid mediator roles, and sphingomyelin is the most common in the outer plasma membrane layer of eukaryotic cells[204]. Ceramides are both precursors and breakdown products of sphingomyelins, and are important for metabolic signalling[204]. Sphingolipid metabolites have been shown to be involved in both mitogenic and apoptotic processes, and ceramides play a role in the regulation of apoptosis stimulated by TNFα and Fas ligand, as well as ionising radiation[205]. Ceramides are also known to regulate the content of diacylglycerol and platelet activating factor[204].

As discussed in Chapter 2, ceramides can be produced either *de novo* from the endoplasmic reticulum or via the breakdown of sphingolipids and are present in lysosomes. Lysosomal ceramide concentrations are dynamically balanced between ceramide generation via hydrolysis of sphingomyelin through sphingomyelinases, and their breakdown catalysed by ceramidases such as N-acylsphingosine amidohydrolase 1 (ASAH1) producing sphingosine and fatty acyl-CoA[177, 206]. Sphingosine can then be further phosphorylated by sphingosine kinase to produce sphingosine 1-phosphate[206], and sphingosine 1-phosphate can be broken down by pyridoxal phosphate-dependent lyase on the endoplasmic reticulum (ER)[205].

In the present study, only one form of ceramide was detected, ceramide (d18:1/14:0, d16:1/16:0), whereas in Chapter 2 four ceramides were detected,

all of which were significantly increased in the IV blood of the women who delivered vaginally. In the present study, ceramide (d18:1/14:0, d16:1/16:0) was significantly higher in the IV blood than in the CV or CA samples with all modes of labour onset. However, there were no significant changes between modes of labour onset in concentrations of this ceramide. This may be because there is no effect, or it may be that the study size did not give the study statistical power to detect these differences, as the non-significant fold change between concentrations of ceramide (d18:1/14:0, d16:1/16:0) in the CA of the IOL group and the SL group, and the CA of the ECS group and the SL group were both 0.66 (non-significant).

Other ceramides that were detected included N-palmitoyl (d18:1/16:0) and N-stearoyl (d18:1/18:0). Again, there were no significant changes detected between the mode of labour onset groups, however the concentration of both in the CV of the SL group were approximately double that of the CV of the IOL and the ECS group. Therefore, in a larger study these ceramides may be found to be significantly changed with spontaneous labour. In a previous study, concentrations of N-palmitoyl (d18:1/16:0) and N-stearoyl (d18:1/18:0) were higher in the placenta of women who had pre-eclampsia when compared with the placenta of age-matched women who had preterm labour (controls), and N-

stearoyl was also increased in the maternal plasma of the group with pre-eclampsia[207]. These ceramides warrant further study.

3.6.2.1 Sphingosine and sphingosine 1-phosphate

Ceramide and sphingosine 1-phosphate play essential but often opposing roles in cellular signalling and metabolism. Whereas ceramide is involved in apoptosis and stress-related responses, sphingosine 1-phosphate stimulates proliferation, cell survival and tissue regeneration. Consequently, the balance between the two is vital for cell fate decisions[206], and altered ceramide concentrations have been implicated in pathologies such as cardiovascular diseases[208] and Alzheimer's disease[209]. Moreover, mitogenesis has been shown to be regulated by sphingosine 1-phosphate in cell lines *in vitro* such as airway smooth muscle. In cell culture of arterial and smooth muscle cells, mitogenic stimuli produce a rapid transient increase in sphingosine 1-phosphate, which is more usually present in low levels[205].

An important downstream effect of sphingosine 1-phosphate is Ca^{2+} regulation, thought to involve Ca^{2+} stores within the endoplasmic reticulum through a pathway independent of inositol triphosphate. In smooth muscle cells, Ca^{2+} mobilised via sphingosine 1-phosphate activation promotes an increase in cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) activation (a different response to that in other cells), and this results in disassembly of actin

and changed cell motility. PKC activates sphingosine kinase which increases levels of sphingosine 1-phosphate [205]. Drugs which increase cAMP temporarily suppress myometrial contractions, but this effect wears off after approximately 24-48 hours[210].

In the present study, both sphingosine and sphingosine 1-phosphate concentrations were greater in the CA of the SL and the IOL groups than the ECS group. This suggests that these metabolites are elevated with labour and may be derived from the fetus, particularly as no such differences were detected in maternal blood samples during pregnancy or in latent phase. Sphingosine concentration was greater in the CV of the SL group than of both the IOL and ECS group which could indicate that the placenta produces more sphingosine with spontaneous labour than with induction of labour. In addition, sphingosine was lower in the CA than the IV samples in the SL group and the ECS group; while sphingosine 1-phophate was higher in the CA than the IV in the SL group and the ECS group, and higher in the CV than the IV in the SL group.

As discussed, sphingosine 1-phosphate can mobilise Ca^{2+} stores[211], and this has been observed to increase cAMP[205]. In the present study, the higher concentrations of sphingosine and sphingosine 1-phosphate observed with labour matched with corresponding increases in cAMP with labour, and cAMP was significantly higher in the latent phase of the SL group than the pre-

operative sample of the ECS group. cAMP was also significantly higher in the CV of the SL group than both the IOL and ECS group. Moreover, cAMP levels were lower in the CV than CA of the IOL and ECS group, whereas the reverse was true for the SL group where concentrations were nearly 3-fold higher in the CV than CA. Further, the concentration of cAMP was more than double in the IV compared with the CV concentrations for the IOL and ECS group, with no change in the SL group.

In summary, these findings could be a reflection of increased placental production of cAMP with spontaneous labour, potentially as a result of increased phosphorylation of sphingosine to sphingosine 1-phosphate by the fetus (as sphingosine was significantly lower in both the CA and CV than the IV in the SL group), that is not observed with induction of labour and no labour. Given sphingosine, sphingosine 1-phosphate and cAMP can affect Ca^{2+} homeostasis and actin assembly, and given the importance of Ca^{2+} in the contraction of myocytes, it is possible that these observed changes represent signals from the fetus with spontaneous labour that stimulate myometrial smooth muscle contractions. However, this is making a lot of assumptions, and further studies would be needed to investigate this, especially the balance between the contractile effects of increased Ca^{2+} and the relaxatory effects of increased cAMP[56].

Bringing this altogether and relating to the hypothesis generated in Chapter 2, these results support the findings that changes in endocannabinoids and sphingolipids appear to be associated with spontaneous labour in humans, including N-stearoyltaurine and N-palmitoyltaurine having a negative correlation with gestational age at delivery, and PEA found in higher concentrations in latent phase of the spontaneous labour group than the ECS group. However, with the cord blood now split into cord artery and cord vein (compared with mixed cord blood in Chapter 2), the hypothesis generated from Chapter 2 that the differences in endocannabinoid production comes from the fetus are not supported. Instead, these changes seem to be more obvious in the maternal blood, although it is still possible that uterine lymphocytes are involved in these changes and warrant further investigation.

3.6.3 Progesterone

There were no significant differences in progestin or oestrogen steroids in latent phase/pre-operative samples between the three groups, which fits with the previous findings that there is no obvious measurable progesterone withdrawal in the maternal blood at the time of labour. Further, for all modes of labour onset and delivery there was consistently higher progesterone in the IV samples compared with the cord blood samples, which is consistent with previous findings of high levels of maternal progesterone at the time of labour. However,

there were differences between the concentrations in the CA and CV of the different modes of labour onset which could give further clues as to the spontaneous onset of labour in humans, and these will be discussed now.

For the IOL group and the ECS group there was more than double the concentration of progesterone in the CV than the CA, as would be expected given the high production of progesterone from the placenta. In contrast, this was found to be reversed in the SL group, with the CV having 0.29-fold the concentration of progesterone as the CA. Further, the CV of the IOL group had 4.27-fold higher mean concentration of progesterone than the SL group, and the CV of the ECS group had nearly double the mean concentration of the SL group. It is not clear what this means, but the lower progesterone in the CV of the SL group than the IOL group could represent a decrease in progesterone production by the placenta associated with spontaneous labour. Further, the progesterone in the CA of the SL group was more than double that of the IOL and ECS groups, therefore there could be a switch in the progesterone production so that the fetus increased production and the placenta decreased production. This warrants more investigation in larger studies.

Previous experimental studies have shown that the action of progesterone on the myometrium include relaxation, inhibition of gap junction formation and blockage of action of oxytocin[212]. One group used radioimmunoassay to

investigate progesterone levels of 67 women delivered with elective or emergency caesarean section. Blood samples were taken from the CA, CV and a peripheral maternal blood sample. The researchers found maternal progesterone levels at delivery were similar whether or not the woman was in labour, and the only differences in the CV progesterone level was a decrease in women with an emergency CS for fetal distress, regardless of whether the CS was elective or emergency (in labour)[70]. Another group found that serum progesterone concentration in the CV was significantly higher in babies born by emergency CS and spontaneous vaginal delivery than ECS and concluded that the fetus produces higher levels of progesterone due to stress in these situations. There were no differences in the maternal serum progesterone levels between the three groups investigated[213]. In the present study, the concentration of progesterone was higher in the CV of the IOL group than the CV of both the spontaneously labouring and the ECS group, and the concentration in the CV of the ECS group was non-significantly greater than in the CV of the SL group. This does not fit with the previous studies as the IOL group and SL group had similar vaginal deliveries, and the ECS deliveries would be presumed to be under the least stress. While it could be argued that the IOL group had a more stressful labour given three of the women had oxytocin infusion to promote contractions as part of the labour induction, it might be surprising to see such a difference

given all of the fetuses were delivered vaginally. The ECS group also had higher progesterone in the CV than the SL group, and this group would have been considered to have the least stress. These observed differences may be because in the present study progesterone was detected on a MS metabolomics platform rather than by radioimmunoassay (which potentially opens to cross-reaction by other interfering steroids).

A further study examined differences in progesterone concentrations between the CV and CA in women who delivered via ECS, and found that the CV concentration was consistently higher than in the CA[69]. This is the same finding as in this present study, with both the ECS and IOL group having higher progesterone concentrations in the CV than CA, and only the SL group having a higher concentration in the CA than CV. The authors found no correlation with progesterone levels in the retroplacental blood, and concluded that progesterone is secreted by the placenta, independent of the mother and fetus[69].

In summary, the shift in the balance in progesterone ratio of the CA and CV between the SL group and the IOL and ECS groups could potentially represent a reduction in progesterone production by the placenta with spontaneous labour, which could be perceived as a progesterone withdrawal by the myometrium. However, a more detailed study would be required to answer this question.

3.6.3.1 17α-hydroxyprogesterone

17α-hydroxyprogesterone is a progesten steroid that is related to progesterone and is a weaker agonist than progesterone at the progesterone receptor. It is produced primarily by the adrenal glands, including the fetal adrenal glands, and also by the ovary and corpus luteum during pregnancy. It is converted from progesterone by the enzyme 17α-hydroxylase; and 21-hydroxylase enzyme, also known as CYP21A2, subsequently metabolises it to 11-deoxycortisol, which is then converted to cortisol. Although not directly related to the present thesis, it is worth noting that 21-hydroxylase deficiency is the most common congenital adrenal hyperplasia, in which 17 α-hydroxyprogesterone levels are elevated, and which results in elevated ACTH levels leading to adrenal hyperplasia and an increase in androgenic hormones which can cause virilisation in females[214, 215].

A previous study investigating maternal 17α-hydroxyprogesterone concentrations in peripheral blood observed an initial rise in early pregnancy, thought to indicate production of progesterone by the corpus luteum. Levels of 17α-hydroxyprogesterone then returned to baseline as the placenta takes over progesterone production, indicating that the placenta has reduced 17α-hydroxylase activity. Levels of 17α-hydroxyprogesterone were then found to increase again at approximately 32 weeks' gestation, thought to be derived from the fetus[216]. A further study investigated steroid concentrations in the CV of very

preterm, preterm and term infants, and found that 17α-hydroxyprogesterone was reduced in the very preterm and preterm babies compared with term, and showed that the concentrations strongly correlated with gestational age at delivery. The authors acknowledge that this may have been secondary to maternal steroid administration prior to delivery of the preterm babies, as is routinely administered to stimulate lung maturation. 17α-hydroxyprogesterone levels were later found to have risen in the preterm neonates on day 3 of life [64].

Interestingly, in the present study the differences in mean concentrations for progesterone discussed above were similar for 17α-hydroxyprogesterone concentrations, which were higher in the CV than CA for both the IOL and ECS group, but lower in the CV than CA for the SL group. Accordingly, the concentration of 17α-hydroxyprogesterone in the CA of the SL group was higher than that for the ECS group, and also the concentration in the CA of the IOL group was higher than that in the ECS group. Conversely, the concentration in the CV was far greater in the IOL group than the CV of the SL group, and higher in the CV of the ECS group than the SL group. This could indicate a major change in production at the time of spontaneous labour that is not replicated with current induction of labour methods.

As discussed in Chapter 1, progesterone has been administered to women with the hope that it may reduce the risk of miscarriage or preterm birth. Large trials

now suggest that progesterone pessaries per vagina may be beneficial in women with three previous miscarriages and who have bleeding in early pregnancy[123], however this treatment does not appear to reduce the risk in other situations[122-125]. Weekly intramuscular injection of 17α hydroxyprogesterone caproate, a synthetic progestogen (the caproate molecule is not made in humans), has also been administered to women in an attempt to reduce the risk of preterm birth, and the efficacy of this is debated[212,217]. A randomised controlled trial (RCT) involving women who had a history of preterm birth showed that weekly injections with 17α-hydroxyprogesterone caproate (a synthetic progestogen) or placebo, found that treatment reduced the rate of preterm births compared with placebo[212]. In a study designed to investigate why 17α-hydroxyprogesterone may have this effect, direct application of 17α-hydroxyprogesterone caproate to human myometrium in an organ bath did not cause myometrial relaxation. The authors suggested that any relaxation effect 17α-hydroxyprogesterone caproate may have on the myometrium could be through altered gene expression[218]. Another study investigating the effect of progesterone and 17α-hydroxyprogesterone caproate on human myometrium in organ bath found that while progesterone did have a relaxing effect, 17α-hydroxyprogesterone caproate did not, and in fact at higher doses was associated with contractility of the myometrium between 1 to 3 hours after

administration[219]. In a further study, researchers investigating the effect of 17α-hydroxyprogesterone caproate on term not in labour myometrium found that it did not stimulate Cox-2 or TNFα production[220]. There are also reports that administration of 17α-hydroxyprogeterone caproate may confer embryo or fetal toxicity, particularly in early pregnancy[221], and others warn that the addition of the caproate means that this drug should not be assumed to be the same as 17α hydroxyprogesterone or progesterone, as some have done[217].

Therefore, it is unclear what the effect, if any, the difference in 17α-hydroxyprogesterone CA/CV ratio between the SL group and the IOL and ECS group may have. It could have an effect similar to progesterone, or it may be that it is related to changes in cortisol production. While progesterone levels are similar in the mother regardless of mode of labour onset or delivery, there is a change in the ratio of progesterone and 17α-hydroxyprogesterone produced by the fetus (CA) and placenta (CV) that could be involved in the trigger for labour.

3.6.4 Oestrogens

There are four oestrogens present in human pregnancy: oestriol, oestradiol, oestrone and oestetrol, with oestriol and oestradiol dominating. All four of these oestrogens have been shown to increase during pregnancy. Oestriol is formed via DHEA-S, which is produced by the fetal adrenal gland upon stimulation of placental corticotrophin releasing hormone (CRH), as discussed in Chapter 1. In

contrast, oestradiol is produced from conversion of DHEA-S produced from DHEA-S from both the maternal and fetal adrenal glands[102]. There are two forms of the oestrogen receptor (ER), namely ERα and ERβ. ERα appears to be the receptor through which the uterotonic actions of oestrogen are driven. Recently the spliced ERα receptor variant ERΔ7 has been shown to dominantly suppress the uterotonic actions of oestrogen, and this receptor withdraws at term in an oestrogen-dependent manner. This mechanism is suggested to be involved in the loss of myometrial quiescence at term[101], and that changing concentrations of oestriol and oestradiol may be responsible for the change in oestrogen receptors[102].

Oestriol is an inhibitor of the action of oestradiol at low concentrations but becomes an agonist when the ratio of oestriol to oestradiol exceeds 10 to 1. One group investigated changes in the ratios of progesterone, oestriol and oestradiol in spontaneously labouring women at 26 weeks' gestation and in the last month of pregnancy. Oestriol was found to increase, resulting in an increase in the oestriol/oestradiol ratio, a decrease in the progesterone/oestriol ratio, and an unchanged progesterone/oestradiol ratio. The authors suggested that this created an oestrogenic environment for the onset of labour[103].

In the present study, mean concentrations of maternal oestriol did not significantly change during pregnancy. However, there were potentially

important differences in the ratio of oestrogen to progesterone between the different types of labouring groups, and in the different vascular compartments. This could therefore indicate different sites of production that may be related to the spontaneous onset of labour. For example, oestriol was 0.18-fold lower in the CA of the ECS group than the SL group, which would fit with the previously described increase in oestriol with spontaneous labour[102]. Oestriol concentrations followed a similar pattern to that of progesterone and were higher in the CA than the CV in the SL group, but higher in the CV than the CA for both the IOL and ECS groups. The mean concentration of oestriol in the CA of the SL group was higher than the mean concentration in both the ECS and IOL group, and the mean concentration of oestriol in the CV of the IOl group was over 6-fold greater than the CV of the SL group, and the concentration in the CV of the ECS group was over 4-fold greater than the CV of the SL. Concentrations in the IV and latent phase samples were similar between the groups. Therefore, this could reflect an increase in oestriol production by the fetus and reduction in oestriol by the placenta with spontaneous labour that does not occur with no labour, but importantly also does not occur with induction of labour. Although this needs to be tested in future studies, these changes are potentially important and could indicate that the change in progesterone and oestriol balance with spontaneous labour is due to changes in fetal production of these hormones.

Oestradiol was not detected in this study, although 17beta-oestradiol did not change between the different modes of labour onset.

3.6.5 Differences between the IOL and SL group

It is worth highlighting here the potential importance of the findings from this study of differences in the ratio of concentrations of some of the discussed metabolites in the CA and CV between the different modes of labour onset, and specifically differences between the IOL and SL groups. One of the potential criticisms of the study described in Chapter 2 is that the differences in metabolites between the labouring and non-labouring groups may just be because of the physiological stress of labour itself. However, the results from this study show that despite the apparently similar preceding events of physiological labour and vaginal delivery of the IOL and SL groups, metabolite levels in the CV and CA were different between these two groups. For example, the heatmap of Figure 3.13 A. shows that in the latent phase, progesterone, oestriol, 17α-hydroxyprogesterone, DHEA-S, sphingosine and sphingosine 1-phosphate were similar in the latent and IV samples between the SL, IOL, and ECS groups. However, Figure 1.13 B. is the heat map for the same metabolites in the CV and CA samples, which shows that the levels are similar for the IOL and ECS group, but that the SL group is different. These differences may have been missed previously because more commonly cord blood is measured as a single

entity, as with the study presented in Chapter 2, rather than separately sampling the CA and CV as in this study.

A previous study investigated the effect of IOL with Dinoprostone (prostaglandin PGE2 analogue as used in this study for induction of labour; trade name is Propess) on maternal serum progesterone, oestradiol and oestriol levels, measured with immunoassays in: women who responded to IOL and women who did not, women who spontaneously laboured, and women who had an ECS. Blood samples were taken at two time points: start of labour or on admission for ECS, and just before delivery. Oestrogen levels were found to be unchanged in all groups. Progesterone levels did not change during spontaneous labour but decreased from admission to delivery in those who had a successful IOL but not those who did not[222].

These results and the results from the present study support a hypothesis that there may be an inherent difference in the signals from the fetus with spontaneous labour compared with induction of labour, despite the intervening event of labour itself. And that with regards to these steroids in the present study, the concentrations in the CV and CA of the IOL group are more like the ECS group than the SL group. It could be interpreted that fetal production of oestriol and progesterone are associated with spontaneous labour but not with induction of labour.

In summary, these findings indicate that IOL labour is different to spontaneous labour and may be due to spontaneous labour resulting from signals from the fetus. Moreover, it could be that because IOL is exogenously imposed upon the mother that the signals that would originate in the fetus with spontaneous labour either do not occur or occur much later. This could in part explain why those with IOL are more likely to have complications such as failure to progress, and an improved understanding of these differences could lead to better targeted management of induction of labour[43].

3.6.6 Correlations with gestational age at delivery

Although this study was not designed to identify metabolites to predict spontaneous labour, there is value in identifying those metabolites that corelated with gestational age at delivery as these may point to the pathways involved in spontaneous labour. Those metabolites for which mean maternal concentrations were significantly correlated with gestational age at delivery will be discussed here.

3.6.6.1 Octadecanedioylcarnitine (C18-DC) and octadecenedioylcarnitine (C18:1-DC)

There is no information in the literature regarding the two metabolites octadecanedioylcarnitine (C18-DC) and octadecenedioylcarnitine (C18:1-DC), both of which were correlated with gestational age at delivery, in humans. These

metabolites have been detected in mouse faeces using MS metabolomics with Metabolon, Inc., following investigation of effect of bacteriophages on a human model of gut bacteria and metabolome[223]. Therefore, it is unclear at present what these metabolites represent other than a potential involvement with fatty acid metabolism.

3.6.6.2 Pregnanediol-3-glucuronide

Pregnanediol-3-glucuronide is a major metabolite of progesterone, and is excreted in the urine[224]. One group found that urinary measurement of pregnanediol-3-glucuronide was lower in ectopic pregnancies compared with ongoing intrauterine pregnancies, and suggested it could be used to predict ectopic pregnancies[225]. In the present study, increased serum pregnanediol-3-glucuronide was correlated with earlier gestational age at delivery at 28 weeks' gestation and 34 weeks' gestation. This could correspond to increased levels of progesterone, or increased levels of progesterone metabolism. It is interesting that mean concentrations of this metabolite were higher CA of the SL and IOL groups than the ECS group, as it could suggest that there is increased metabolism of progesterone within the fetus during labour.

3.6.6.3 17alpha-hydroxypregnanolone glucuronide

17alpha-hydroxypregnanolone glucuronide was significantly negatively correlated with gestational age at delivery at 34 weeks' gestation and latent

phase. It was also significantly lower in the IV blood of the SL group when compared with the ECS group, which would not necessarily be expected given the correlation results. Moreover, it was significantly lower in the CV than CA in the SL group, which could indicate that its source is fetal. The opposite was found for the ECS group, where concentration of 17alpha-hydroxypregnanolone glucuronide in the IV blood was almost double that in both the CV and CA , which could be a result of the ECS group having higher maternal levels than the SL group.

3.6.6.4 N,N,N-trimethyl-alanylproline betaine

N,N,N-trimethyl-alanylproline betaine (TMAP) was significantly negatively correlated with gestational age at delivery at 34 weeks' gestation and in latent phase, and was lower in the latent phase of the SL and IOL groups when compared with the pre-operative sample for the ECS group. TMAP was also significantly higher in the CV of the SL group compared with the ECS group. Thereby higher mean concentrations of TMAP was associated with earlier gestational age at delivery, and maternal levels were higher at term in women in latent phase than in women at term but not labouring. There is relatively little known about TMAP, however in a study involving untargeted metabolomics, TMAP was identified as a biomarker of reduced kidney function in people

undergoing dialysis[226]. It is possible that this metabolite is involved in vascular function, and it would warrant further investigation.

3.6.6.5 DHEA-S

In Part 2, DHEA-S levels were positively correlated with gestational age at delivery at all time points, although only significantly in latent phase. This could indicate that decreased levels are associated with an earlier gestational age at spontaneous labour. In addition, mean concentrations of DHEA-S were significantly decreased at 34 weeks' gestation compared with 28 weeks', and between latent phase and 28 weeks' gestation. It was also significantly increased in the CA of placenta taken from women who had an ECS compared with the levels in the maternal blood and was doubled in the CV of women compared with maternal blood in this group, although not significant. This was not the case for other deliveries.

This suggests that higher levels of DHEA-S in the maternal blood may be associated with later gestational age at delivery. It is not clear why the DHEA-S in the non-labouring women may be so much higher in the CA and CV than in the maternal blood, and that this difference isn't present in the labouring group, other than to say that in non-labouring fetuses the levels are significantly different to the maternal blood, but that this difference reduces during labour.

As mentioned in the introduction[74-76], in sheep the fetal hypothalamic-pituitary-adrenal (HPA) axis maturation is involved in the trigger for parturition, where increased fetal cortisol results in increased maternal oestrogen and resultant decreased progesterone and labour. In non-human primates, parturition without progesterone withdrawal is preceded by an increase in fetal DHEA-S. DHEA-S is a precursor to oestriol during pregnancy[65]. Oh *et al.* (2006)[65] measured cord cortisol and DHEA-S levels in utero and at preterm, term, labouring and non-labouring foetuses/neonates and found that changes in fetal cord cortisol levels did not change until 36 weeks' gestation, after which the levels increased towards term, and that active labour was significantly associated with an increase in cord cortisol. DHEA-S was found to decrease until 36 weeks' gestation, after which point it also increased.

In addition, 16alpha-hydroxy DHEA-3-sulfate, which was found to be reduced in the cord and IV blood of the labouring group compared with the non-labouring group in Chapter 2, had changes in the opposite direction to DHEA-S in the present study. This metabolite significantly increased between 28 weeks' gestation and 34 weeks' gestation, and again between 34 weeks' gestation and latent phase, although there was no correlation between maternal concentrations of 16alpha-hydroxy DHEA-3-sulfate and gestational age at

spontaneous delivery. In summary, DHEA-S is an important adrenal product and may reflect fetal adrenal gland maturation towards term.

3.6.7 Perfluorooctane sulfonate

Interestingly, perfluorooctane sulfonate (PFOS) was negatively correlated with gestational age at each time point. PFOS is a perfluorinated chemical that has been used in industry including hydraulic fluids in the aviation industry, and previously for coating for textiles, carpets and leather. It was banned in the European Union under the 2006/122/EC directive which came into force in 2008. Public Health England reported in 2009 that the general public are only exposed to trace levels of PFOS, but because of the potential risk to both humans and the environment it has been banned in the EU. Public Health England reported that PFOS does not appear to be mutagenic or a cancer risk at the levels the general public are exposed to[227]. The half-life of PFOS in human serum is approximately five years[228]. Perfluorooctanoate (PFOA) is another perfluorinated chemical[227] that was detected in the maternal and cord serum, but was not correlated with gestational age at delivery in this study. A population study in an area of the United States that is highly exposed to PFOA showed no association between serum PFOS (5262 pregnancies) levels or PFOA (1845 pregnancies) and self-reported miscarriage or preterm delivery in the five years prior to the sample measurements. Both PFOS and PFOA were weakly associated with pre-

eclampsia; and low birth weight was weakly associated with PFOS; and birth defects weakly associated with PFOA[229]. The issue with this study is that all outcomes were self-reported and therefore there may be inaccuracies. In addition, as all were from the high-exposure area, it may be that there was not enough comparison with people with low or normal levels.

There have been some reports that PFOS may affect oestrogen haemostasis in pregnancy[230]. Moreover, in cell culture of porcine ovarian theca and granulosa cells, PFOS was found to inhibit the steroids released by these cells in response to luteinising hormone and follicle stimulating hormone, respectively[231]. Progesterone and androstenedione secretion by theca cells in response to luteinising hormone was reduced in these cells[231]. The authors concluded that PFOS appears to act as an endocrine disruptor in ovarian cells[231]. Similar conclusions were drawn from a study in H295R cell culture (an angiotensin-II responsive steroid producing adrenocortical cell line), where exposure to PFOS increased supernatant oestradiol concentrations and decreased supernatant testosterone. Exposure to PFOS of zebra fish embryos resulted in an increased gene expression of *esr1* (for ERα) and a decrease in expression of *esr2b* (ERβ1)[232].

The present study was performed in Bristol, UK in 2018/2019. In a sub-study of 457 mother-son pairs within the Avon Longitudinal Study of Parents and Children (ALSPAC), also based in Bristol and surrounding areas, PFAS was

detected in all maternal pregnancy samples taken at around 30 weeks' gestation, and an inverse association between PFOS and birth weight, crown to heel length and head circumference was observed[233]. And in a similar study with ALSPAC of 447 mother-daughter pairs, an association was found between higher levels of PFOS at birth and lower birth weight, but with greater weight at 20 months of age[234].

In further analysis performed by the thesis author but not included in this thesis, 1000 women (from the Born in Bradford cohort study, described in Chapter 4) had MS metabolomics analysis performed on fasting blood samples collected during the second trimester. Unadjusted linear regression analysis showed that a 1 standard deviation increase in maternal blood mean PFOS concentration was associated with a 0.514 day reduction in mean gestational age at delivery (95% confidence interval: -1.014 to -0.015 p=0.044).

Thus, multiple lines of evidence from different studies in this thesis support an inverse association of PFOS with gestational age at delivery. This effect is still present despite production of PFOS being effectively banned by the EU over a decade ago and warrants further study.

3.6.8 Over-representation analysis

The over-representation analysis indicated that the alpha linolenic acid and linoleic acid metabolism pathway was significantly over-represented among

those metabolites which were significantly different between the CV of the IOL group and the CV of the SL group. Linoleate is the conjugate base of linoleic acid, and in this study linoleate was significantly higher in the latent phase samples of the SL group than the pre-operative samples of the ECS group. Further, linoleate was significantly higher in the CV than CA of the SL group, whereas for both the IOL and ECS groups it was significantly higher in the CV than the CA. Further, linoleate was significantly higher in the CA of the SL group than the CA of both the IOL and ECS groups, with no significant difference between the CA of the IOL and ECS groups.

Linoleic acid is a polyunsaturated fatty acid (PUFA) and has become the most common n-6 PUFA in the human diet over recent decades. Interestingly, following elongation and desaturation, it can produce arachidonic acid[235]. Arachidonic acid, as has been discussed previously, is a precursor to prostaglandins as well as other eicosanoid mediators such as leukotrienes and thromboxanes, which are associated with inflammatory processes[235]. One group investigated the effect of a high linoleic acid maternal diet in mice on the heart function of offspring, and specifically whether such a diet was associated with changes in circulating fatty acids and cardiac function. The researchers found that a high linoleic acid diet: decreased the circulating total- and HDL-cholesterol in female offspring but not in male offspring; in female and male offspring

decreased total plasma n-3 PUFA; and did affect overall cardiac function of the offspring[235]. In addition, in Chapter 4 of this thesis, an increase in 18:2 linoleic acid was associated with a shorter gestational length, although an increase in the ratio of 18:2 linoleic acid to total fatty acids was associated with a longer gestational length (Figures 4.7 and 4.8). When taken in the context of the previously conducted study, the former observation supports the hypothesis that there is an association between maternal dyslipidaemia and a shorter gestational age at spontaneous labour, which will be discussed in more detail in Chapter 4. Further, in a previous study involving the Born in Bradford cohort, linoleic acid was negatively associated with pre-eclampsia[236].

In addition, previous research has shown that linoleic acid intake is important for production of anandamide (AEA), as the production of AEA is dependent upon the metabolic derivatives of arachidonic acid in phospholipids. In a mouse study, the effect of a diet in which linoleic acid made up 1% of the energy intake was compared to the effect of a diet in which linoleic acid made up 8% of the energy intake. The 8% version was found to increase the tissue concentration of arachidonic acid, with an associated increase in AEA and diet-induced obesity[237]. Arachidonic acid is part of the arachidonate cascade, and arachidonate is formed following removal of a proton from the arachidonic acid carboxyl group[238]. In this study, arachidonate was lower in the CV of the SL group than the CV of the

IOL and ECS groups, and conversely higher in the CA of the SL group than the CA of the IOL and ECS groups. In addition, arachidonate increased between 28-weeks' gestation and in latent phase and was significantly lower in the pre-operative samples of the ECS group when compared with the samples from the latent phase in the SL group.

In summary, both arachidonate and linoleate were higher in the CA than CV in the SL group, but lower in the CA than CV in both the IOL and elective CS groups. This again shows differing ratios of metabolite concentrations between the CA and CV, and that this ratio for the IOL group is more alike the ECS group than the SL group, despite the intervening labour. In addition, both metabolites were higher in the latent phase of the SL and IOL group than the pre-operative sample of the ECS group, with similar levels in the intervillous blood. Therefore, this supports the idea suggested in section 3.6.1.2 that it may be that the elevated arachidonate is a result of the metabolism of AEA by FAAH in the CA, resulting in increased arachidonate, and that endocannabinoid activation, potentially from the fetus (CA in the SL group) may be involved in the trigger for human labour.

Regarding further findings of the ORA, although not significant, over-represented pathways for metabolites significantly correlated with gestational age at 28- and 34-weeks' gestation, and which were different between the SL

group and both the IOL and ECS groups in the CA and CV, included beta-oxidation of very long chain fatty acids, and fatty acid biosynthesis. This suggests that mobilisation of fatty acids is important for spontaneous labour and corresponds to the findings presented in Chapter 4 (discussed further in section 4.7.6).

3.7 Conclusions

This study has highlighted metabolites that correlate with gestational age at delivery and warrant further investigation. Importantly, by analysing the cord artery and cord vein separately, and between different modes of labour onset, the results of this study indicate that there are differences in the ratio of metabolites in the cord vein and cord artery between women who spontaneously labour and women who have induced labour or elective caesarean section. This includes progesterone and oestriol and potentially endocannabinoid metabolism. This is interesting because it shows that despite the apparently similar physiological events of labour that women who spontaneously labour and women who have induced labour go through, there are persistent differences in the cord artery and cord vein.

It is possible that these results represent changes in the production of certain metabolites by the fetus, which may coincide with maturation of the fetal pituitary-adrenal axis, and may contribute to a change in the balance between

progesterone and oestrogen with spontaneous labour. These differences may also in part account for induced labour having higher risk for adverse pregnancy outcomes, including emergency caesarean section and instrumental delivery, because without the signals from the fetus the myometrium may not contract as efficiently. These findings warrant further investigation.

3.8 Limitations

Limitations for this study include that the numbers are relatively small, and larger studies are required to confirm the hypotheses discussed. In addition, most of the blood tests were taken in a non-fasted state and this may have affected results. However, despite this, the strengths of this study include that because the numbers are small, detailed information was known about the women and the pregnancies, and samples were taken at multiple time points. In addition, despite the small numbers, the relatively tight 95% confidence intervals suggest that this is a useful method for comparing levels of metabolites in pregnancy, and that metabolite levels are consistent between the groups studied. There is also the potential to use bioinformatic pipelines to extract more information from the data presented in this thesis.

CHAPTER 4. MATERNAL METABOLIC PROFILE IN THE SECOND TRIMESTER AND GESTATIONAL AGE AT DELIVERY: FINDINGS FROM THE BORN IN BRADFORD COHORT STUDY

4.1 Background

While smaller clinical cohort studies, such as those of the previous three chapters of this thesis, can be designed to investigate specific pathways at specific time points during pregnancy, the size of these studies can limit statistical power and generalisation of results to other settings. On the other hand, epidemiological studies which incorporate data from large population-based cohorts can maximise statistical power and provide information that can potentially be more relevant to wider populations. Triangulation is a methodology which advocates approaching a question from multiple angles using different methodologies with contrasting biases, rather than depending upon a single method[20]. If these different methodologies give consistent results, this improves confidence in the validity of the results. In the previous chapters of this thesis, findings from studies using mass spectrometry (MS) metabolomic profile analysis of repeat sampling at different time points in pregnancy, including during labour and immediately following birth, have been presented. This chapter complements those by using an epidemiological approach to address the over-riding aim of this thesis that is improving understanding of the putative mechanisms involved in spontaneous labour in humans. Specifically,

this study explores the association between a gestational Nuclear Magnetic Resonance (NMR) serum determined metabolomics profile and gestational length in a prospective birth cohort. Data from the Born in Bradford (BiB) cohort has been analysed, in which fasting serum samples were collected as part of an oral glucose tolerance test (OGTT) for gestational diabetes at 26-28 weeks of gestation. BiB is a large multi-ethnic cohort, and 7440 women (see methodology section and Figure 4.1 for those pregnancies excluded) were included in the analyses presented in this chapter.

4.2 Maternal metabolic traits and potential link to gestational age of delivery

Previous studies have investigated maternal and cord metabolomic profiles and potential association with conditions such as preterm birth and small for gestational age. One group performed a retrospective case-control study comparing plasma sampled from women who delivered preterm (between 22 weeks and 36 weeks gestation) (n=57) or at term (between 38 and 41 weeks) (n=25), as well as women with threatened preterm labour who went on to deliver at term (n=49). Plasma samples were taken within 7 days of delivery for the preterm group, and during labour in the threated preterm and term delivery groups. Blood was centrifuged and plasma stored at -80 degrees Celsius until metabolomics analysis using liquid chromatography quadrupole time of flight mass spectrometry. This group found that fatty acids were the metabolites that

were most differentiated between the three groups, and the authors concluded that there were differences in the metabolome between women who delivered preterm and those who delivered at term. However, potential issues with this study design include that the samples were taken at different time points of labour, including up to seven days after delivery in the preterm group. These factors mean that any differences between the metabolomic profiles of the three groups are not necessarily related to gestational age at delivery[239]. Therefore, while these results are potentially interesting, and point towards differences in fatty acids, these findings require further testing. Another group performed a nested case-control study using LC-MS metabolomics to investigate the potential for obtaining biomarkers for prediction of spontaneous preterm birth in asymptomatic women at 15 and/or 20 weeks' gestation. 50 infants were delivered before 37 weeks' gestation, and 55 delivered after 41 weeks' gestation. Samples were collected during antenatal clinics and blood processed within 3 hours of collection and stored at -80degrees Celsius. Participants were enrolled in the Screening for Obstetric and Pregnancy Endpoints (SCOPE) study, based in Cork, Ireland. The authors found no significant differences between the two groups when using standard t-tests, even with an FDR cut-off of 0.1, which the authors put down to heterogeneity in the disease group. Instead, the authors ranked candidate biomarkers from multivariate and univariate analyses, and

developed a biomarker panel for future investigation which included bile acids, prostaglandins, vitamin D and fatty acids[240]. Issues with this study include the up to three hours between sample taking and processing, during which time fast-acting metabolites may metabolise, and the highlighted heterogeneity in the participants. Further studies include a group which analysed maternal blood, cord artery and cord vein blood at the time of delivery for women delivering babies preterm (cases n=7) or term (n=8). These findings suggested enhanced β-oxidation of short- and medium-chain fatty acids in the preterm group[67]. Another group performed metabolomic analysis of newborn blood sampled for metabolic screening, and created a model that explained 52.8% of the variation in gestational age at delivery, that included 37 metabolite measurements[241]. There remains a gap in the literature regarding the pathways for spontaneous labour in humans, and the use of metabolomics in this area does not appear to have been utilised to its full potential as yet, particularly in larger cohort studies as with the BiB cohort.

4.3 Epidemiology and public health consequences of pre-term and post-term births

Approximately 7% of pregnancies deliver preterm (<37 completed weeks' gestation)[39]. Preterm birth is responsible for 16% of global deaths in children under five years of age[37], and approximately 35% of babies who are born

preterm are later diagnosed with cerebral palsy[41]. In contrast, current management options for pregnancies which are clinically recommended to be artificially shortened by inducing labour are frequently inefficient, with an increased risk for complications of labour including fetal distress, instrumental delivery, emergency caesarean section and post-partum haemorrhage [4, 43]. Induction rates in the UK are currently increasing, from 20% in 2006-7 to 29% in 2016-17 [45], and therefore there is need for improved management options.

While recently published metabolomics studies have pointed towards useful biomarkers for prediction of pathologies of pregnancy such as small for gestational age, gestational diabetes, and preterm labour [15, 16], such biomarkers may not reflect the relevant causal pathways[21, 22]. Therefore, in order to better understand the causal pathways, and to identify better targets for drug development, studies which investigate association and potential causal pathways are required, and hence the work presented in this thesis. This chapter will specifically investigate potential associations of maternal metabolites in the second trimester and gestational age at delivery in order to provide direction for future research in studies investigating causation.

4.4 Aim

The aim for this chapter was to assess potential associations between the maternal circulating metabolites measured in the second trimester for women

participating in the BiB study and gestational age at delivery (continuous variable, preterm and post-term birth).

4.5 Methodology

4.5.1 Data source

The BiB cohort was set up in 2007 to investigate the effect of socioeconomic, behavioural, genetic and nutritional factors, on childhood development and health within a deprived multi-ethnic population [242]. Recruitment took place between March 2007 and November 2011. At the time of recruitment, Bradford was the sixth largest city in the UK, with high levels of deprivation, and the maternity unit had 6000 deliveries per year. Because of their background, many women in Bradford are considered at high risk for development of gestational diabetes. Therefore, all women in Bradford, except those with known diabetes, are offered a second trimester fasting oral glucose tolerance test (OGTT) between 26- and 28-weeks' gestation. Women who had their pregnancies booked in Bradford were invited to participate in the BiB cohort at the time of their OGTT and over 80% consented[242]. Overall, 12 453 women with 13 776 pregnancies were recruited to the study; and 12 331 pregnancies (89% of total pregnancies) completed the OGTT and had stored fasting samples which were subsequently used for the NMR metabolomics profiling used in this chapter.

4.5.2 Ethics and informed consent

All participants gave written informed consent. Ethical approval for the BiB data collection was provided by Bradford Research Ethics Committee (Ref 07/H1302/112).

4.5.3 Data collection

Most women were recruited at the time of their OGTT as described above, however for 17%[242] of the pregnancies included in the BiB cohort (2334/13776 pregnancies), women were offered an OGTT but did not attend the appointment. These women were subsequently recruited at hospital visits attended later in pregnancy, or during the hospital stay for birth. For those women who consented to participate in BiB at the time of the OGTT, an extra 20ml of fasting blood sample was taken at the same time as the fasting OGTT blood sample draw for future analysis. Trained phlebotomists drew the blood samples in serum separating blood collecting tubes which were then transferred immediately to the laboratories at the Bradford Royal Infirmary. The samples were centrifuged at 1000 x g for ten minutes at room temperature. Automated pipettes were used to aliquot 1ml of serum into 1.5 microtubes vials. Care was taken throughout to track and confidentially label each sample with the relevant mother and pregnancy unique identifiers, and the serum samples were stored at -80 degrees Celsius until further analysis. Samples were stored within two and

a half hours of the sample being obtained. There were no freeze-thaw cycles for the samples on which subsequent NMR metabolomics analysis was performed[236]. In summary, 12 453 women across 13 776 pregnancies were recruited into the BiB cohort, and there were OGTT results for 12 331 pregnancies (11 231 women), and NMR data for 11 479 pregnancies (10 574 women).

At recruitment, women had their weight and height measured, and a baseline questionnaire was completed via interview. Over 50% of the obstetric population in Bradford are of South Asian origin, the majority of which are of Pakistani origin and specifically from the city of Mirpur (Kashmir region). Interviews were therefore undertaken in English, Urdu or Mirpuri. Mirpuri is not a written language hence the verbal interview was essential. Further details regarding labour, delivery, and baby outcomes were obtained from electronic and paper hospital records.

4.5.4 Data access

The custodians of BiB are committed to making study data widely available to international researchers, and many documents are freely available online (https://borninbradford.nhs.uk/about-us/). Full details of how to apply to access existing data for analyses are available from https://borninbradford.nhs.uk/research/how-to-access-data/. An application to

use the BiB data to undertake the study presented here, alongside an application to undertake analyses by having access to the relevant data files for this study using the BiB data files held in Bristol, was submitted via this pathway and access was granted on 18th June 2018.

4.5.5 Study eligibility criteria

Women with singleton pregnancies recruited into the BiB cohort were eligible for inclusion in the study presented in this chapter. Only one pregnancy per woman was included.

Multi-fetal pregnancies were excluded as it is plausible that carrying two or more fetuses would alter maternal gestational metabolite concentrations more so than with single-fetal pregnancies, and most multi-fetal pregnancies are iatrogenically delivered preterm. Only one pregnancy was included per mother to avoid including multiple observations from the same mother in the analysis, important as the statistical methods used assume independence of observations between participants. The first pregnancy recruited into the cohort for each woman was included, and parity was controlled for in the analysis.

Figure 4.1 shows the flow of BiB participants from all pregnancies recruited, to the eligible dataset (all singleton pregnancies and only one pregnancy per mother), and the final analysis dataset. Of the 13776 pregnancies recruited into BiB (across 12 453 women), 159 were excluded as twin pregnancies and three

excluded as triplet pregnancies (Figure 4.1 and Table 4.1). A further 1306 were removed in order to include only one pregnancy per mother (this would have been 1323 pregnancies if the multi-fetal pregnancies had not already been removed as 17 of the women with multi-fetal pregnancies also had one other pregnancy included in the BiB cohort) (Figure 4.1 and Table 4.2). The eligible sample included 12 308 (89% of total recruited sample) mothers with one singleton pregnancy. Women with pregnancies who had any data missing among the eligibility variables, exposure variables, outcome variable or co-variables were then removed (n=4868), leaving 7440 women with one singleton pregnancy and no missing data in the complete analysis sample.

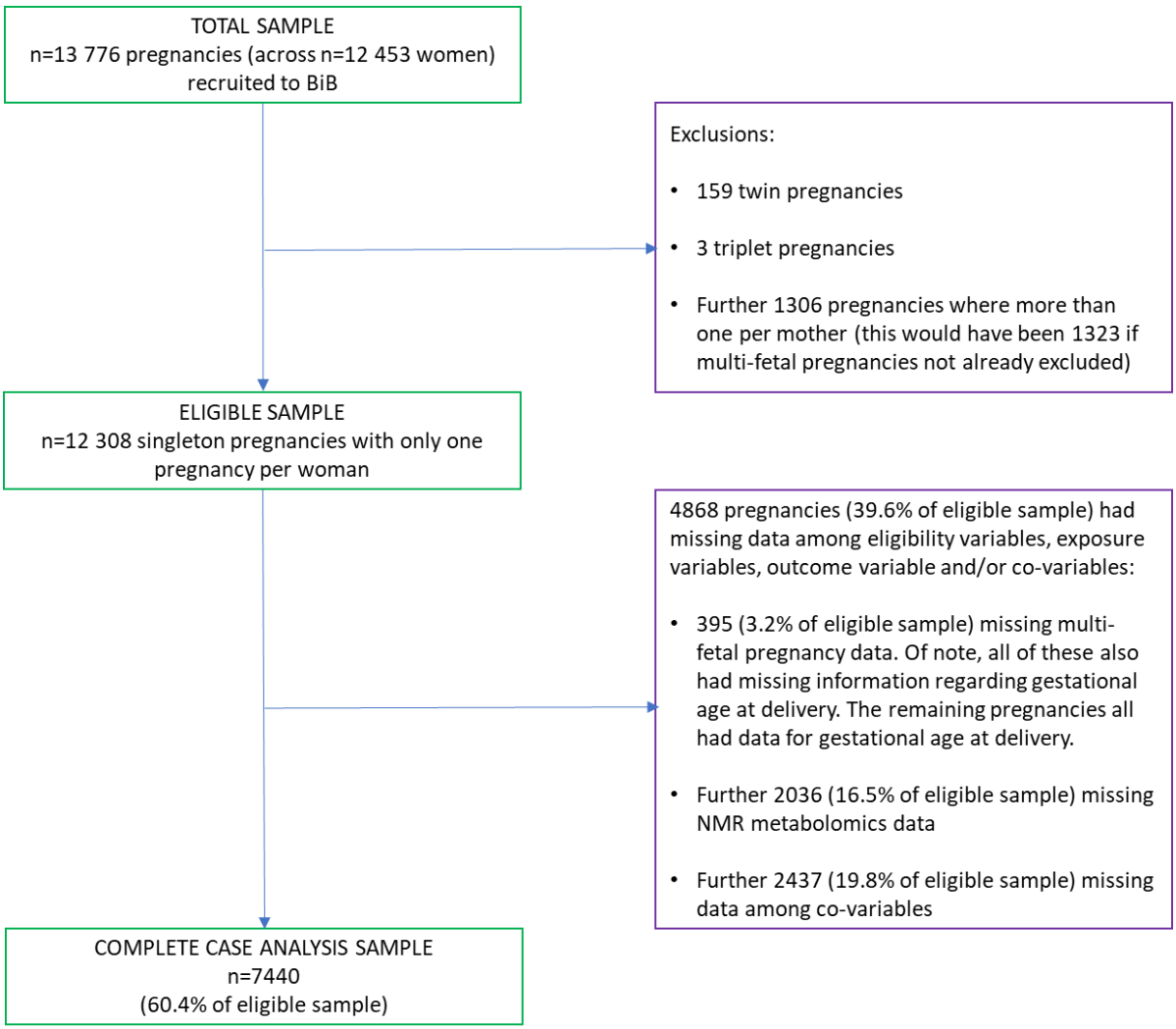

Figure 4.1: Flow chart for participant inclusion: Total sample = all women and pregnancies recruited to BiB; Eligible sample = multi-fetal pregnancies excluded and only one pregnancy per mother included; Analysis sample = only one pregnancy per mother, only singleton pregnancies, no missing data.

Table 4.1: Distribution of multi-fetal pregnancies for mothers enrolled in BiB.

Number of multi-fetal pregnancies	Number of pregnancies total dataset
Singleton	13199
Twins	159
Triplets	3
Missing	415
Total	13776

Table 4.2: Distribution of pregnancies for mothers enrolled in BiB.

Number of pregnancies (PregnancyID) per mother (MotherID) recorded in BiB	Number of pregnancies in total dataset	Number of pregnancies in eligible dataset	Number of pregnancies in analysis dataset
1	11 168	12 308	7440
2	2494	0	0
3	114	0	0
Total	13776	12 308	7440

4.5.6 Exposure measures: maternal gestational metabolite concentrations

The exposures for this study were the NMR serum metabolites measured in fasted serum samples taken from women at 26 to 28 weeks' gestation. Up to 157 metabolic traits were measured using NMR at the University of Bristol Bioresource Laboratories and NMR metabolite facilities. Measurements were then quantified by Nightingale Health (Nightingale Health©(Helsinki, Finland)). The Nightingale Health NMR metabolomics platform involves the use of three molecular windows for metabolite quantification, with two applied to native serum and one applied to lipid extract from the serum using minimal preparation. For metabolite quantification, two spectra (LIPO and LMWM) were obtained from the two native serum samples, and one (LIPID window) from the lipid extract. A Bruker AVANCE III spectrometer was used to measure the NMR spectra, operated at 500 or 600 MHz. The native serum samples were measured at 37 degrees Celsius, and the lipid extract at 22 degrees Celsius. The LIPO window provides a standard human serum spectrum, with lipid molecules within lipid particles causing broad overlapping resonances. Recording of the LIPO data was achieved through use of eight transients gained using NOESY-presat pulse sequence with water peak suppression and a 10-millisecond mixing time. Spectra from low-molecular weight molecules (LMWM) were detected

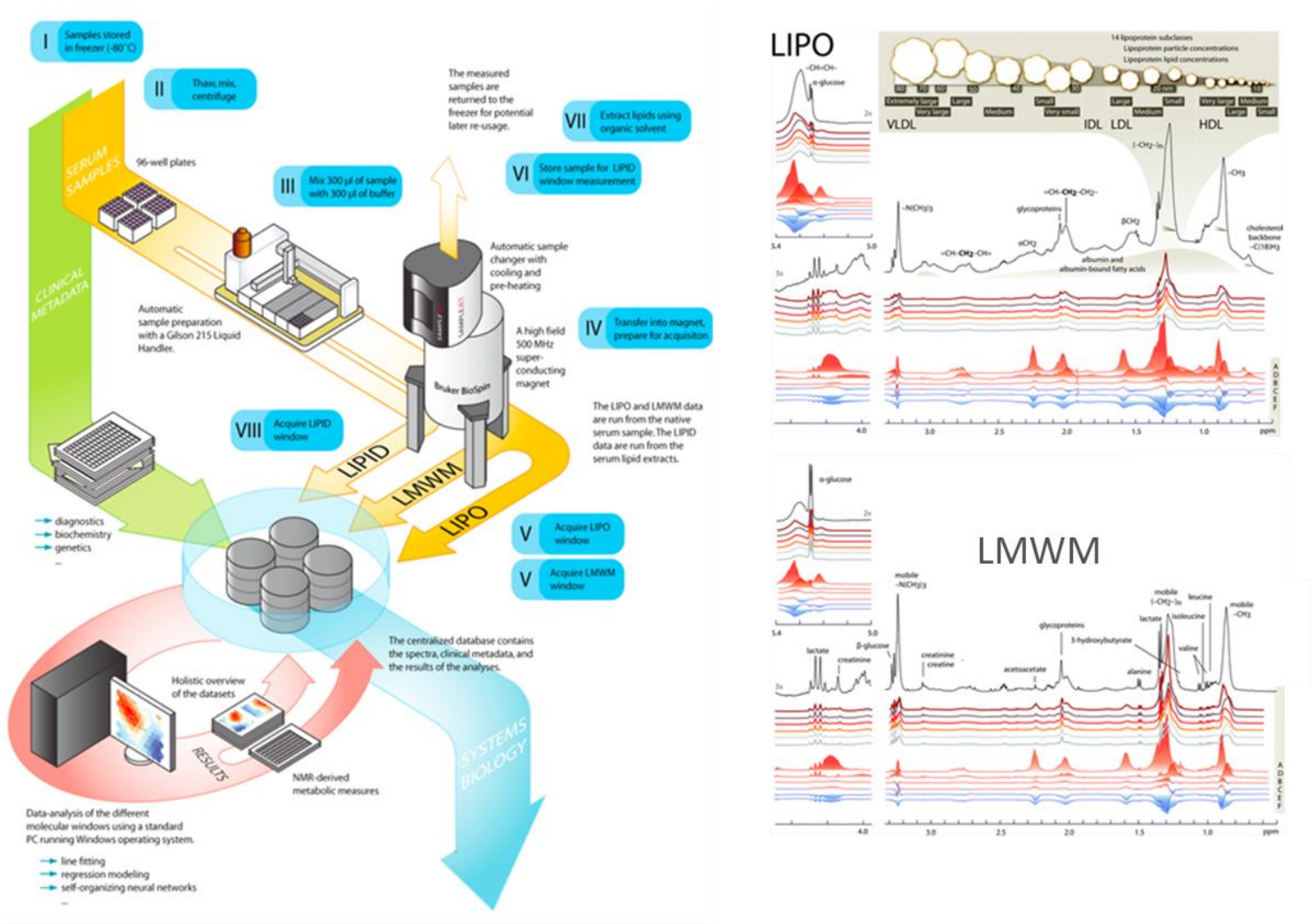

Figure 4.2: Illustration of processes involved in the Nightingale NMR metabolomics platform used to produce the metabolomics data analysed in this chapter (Reproduced from Mills *et al.* (2019)[243] who adapted from Wurtz *et al.* (2017)[136])

using the LMWM window and recorded via a relaxation-filtered pulse sequence which allowed enhanced detection of the smaller solutes through suppression of broad signals from macromolecules and lipids. A Car-Purcell-Meiboom-Gill (CPMG) pulse sequence with fixed echo delay of 403 microseconds and a 78 milliseconds T_2 filter applied with 24 transients was used. Finally, a standard 1D spectrum using 32 transients was used to acquire the LIPID window[243, 244] (See Figure 4.2).

Automated NMR spectra analysis was performed to obtain metabolite molar concentrations. To account for the difficulties of overlapping spectral data, a ridge regression was applied for each metabolite. Calibration of lipoprotein lipid data quantification models involved high-performance LC methods, followed by cross-validation against lipid data acquired from non-NMR analysis. Quantification of LMW molecules and molecules from serum lipid extract (quantified as mmol per litre) was based on regression modelling and calibrated against metabolite measures that were manually applied. Iterative line-shape with PERCH NMR (PERCH solutions ltd, Kupio, Finland) software was used for calibration data quantification. Due to variation in the lipid extraction protocol it is not possible to directly establish absolute quantification for the lipid extract measures, and instead these metabolites were scaled against the total

cholesterol that was quantified from the LIPO spectrum results of the native serum [243, 244].

4.5.7 Outcome: gestational age at delivery

As described previously, preterm birth is birth which occurs prior to 259 days gestation (37 completed weeks), and post-term is defined as pregnancy which exceeds 294 days (more than 42 completed weeks). Term pregnancy is defined as 259 days to 294 days inclusive (37-42 completed weeks' gestation)[29]. The World Health Organisation **(**https://www.who.int/news-room/fact-sheets/detail/preterm-birth) further classifies preterm birth as extremely preterm (less than 196 days' or 28 completed weeks' gestation), very preterm (196 days or more but less than 224 days' gestation, or 28 to <32 weeks' gestation), and moderate to late preterm (224 days or more but less than 259 days' gestation, or 32 to <37 weeks' gestation)[29]. These definitions were used for analysis in this study.

The primary outcome for this study was gestational age at delivery, and the gestational age at delivery for each of the pregnancies was obtained from electronic and paper medical records. Gestational age (outcome) was analysed in the following ways:

1. Gestational age at delivery in days as a continuous outcome;

2. Preterm birth defined as less than 259 days (<37 completed weeks gestation) compared to term birth, defined as 259 days to 294 days, inclusive (37 to 42 completed weeks' gestation);
3. Post-term birth, defined as more than 294 days (over 42 completed weeks' gestation) compared to term birth;
4. Extremely preterm defined as less than 196 days (<28 completed weeks') compared to term birth;
5. Very preterm, defined as 196 days to less than 224 days (≥28 completed weeks' to <32 completed weeks) compared to term birth;
6. moderate to late preterm, defined as 224 days to less than 259 days (32 weeks to less than 37 weeks' gestation) compared to term birth.

Analyses with the outcomes described in 3, 4, and 5 above were considered exploratory as statistical power would be relatively limited for those outcomes.

4.5.8 Data analysis

4.5.8.1 Directed Acyclic Graphs

When the aim of an analysis using observational data is to understand causal relations, as in the present study, a major concern is the potential that the results obtained may be biased because of residual confounding. Confounding occurs when one or more characteristics influence both the exposure of interest (here maternal gestational metabolite levels) and the outcome (here gestational

age at delivery). Causal effect estimates from multivariable adjusted analyses in observational data assume that all confounders have been thought of, accurately measured and appropriately adjusted for. For the present study, the intention was to take a systematic approach in deciding what may confound the analyses.

Directed Acyclic Graphs (DAGs) are a type of causal diagram that have been increasingly used to improve study design and data analysis for causal inference. DAGs represent expert knowledge and a priori assumptions about the causal structure underlying the data, and can be used to guide data analysis. Understanding DAG theory can aid identification of what needs to be adjusted for and what should not be adjusted for. This can be useful if potential confounders are identified and not all are measured in the dataset. If relationships between confounders are correctly presented in the DAG (assuming all confounders are measured with minimal or no error), the DAG can be used to identify whether all relevant confounders are included in a proposed regression model[19, 245]. For the present study, a priori knowledge was used to identify potential confounders and a DAG was created to represent known or plausible relationships between these confounders, the study exposure and study outcomes.

Within a DAG, nodes represent variables and are connected by unidirectional arrows pointing in the direction that a causal relationship is known or plausible. Paths between several nodes must be acyclic, i.e. no path can return to a node from whence it originated[246,19]. DAGs are completely dependent upon expert knowledge on the underlying causal structure, and could therefore be developed on the basis of wrong assumptions about the data given our incomplete knowledge of the relationships between many human characteristics[19].

For this study, potential confounders (or covariables) were identified following a review of relevant literature, clinical knowledge, and through discussion with the supervisors of this PhD. The Dagitty web interface (www.dagitty.net) was used to draw a DAG with the assumed underlying causal assumptions for this study, and to determine which variables should be adjusted for in the analysis[19]. Figure 4.3 shows the final DAG used for this study. Initial iterations of the DAG also included medications that participants were recorded as taking during pregnancy. However, after investigation of the data and further discussion it was decided that the recorded medications were unlikely to influence gestational age at delivery and were therefore not adjusted for in the models used. Conversely, parity was not included in the first iteration of the DAG, however following further discussion it was agreed that this could plausibly influence

both metabolite levels and gestational age at delivery[236, 247, 248] and was adjusted for. All variables in the DAG were included in the subsequent analyses as covariates. For each of these covariables, the reason for identifying it as a potential confounder and the method by which the relevant data was collected within the BiB cohort study are described below.

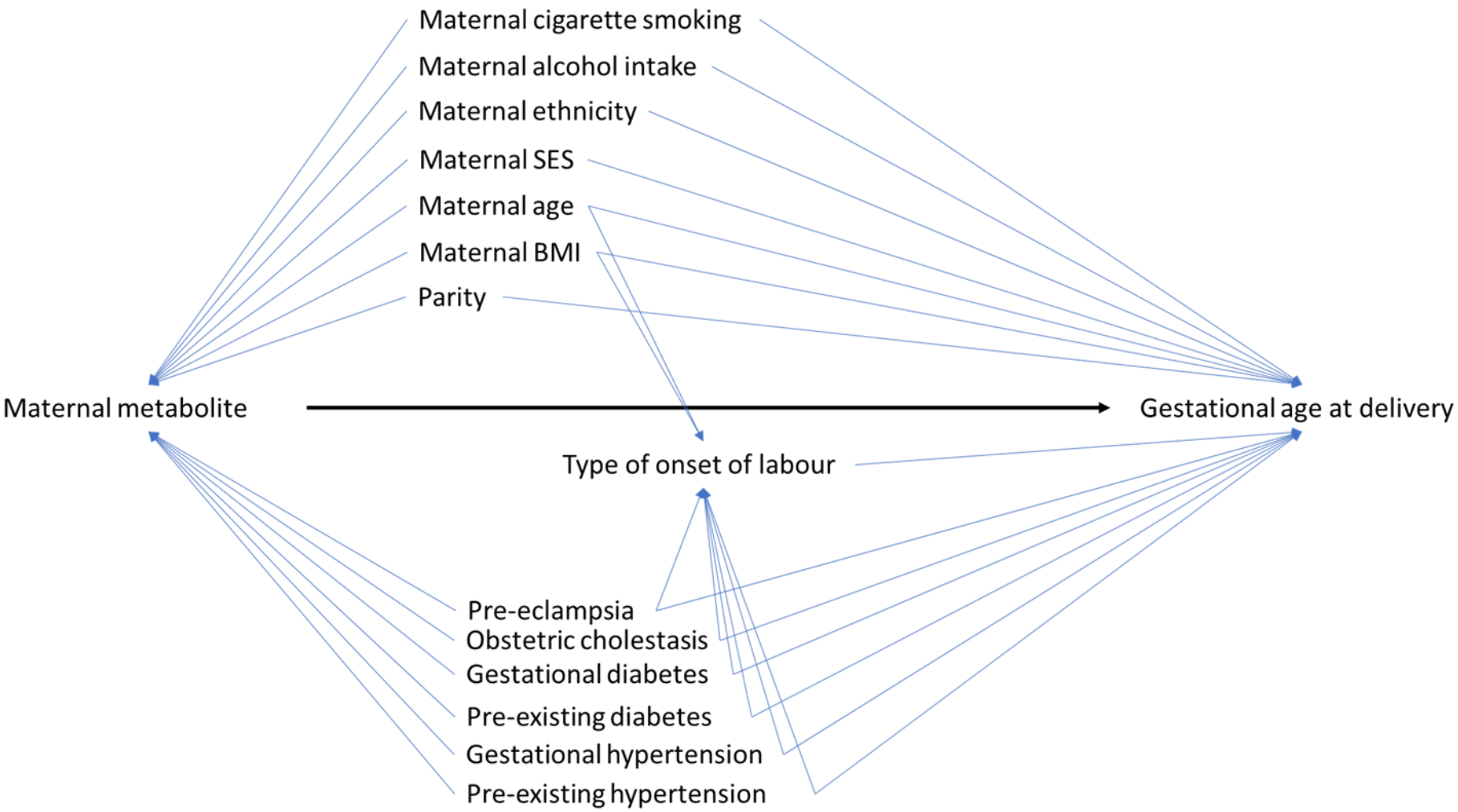

Figure 4.3: Directed acyclic graph for analysis of the relationship between maternal metabolites measured during the second trimester and gestational age at delivery.

4.5.8.2 Assessment of confounders

Maternal age has previously been shown to affect both gestational age at delivery[249] and pregnancy metabolic profile[236]. Women reported their date of birth at the time of the baseline interview, and with the date of the interview this was used to calculate maternal age and recorded as the mother's age in years at the time of the baseline questionnaire.

Maternal body mass index (BMI) has been associated with an altered pregnancy metabolomic profile[236, 250] and gestational age at delivery[251]. At the time of the baseline interview, women were weighed and had their height measured unshod and in light clothing, following a protocol that all assessors were trained to follow. As the women at baseline questionnaire were 26-28 weeks' gestation, the fetus, amniotic fluid and physiological changes of pregnancy would influence their weight. Therefore, early pregnancy BMI was calculated using weight measurement extracted from that measured at the booking antenatal clinic generally attended in the first trimester. Therefore the maternal BMI used in this study was calculated as: (booking weight in kilograms)/(height measured at time of recruitment in metres)^2.

Maternal ethnicity may also influence both maternal metabolic profile[236] and gestational age at delivery[252]. Ethnicity data in BiB was derived from both self-

reported data and linked data from General Practitioners, and ethnicity was categorised as White European, South Asian or Other.

Socioeconomic position (SEP) has been associated with pregnancy metabolic profile[236] as well as gestational age at delivery[253, 254,255]. Within the BiB cohort, information regarding different components of SEP was collected at the time of recruitment to BiB and recorded in the mother and father baseline questionnaires. Two potential methods for assessing SEP are: to use one or more individual assessment points, such as education and information regarding index of multiple deprivation; or to combine multiple factors of SEP into one score[255]. Latent class analysis (LCA) is an example of the latter, and is a statistical method for classifying individuals into groups on the basis of conditional probabilities that incorporate multiple factors related to SEP. This means that individuals within each group will have similar responses to categorical variables[255]. LCA has previously been successfully applied to the BiB data, for which the following SEP factors were included in the analysis: employment status of the mother and father; education of the mother and father; subjective poverty; receiving means tested benefits; being up to date with bills; housing tenure; able to afford a holiday for at least a week at least once a year; to have food and drink with family and friends at least once a month; to afford two pairs of all-weather shoes; house contents insurance; keep house decorated; replace worn out

furniture; replace or repair major electrical goods; a hobby or leisure activity; keep home warm; to spend money on themselves[255]. Following this analysis, the following five latent classes were derived: Least socioeconomically deprived and most educated, employed not materially deprived, employed no access to money, benefits and not materially deprived, and most economically deprived. For the present study, the derived variables from the LCA described above were used to adjust comprehensively for SEP [255].

Alcohol intake during pregnancy has previously been found to be associated with preterm labour, although the evidence is not strong [256], and has been shown to alter the metabolic profile of non-pregnant individuals[257]. Data for alcohol intake during pregnancy and in the three months before pregnancy was also recorded in the maternal baseline questionnaire and was dependent upon the women remembering and reporting that they had consumed alcohol. This was categorised into yes/no/can't remember.

Cigarette smoking could also plausibly influence maternal metabolites and gestational age at delivery[258-260]. Whether or not a woman smoked cigarettes during pregnancy was derived from the questions in the maternal baseline questionnaire, categorised as yes or no to smoking during pregnancy.

The type of onset of labour would also influence the gestational age at delivery, as those induced or with elective caesarean section would have iatrogenically

shortened gestations, and it is plausible that the type of onset of labour may have had an influence on the maternal metabolite level. Information on labour onset was extracted from electronic records, and categorised as spontaneous, induction or elective caesarean section (no labour).

Parity has been associated with differences in gestational age at delivery [247, 248] and pregnancy metabolomic profiles [236]. Data regarding parity was derived from the hospital paper notes or electronic records, and was recorded as an integer value.

Pre-existing diabetes (type 1 and type 2) and gestational diabetes (GDM) have been associated with altered metabolome[261,236, 262] and preterm birth[263,264]. Pre-existing diabetes data was obtained from electronic records and paper notes and categorised as yes or no to diabetes prior to pregnancy. Data regarding GDM was obtained from the OGTT results. GDM was categorised as being diagnosed, not diagnosed or not documented.

Pre-existing hypertension is associated with altered metabolome[265], as is pregnancy induced hypertension and pre-eclampsia[236, 266], and both have been associated with preterm birth[267-269]. Data regarding pre-existing hypertension was obtained from electronic and paper medical records, categorised as no or yes. Data regarding pre-eclampsia and gestational hypertension were

abstracted from the maternal paper records, categorised as normotensive, gestational hypertension, or pre-eclampsia.

A previous study found concentrations of total cholesterol, LDL cholesterol, and apolipoprotein B-100 were raised in the plasma of women with intrahepatic cholestasis of pregnancy (also-known-as obstetric cholestasis) when compared with controls [270]. Obstetric cholestasis is also associated with earlier delivery of pregnancy, although this may be iatrogenic[271]. Prevalence of OC varies worldwide, and in the UK affects 0.7% of pregnancies in multi-ethnic populations, but 1.2-1.5% of women of Pakistani-Asian background, and 5% in women of Araucanian-Indian origin [272]. Obstetric cholestasis was not documented in the BiB study, so it was not possible to control for this in the analysis.

4.5.9 Creation of analysis sample

In this study, complete case analyses were used in order to deal with the issue of missing data. Complete case analyses involve performing all regression models on the same sub-sample, which includes only those participants with no missing data for exposure, outcome or identified covariables (confounders). In general, complete case analysis is expected to be unbiased if missingness is not related to the outcome in the main analysis model conditioned on exposure, and that all covariables are included in the main analyses[273]. For the present study, the final complete case analysis sample therefore only included women with

complete data on serum NMR, gestational age and all confounders (see Figures 4.1 and 4.4 for numbers for each of the different main analyses). As this method requires the same sample set to be included in both the unadjusted and confounder adjusted models, any differences between these estimates would likely be due to confounding.

4.5.10 Statistical analysis

The metabolic traits assessed in the second trimester of pregnancy were transformed to standard deviation (SD) units. Table 4A1 (Appendix) shows each metabolite measured with units and standard deviation for the analysis sample. SD units were used to allow for comparisons across the association of individual metabolites and gestational age at delivery.

Figure 4.4 summarises the statistical analyses performed. Linear regression was used to estimate the association between each of the maternal metabolites and gestational age at delivery where gestational age was measured as a continuum in days. In order to further analyse whether there may be differences in maternal metabolites between different extremes of gestational age at delivery, logistic regression was performed to compare metabolite concentrations of those pregnancies which delivered preterm (<259 days, n=392) with those that were born at term (259-294 days gestation, n=6994); and similarly to compare metabolite concentrations for those pregnancies which delivered post-term

(>294 days, n=54) with those that were delivered at term. Following discussion with supervisors, it was intended that all models would be presented with no adjustment and with adjustment for the minimal sufficient set of confounders as described above. Further sensitivity analysis was then performed to determine if there were differences between the categories of preterm birth, where those which were born extremely preterm (<196 days, n=12), very preterm (≥196 to <224 days, n=51), or moderate to late preterm (≥224 <259 days, n=329) were separately compared with those born at term.

In order to account for multiple testing, a p-value threshold calculation was conducted according to previous work using this NMR data[250]. Principal component analyses were conducted on z-scored NMR data, including data from the BiB cohort, to estimate the number of independent dimensions in the data and it was determined that the first 17 principal components explained 95% of the variance in the metabolic measures. Therefore, for the present study a corrected p-value was calculated through dividing 0.05 by 17 (p=0.05/17= 0.0029).

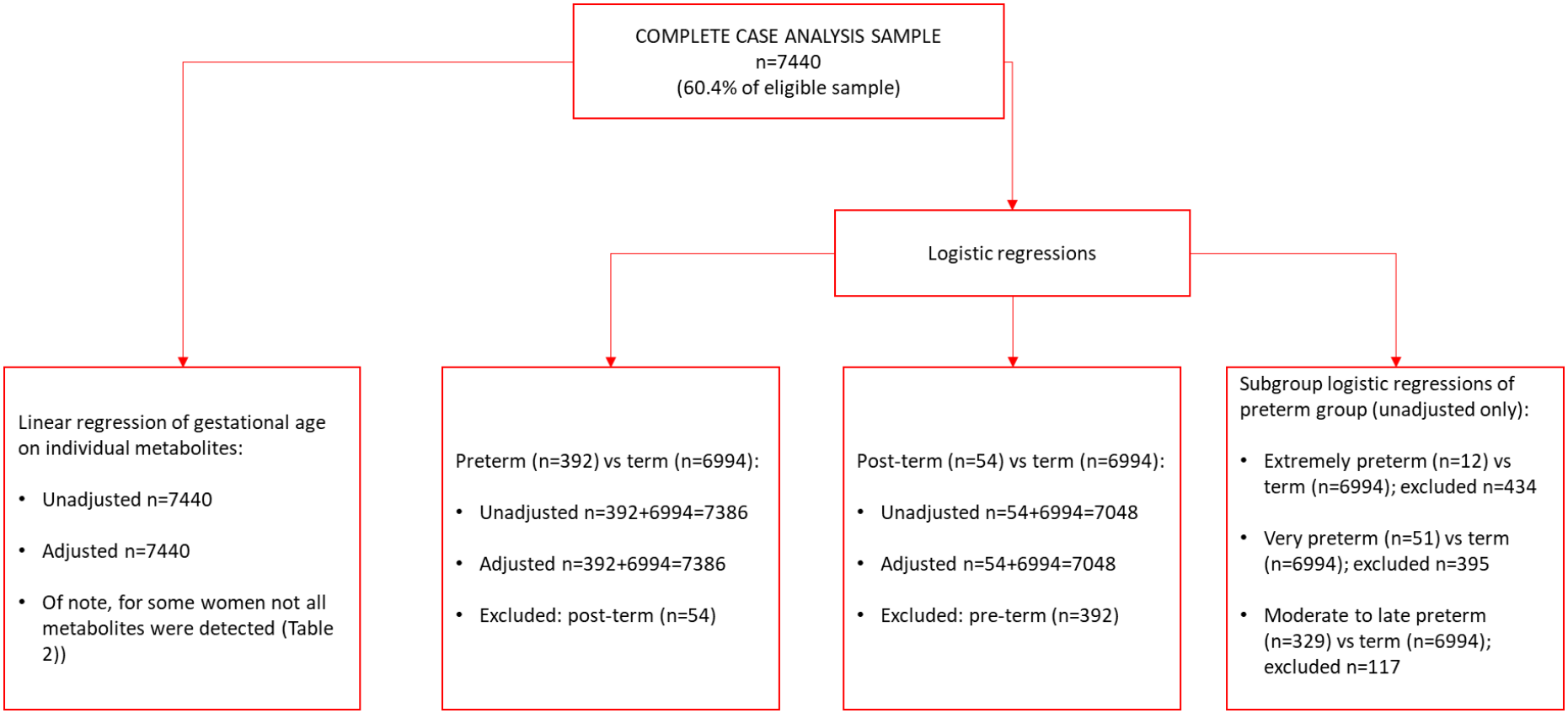

Figure 4.4: Flow of participants included in complete cases analysis sample for statistical analyses performed.

4.6 Results

4.6.1 Participants' characteristics

Figure 4.1, and Tables 4.3-4.5 and 4A2 (Appendix) show the differences in pregnancy numbers and missing data between the total recruited pregnancies (n=13776), the eligible sample (n=12308) and the analysis sample (n=7440). Specifically, Table 4.3 shows the median, minimum and maximum gestational age values in days, and the number of missing values for the total recruited pregnancies, the eligible pregnancies and the analysis sample pregnancies; Table 4.4 shows the median, maximum, minimum and missing values for the continuous co-variables maternal age and maternal BMI among all recruited pregnancies, all eligible pregnancies, and within the analysis sample; and Table 4.5 shows the total number for each category and total missing values for each of the remaining covariables other than obstetric cholestasis, again among the total recruited sample, eligible pregnancies sample and analysis sample.

7440 pregnancies (60.5% of eligible) had no missing data for NMR, gestational age or the confounder variables, and comprised the analysis sample. Despite the difference in number of pregnancies, the three samples had very similar distributions of exposures (Table 4A2, Appendix), outcome (Table 4.3) and measured confounders (Table 4.4). The one exception was pre-existing maternal diabetes for which there were 61 pregnancies in the eligible sample group but

only four in the analysis group. This is likely due to the NMR sample being taken at the time of the OGTT, and those with pre-existing diabetes would presumably be less likely to attend for this test.

Table 4.6 shows the distribution of co-variables for the 7440 pregnancies included in this study according to whether the pregnancies delivered preterm, term or post-term. 94% (6994/7440) of the pregnancies were delivered at term, while 5% (392/7440) delivered preterm, and 1% (54/7440) delivered post-term. The median gestational age at delivery in the preterm group was 247 days (range 176-258 days), 280 days in the term group (range 259-294 days), and 296 days in the post-term group (range 195-313 days).

There were no substantial differences in median maternal age at enrolment, maternal BMI or parity across the three categories of gestational age at delivery. The distribution of ethnicity was similar among women who delivered preterm and term, however post-term delivery seemed more common in white European women. The proportion reporting cigarette smoking in pregnancy were slightly higher in those delivering pre-term than at term or post-term. There were no substantial differences in the distribution across the socioeconomic quintiles for pregnancies delivering preterm and term, however a higher proportion of women who delivered post-term were in the two least deprived groups (Table 4.5). Further, women in the most deprived group had

the highest proportion of preterm birth (6.1%), and those in the 3rd quintile had the lowest proportion (4.7%).

Regarding labour onset, there was a higher proportion of spontaneous labour in the term group, and the post-term group had the lowest proportion of spontaneous labour and the highest proportion of induced labour, which is in keeping with national guidelines regarding induction of labour for post-term pregnancies. The caesarean section rate was highest in the pre-term group and was considerably lower than the national average, reported by NHS Digital as 20-25% for the years 2016-17[45], in both the term and post-term groups.

Table 4.3: Summary of outcome variable (gestational age at delivery) for: all pregnancies recruited to BiB; all eligible pregnancies (women with singleton pregnancies and only one pregnancy per mother); and Analysis sample (all eligible pregnancies with no missing data).

Gestational age at delivery (days)	All recruited pregnancies	All eligible pregnancies (singleton pregnancies and one pregnancy per mother)	Analysis sample (all eligible with no missing data)
Median	**279**	**279**	**279**
Minimum	**174**	**174**	**176**
Maximum	**313**	**313**	**313**
Missing	**415**	**395**	**0**
Total	**13776**	**12308**	**7440**

Table 4.4: Summary of continuous covariables for: all pregnancies recruited to BiB (12453 women across 13776 pregnancies); all eligible pregnancies (women with singleton pregnancies and only one pregnancy per mother) n=12308; Analysis sample (all eligible pregnancies with no missing data) n= 7440.

Variable	All recruited pregnancies n=13776 (12453 mothers)	All eligible pregnancies (singleton pregnancies and one pregnancy per mother) n=12308	Analysis sample (all eligible with no missing data for variables or NMR) n=7440
Mother age in years at baseline questionnaire			
Number of observations recorded	**11395**	**10247**	**7440**
Median	**27**	**27**	**27**
Minimum	**14**	**14**	**15**
Maximum	**49**	**49**	**49**
Missing	**2381**	**2061**	**0**
Total	**13776**	**12308**	**7440**
Body Mass Index			
Number of observations recorded	**10495**	**9445**	**7440**
Median	**25.0**	**25.0**	**25.0**
Minimum	**12.9**	**12.9**	**14.7**
Maximum	**57**	**57.0**	**57.0**
Missing	**3281**	**2863**	**0**
Total	**13776**	**12308**	**7440**

NMR=nuclear magnetic resonance metabolomics; GA = gestational age

Table 4.5: Summary of categorical covariables for: all pregnancies with questionnaire data; all pregnancies with NMR metabolomics data; all eligible pregnancies with NMR metabolomics data (singleton pregnancies and one pregnancy per mother, randomly selected); and all eligible pregnancies with NMR metabolomics data and gestational age at delivery recorded.

	All recruited pregnancies n=13776 pregnancies (12453 mothers)		All eligible pregnancies (singleton pregnancies and one pregnancy per mother) n=12308		Analysis sample (all eligible with no missing data for variables or NMR) n=7440	
Variable	**Number**	**(%)**	**Number**	**(%)**	**Number**	**(%)**
Ethnicity:						
White European	**5,474**	**(39.7)**	**4975**	**(40.4)**	**3341**	**(44.9)**
South Asian	**6,982**	**(50.7)**	**6110**	**(49.6)**	**3640**	**(48.9)**
Other	**910**	**(6.6)**	**833**	**(6.8)**	**459**	**(6.2)**
Missing	**410**	**(3)**	**390**	**(3.2)**	**0**	
Total	**13,776**	**(100)**	**12308**		**7440**	
Socioeconomic status:						
Least socioeconomically deprived and most educated	**2231**	**(16.2)**	**2027**	**(16.5)**	**1468**	**(19.7)**
Employed not materially deprived	**2248**	**(16.3)**	**2100**	**(17.1)**	**1594**	**(21.4)**
Employed no access to money	**1721**	**(12.5)**	**1538**	**(12.5)**	**1118**	**(15.0)**
Benefits and not materially deprived	**3325**	**(24.1)**	**2910**	**(23.6)**	**2110**	**(28.4)**
Most economically deprived	**1800**	**(13.1)**	**1605**	**(13.0)**	**1150**	**(15.5)**
Missing	**2451**	**(17.8)**	**2128**	**(17.3)**	**0**	
Total	**13776**		**12308**		**7440**	
Parity:						
0	**5101**	**(37.0)**	**5032**	**(40.9)**	**3376**	**(45.4)**
1	**3727**	**(27.0)**	**3015**	**(24.5)**	**1992**	**(26.8)**
2	**2201**	**(15.9)**	**1859**	**(15.1)**	**1132**	**(15.2)**
3	**1098**	**(7.9)**	**926**	**(7.5)**	**561**	**(7.5)**
4	**458**	**(3.3)**	**401**	**(3.3)**	**249**	**(3.4)**
5	**195**	**(1.4)**	**150**	**(1.2)**	**97**	**(1.3)**
6	**50**	**(0.4)**	**43**	**(0.35)**	**20**	**(0.3)**

7	25	(0.2)	21	(0.17)	7	(0.09)
8	10	(0.07)	8	(0.06)	3	(0.04)
9	5	(0.04)	5	(0.04)	3	(0.04)
10	4	(0.03)	2	(0.02)	0	(0)
Missing	903	(6.6)	846	(6.9)	0	(0)
Total	13776		12308		7440	
Alcohol during pregnancy or three months prior:						
Yes	3480	(25.3)	3237	(26.3)	2440	(32.8)
No	7882	(57.2)	6981	(56.7)	4993	(67.1)
Don't remember	7	(0.05)	7	(0.06)	7	(0.1)
Missing	2407	(17.5)	2083	(16.9)	0	
Total	13776		12308		7440	
Cigarette smoking during pregnancy:						
Yes	1870	(13.6)	1724	(14)	1312	(17.6)
No	9504	(70.0)	8504	(69.1)	6128	(82.4)
Missing	2402	(17.4)	2080	(16.9)	0	
Total	13776		12308		7440	
Pre-existing diabetes:						
Yes	70	(0.5)	61	(0.5)	4	(0.1)
No	12705	(92.2)	11353	(92.2)	7436	(99.9)
Missing	1001	(7.3)	894	(7.3)	0	
Total	13776		12308		7440	
Gestational Diabetes Mellitus:						
Yes	884	(6.4)	775	(6.3)	568	(7.6)
No	10037	(72.9)	9004	(73.2)	6872	(92.4)
Missing	2855	(20.7)	2529	(20.5)	0	
Total	13776		12308		7440	
Pre-existing hypertension:						
Yes	116	(0.8)	108	(0.9)	70	(0.9)
No	12648	(91.8)	11298	(91.8)	7370	(99.1)

Missing	**1012**	**(7.4)**	**902**	**(7.3)**	**0**	
Total	**13776**		**12308**		**7440**	
Hypertensive disorders of pregnancy:						
Normotensive	**9840**	**(71.4)**	**8778**	**(71.3)**	**6678**	**(89.8)**
PIH	**776**	**(5.6)**	**716**	**(5.8)**	**564**	**(7.6)**
PET	**279**	**(2.0)**	**264**	**(2.1)**	**198**	**(2.6)**
Missing	**2881**	**(20.9)**	**2550**	**(20.72)**	**0**	
Total	**13776**		**12308**		**7440**	
Type of labour onset:						
Spontaneous	**9,234**	**(67)**	**8264**	**(67.1)**	**5190**	**(69.8)**
Caesarean section (no labour)	**1,413**	**(10.3)**	**1193**	**(9.7)**	**670**	**(9.0)**
Induction of labour	**2,702**	**(19.6)**	**2448**	**(19.9)**	**1580**	**(21.2)**
Missing	**427**	**(3.1)**	**403**	**(3.3)**	**0**	
Total	**13,776**		**12308**		**7440**	

NMR=nuclear magnetic resonance metabolomics; GA = gestational age

Table 4.6: Distribution of the characteristics of those in the analysis group according to whether delivered preterm, term or post-term (n=7440). Of note there are no missing values for these co-variables within this complete analysis sample.

Demographic variable		Preterm (< 259 days)	Term (259 - 294 days)	Post-term (>294 days)
	Total Number	392	6994	54
	Percentage of total	(5%)	(94%)	(1%)
Maternal age (years):				
	Median	27	27	26
	Range	15-43	15-49	16-39
Maternal BMI (kg/m2):				
	Median	24.5	25.1	26.7
	Range	16.2-49.9	14.7-57.0	19.3-41.5
Gestational age at delivery (days):				
	Median	247	280	296
	Range	176-258	259-294	295-313
Parity:				
	Median	0	1	0
	Range	0-7	0-9	0-5
Maternal ethnicity:				
White European				
	Number	180	3129	32
	Percentage	(46)	(45)	(59)
South Asian				
	Number	191	3431	18
	Percentage	(49)	(49)	(33)
Other				
	Number	21	434	4
	Percentage	(5)	(6)	(7)
Maternal smoking during pregnancy:				
Yes				
	Number	91	1212	9
	Percentage	(23)	(17)	(17)
No				
	Number	301	5782	45
	Percentage	(77)	(83)	(83)
Maternal socioeconomic status:				
Least deprived & most educated				
	Number	81	1374	13
	Percentage	(21)	(20)	(24)
Employed not materially deprived				
	Number	88	1493	13
	Percentage	(22)	(21)	(24)
Employed but no access to money				
	Number	46	1062	10
	Percentage	(12)	(15)	(19)

Benefits but coping				
	Number	**111**	**1990**	**9**
	Percentage	**(28)**	**(28)**	**(17)**
Most deprived				
	Number	**66**	**1075**	**9**
	Percentage	**(17)**	**(15)**	**(17)**
Labour onset:				
Spontaneous				
	Number	**242**	**4921**	**27**
	Percentage	**(62)**	**(70)**	**(50)**
Caesarean section (no labour)				
	Number	**90**	**578**	**2**
	Percentage	**(23)**	**(8)**	**(4)**
Induction of labour				
	Number	**60**	**1495**	**25**
	Percentage	**(15)**	**(21)**	**(46)**

4.6.2 Association of maternal metabolites with gestational age at delivery

Table 4A1 (Appendix) lists the total metabolites measured, along with the units, mean values and SD for the total analysis sample. Estimates for the variation in gestational age at delivery per SD-unit increase in maternal metabolites with associated 95% confidence intervals can be found in Figures 4.5-4.26 and Tables 4A2-7 (Appendix). In each Figure, panel A displays the difference in gestational days per 1 SD change in metabolite, both unadjusted and adjusted for the available confounders; panel B shows the odds ratios for preterm birth compared to term, and post-term birth compared to term, again unadjusted and adjusted; and panel C presents the odds ratios for extremely preterm, very preterm, and moderate to late preterm compared to term. For the latter sensitivity analysis, only the unadjusted model was possible due to the limited number of cases. The same numbers of pregnancies were used for the adjusted

and unadjusted models for the linear and logistic regressions. Numerous maternal metabolic traits were associated with gestational age at delivery. The description of results will focus on estimates for the adjusted models, although unadjusted estimates are also presented in the Figures for comparison.

4.6.2.1 Multivariable linear regression analysis

Figures 4.5-4.26A show the results for the adjusted and unadjusted linear regressions of gestational age at delivery as a continuous variable on the 157 metabolites measured. For the majority of metabolites, a greater metabolite concentration was associated with a lower mean gestational age at delivery. After taking account of multiple testing (see above), there was statistical evidence for a positive association between three of the metabolites (Table 4.7) and a negative association between 76 of the metabolites (Table 4.8) with mean gestational age at delivery.

Triglycerides in medium HDL (Figure 4.14A), glycerol (Figure 4.11A) and glycoprotein acetyls (a1-acid glycoprotein) (Figure 4.9A) were the metabolites associated with the largest change in gestational age, with nearly a day decrease in gestational age at delivery per SD-unit increase in metabolite. Triglycerides and VLDLs were also highly represented in the metabolic traits significantly associated with gestational age at delivery (Table 4.7). Higher VLDL particle size and concentration and higher content of several lipids (from small to very large

VLDLs) were associated with lower mean gestational age at delivery. Higher concentration of triglycerides across lipoproteins (ie VLDL, LDL and HDL) was also related to lower mean gestational age at delivery. Higher particle and lipids concentration in small HDL particles were also related to lower gestational age at delivery.

Figure 4.5A shows that 1SD-increases in alanine, apolipoprotein B, phenylalanine and leucine concentrations were associated with shorter mean gestational age at delivery by approximately half a day. Figure 4.10A shows that a 1SD increase in the majority of glycerides and phospholipids (Figure 4.10A) concentrations were associated with a decrease in mean gestational age at delivery by half to one day. Similar associations were observed for creatinine, glycoprotein acetyls (mainly a1-acid glycoprotein) (Figure 4.9), fatty acids (Figure 4.8), citrate, glucose and glycerol (Figure 4.11).

Table 4.7: Metabolites with a significant (adjusted) negative association with gestational age at delivery (p<0.0029).

Metabolite	Group	Units	Change in GA per SD-unit increase in metabolite	SD	Lower 95%CI	Upper 95%CI	p-value	Total N
Triglycerides in medium HDL	Lipoprotein subclasses HDL	mmol/l	-0.914	0.013	-1.200	-0.628	3.818E-10	7440
Glycerol	Glycolysis related metabolites	mmol/l	-0.858	0.016	-1.150	-0.566	8.685E-09	7421
Glycoprotein acetyls (a1-acid glycoprotein)	Fluid balance and inflammation	mmol/l	-0.857	0.198	-1.148	-0.566	8.381E-09	7436
Phospholipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.825	0.044	-1.107	-0.544	9.412E-09	7440
Free cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.783	0.031	-1.063	-0.503	4.266E-08	7440
Serum total triglycerides	Glycerides and phospholipids	mmol/l	-0.770	0.580	-1.049	-0.490	7.125E-08	7435
Triglycerides in small VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.767	0.097	-1.048	-0.487	8.633E-08	7440
Triglycerides in VLDL	Glycerides and phospholipids	mmol/l	-0.758	0.460	-1.038	-0.478	1.133E-07	7435
Triglycerides in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.756	0.042	-1.037	-0.476	1.282E-07	7440
Concentration of small VLDL particles	Lipoprotein subclasses VLDL	mol/l	-0.751	0.000	-1.030	-0.472	1.400E-07	7440
Phospholipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.749	0.037	-1.027	-0.470	1.471E-07	7440
Triglycerides in large VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.745	0.117	-1.024	-0.466	1.739E-07	7440
Free cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.744	0.039	-1.023	-0.465	1.730E-07	7440

Total lipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.744**	0.215	-1.023	-0.465	**1.770E-07**	7440
Triglycerides in small LDL	Lipoprotein subclasses LDL	mmol/l	**-0.743**	0.013	-1.021	-0.465	**1.639E-07**	7440
Free cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.741**	0.023	-1.020	-0.463	**1.885E-07**	7440
Concentration of large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**-0.737**	0.000	-1.016	-0.458	**2.299E-07**	7440
Phospholipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.737**	0.058	-1.016	-0.458	**2.228E-07**	7440
Total lipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.735**	0.199	-1.014	-0.456	**2.444E-07**	7440
Triglycerides in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.731**	0.163	-1.010	-0.452	**2.977E-07**	7440
Concentration of medium VLDL particles	Lipoprotein subclasses VLDL	mol/l	**-0.722**	0.000	-1.001	-0.444	**3.826E-07**	7440
Triglycerides in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.721**	0.035	-1.000	-0.443	**3.862E-07**	7440
Total lipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.721**	0.298	-0.999	-0.442	**3.988E-07**	7440
Phospholipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.717**	0.009	-0.994	-0.440	**4.053E-07**	7440
Concentration of very large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**-0.710**	0.000	-0.988	-0.432	**5.616E-07**	7440
Triglycerides in small HDL	Lipoprotein subclasses HDL	mmol/l	**-0.705**	0.016	-0.991	-0.420	**1.300E-06**	7440
Total lipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.705**	0.055	-0.983	-0.428	**6.487E-07**	7440
Total cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.692**	0.046	-0.970	-0.415	**1.050E-06**	7440
Phospholipids in cms & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.686**	0.002	-0.961	-0.410	**1.117E-06**	7440

Free cholesterol in cms & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.681	0.002	-0.958	-0.405	1.408E-06	7440
Free cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.674	0.005	-0.950	-0.398	1.740E-06	7440
Total cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.666	0.080	-0.943	-0.390	2.382E-06	7440
Concentration of small HDL particles	Lipoprotein subclasses HDL	mol/l	-0.666	0.000	-0.945	-0.387	2.983E-06	7440
Monounsaturated fatty acids; 16:1, 18:1	Fatty acids and saturation measures	mmol/l	-0.652	0.997	-0.936	-0.368	6.845E-06	7386
Total lipids in small HDL	Lipoprotein subclasses HDL	mmol/l	-0.646	0.142	-0.925	-0.368	5.418E-06	7440
Cholesterol esters in large VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.636	0.022	-0.913	-0.359	6.795E-06	7440
Total cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.633	0.011	-0.909	-0.357	6.879E-06	7440
Triglycerides in LDL	Glycerides and phospholipids	mmol/l	-0.632	0.070	-0.909	-0.355	7.901E-06	7435
Triglycerides in cms & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.629	0.012	-0.904	-0.354	7.483E-06	7440
Total lipids in cms & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.629	0.018	-0.904	-0.354	7.457E-06	7440
Conc. Of cms & extremely large VLDL particles	Lipoprotein subclasses VLDL	mol/l	-0.628	0.000	-0.903	-0.353	7.641E-06	7440
Triglycerides in LDL	Lipoprotein subclasses LDL	mmol/l	-0.622	0.042	-0.899	-0.344	1.139E-05	7440
Triglycerides in medium LDL	Lipoprotein subclasses LDL	mmol/l	-0.612	0.020	-0.889	-0.336	1.447E-05	7440
Triglycerides in HDL	Glycerides and phospholipids	mmol/l	-0.601	0.042	-0.879	-0.323	2.276E-05	7435
Total cholesterol in VLDL	Cholesterol	mmol/l	-0.600	0.276	-0.876	-0.325	1.939E-05	7435

Cholesterol esters in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.596**	0.006	-0.872	-0.321	**2.236E-05**	7440
Total cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.594**	0.082	-0.869	-0.319	**2.332E-05**	7440
Monounsaturated fatty acids to total fatty acids	Fatty acids (%)	%	**-0.586**	2.415	-0.902	-0.270	**2.791E-04**	7386
Total cholesterol in cms & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.583**	0.004	-0.857	-0.309	**3.117E-05**	7440
Triglycerides in large LDL	Lipoprotein subclasses LDL	mmol/l	**-0.579**	0.039	-0.856	-0.303	**4.031E-05**	7440
Cholesterol esters in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.577**	0.043	-0.852	-0.302	**3.869E-05**	7440
Creatinine	Fluid balance and inflammation	mmol/l	**-0.575**	0.006	-0.853	-0.296	**5.314E-05**	7431
Phenylalanine	Aromatic amino acids	mmol/l	**-0.569**	0.011	-0.861	-0.278	**1.283E-04**	7436
Leucine	Branched--chain amino acids	mmol/l	**-0.569**	0.010	-0.851	-0.286	**8.260E-05**	7433
Total phosphoglycerides	Glycerides and phospholipids	mmol/l	**-0.555**	0.399	-0.831	-0.280	**7.897E-05**	7409
Total fatty acids	Fatty acids and saturation measures	mmol/l	**-0.542**	2.779	-0.817	-0.268	**1.088E-04**	7386
Apolipoprotein B	Apolipoproteins	g/l	**-0.536**	0.228	-0.810	-0.263	**1.223E-04**	7435
Saturated fatty acids	Fatty acids and saturation measures	mmol/l	**-0.536**	1.044	-0.811	-0.262	**1.305E-04**	7386
Phosphatidylcholine and other cholines	Glycerides and phospholipids	mmol/l	**-0.531**	0.459	-0.806	-0.256	**1.525E-04**	7407
Mean diameter for VLDL particles	Lipoprotein particle size	nm	**-0.525**	1.009	-0.804	-0.245	**2.374E-04**	7435
Ratio of triglycerides to phosphoglycerides	Glycerides and phospholipids	#N/A	**-0.524**	0.160	-0.807	-0.241	**2.849E-04**	7409
Free cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**-0.517**	0.017	-0.795	-0.239	**2.658E-04**	7440

Total cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	-0.516	0.067	-0.790	-0.242	2.243E-04	7440
Total cholines	Glycerides and phospholipids	mmol/l	-0.500	0.485	-0.775	-0.225	3.606E-04	7407
Cholesterol esters in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.493	0.002	-0.766	-0.220	3.945E-04	7440
Alanine	Amino acids	mmol/l	-0.485	0.048	-0.758	-0.213	4.849E-04	7408
Concentration of very small VLDL particles	Lipoprotein subclasses VLDL	mol/l	-0.477	0.000	-0.751	-0.203	6.396E-04	7440
Remnant cholesterol (non-HDL, non-LDL)	Cholesterol	mmol/l	-0.477	0.467	-0.750	-0.204	6.211E-04	7435
Phospholipids in small HDL	Lipoprotein subclasses HDL	mmol/l	-0.475	0.094	-0.752	-0.198	7.814E-04	7440
Cholesterol esters in small VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.465	0.054	-0.738	-0.192	8.468E-04	7440
Phospholipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	-0.451	0.087	-0.726	-0.176	1.295E-03	7440
Phospholipids in small LDL	Lipoprotein subclasses LDL	mmol/l	-0.447	0.034	-0.721	-0.173	1.380E-03	7440
Total lipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	-0.442	0.161	-0.716	-0.169	1.522E-03	7440
Cholesterol esters in small HDL	Lipoprotein subclasses HDL	mmol/l	-0.437	0.059	-0.710	-0.165	1.676E-03	7440
Apolipoprotein B:apolipoprotein A-I	Apolipoproteins	#N/A	-0.434	0.121	-0.708	-0.159	1.945E-03	7435
Phospholipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	-0.420	0.050	-0.693	-0.147	2.598E-03	7440

Table 4.8: Metabolites with a significant (adjusted) positive association with gestational age at delivery (p<0.0029).

Metabolite	Group	Units	Change in GA per SD-unit increase in metabolite	Standard Deviation	Lower 95%CI	Upper 95%CI	p-value	Total n
Polyunsaturated Fatty Acids to total Fatty Acids	Fatty acids (%)	%	0.548	2.386	0.254	0.842	2.60E-04	7386
Omega-6 Fatty Acids to total Fatty Acids	Fatty acids (%)	%	0.520	2.205	0.224	0.816	5.67E-04	7386
Mean diameter for HDL particles	Lipoprotein particle size	nm	0.474	0.205	0.194	0.754	9.09E-04	7435

4.6.4 Multivariable logistic regression analysis

The adjusted and unadjusted results of the two separate multivariable logistic regressions of metabolite concentration in pregnancies delivered preterm and post-term compared with term are presented in Panel B of Figures 4.5-4.26. The results of the logistic regression comparing post-term with term indicate that there is no strong evidence that any of the 157 metabolites tested is associated with post-term delivery (Panel B Figures 4.5-4.26 and Table 4A4).

For the analysis comparing preterm with term, the results are similar to those for the linear regressions with gestational age as a continuous variable. An increase of 1SD in mean diameter of HDL particles was associated with reduced odds for delivering preterm (Figure 4.12B, 4.13B and 4.16B) while an increase of 1SD in mean diameter of VLDL particles was associated with higher odds for delivering preterm (Figures 4.12B, 4.15B, 4.17B, 4.20B). VLDL concentrations were particularly associated with higher odds for delivering preterm (Figures 4.21-4.26B).

The volcano plot 4A8 (Appendix) presents the logOR values derived from the logistic regression outputs of both post-term (red dots) and preterm (blue dots) with term for the 157 metabolite conditions measured, plotted against -log10(p-value). This plot illustrates that for the post-term analyses, p-values were high even at the extreme of the logOR distribution, which was not the case for

preterm. This would be expected as the number of cases was low in comparison to preterm.

4.6.5 Sensitivity analysis

A sensitivity analysis was performed where those pregnancies which delivered preterm were further categorised into extremely, very, and moderate to late preterm. Metabolite concentrations of those pregnancies which delivered extremely preterm, those which delivered very preterm, and those which delivered moderate to late preterm, were compared with those which delivered at term. Table 4.9 shows the number of pregnancies included in these three categories of preterm birth and the mean gestational age at delivery. As previously discussed, because of the limited number of cases this analysis is considered exploratory and only unadjusted results are presented in Panel C of Figures 4.5-4.26.

Although unadjusted, the results indicate that there may be a dose-response association between concentrations of many of the metabolites and degree of prematurity at delivery. This includes concentrations of glycoprotein acetyls (a1-acid glycoprotein), creatinine (Figure 4.9C), phenylalanine, leucine (Figure 4.5C), polyunsaturated fatty acids to total fatty acids ratio, monounsaturated fatty acids to total fatty acids (Figure 4.7C), and estimated degree of unsaturation (Figure 4.8C). As with the previous analyses, an increased mean diameter of HDL

particles was associated with decreased odds for more extreme preterm, while increased diameter of VLDL particles was associated with increased odds (Figure 4.12C). In addition, increased concentration of very large HDL was associated with decreased odds for extremely and very preterm birth (Figure 4.16C), while increasing concentrations of VLDLs was associated with increased odds for extremely preterm birth (Figures 4.21 to 4.26C).

Table 4.9: Number of pregnancies within the preterm categories, with mean and range of gestational age at delivery (days) within each of these groups.

Preterm category	Number of pregnancies	Mean gestational age at delivery (days)	Range of gestational age at delivery (days)
Extremely preterm (<28 weeks gestation)	12	187.8	176-195
Very preterm (28 to 31+6 weeks' gestation)	51	210.9	196-223
Moderate to late preterm (31+6 weeks to 36+6 weeks gestation)	329	247.1	224-258

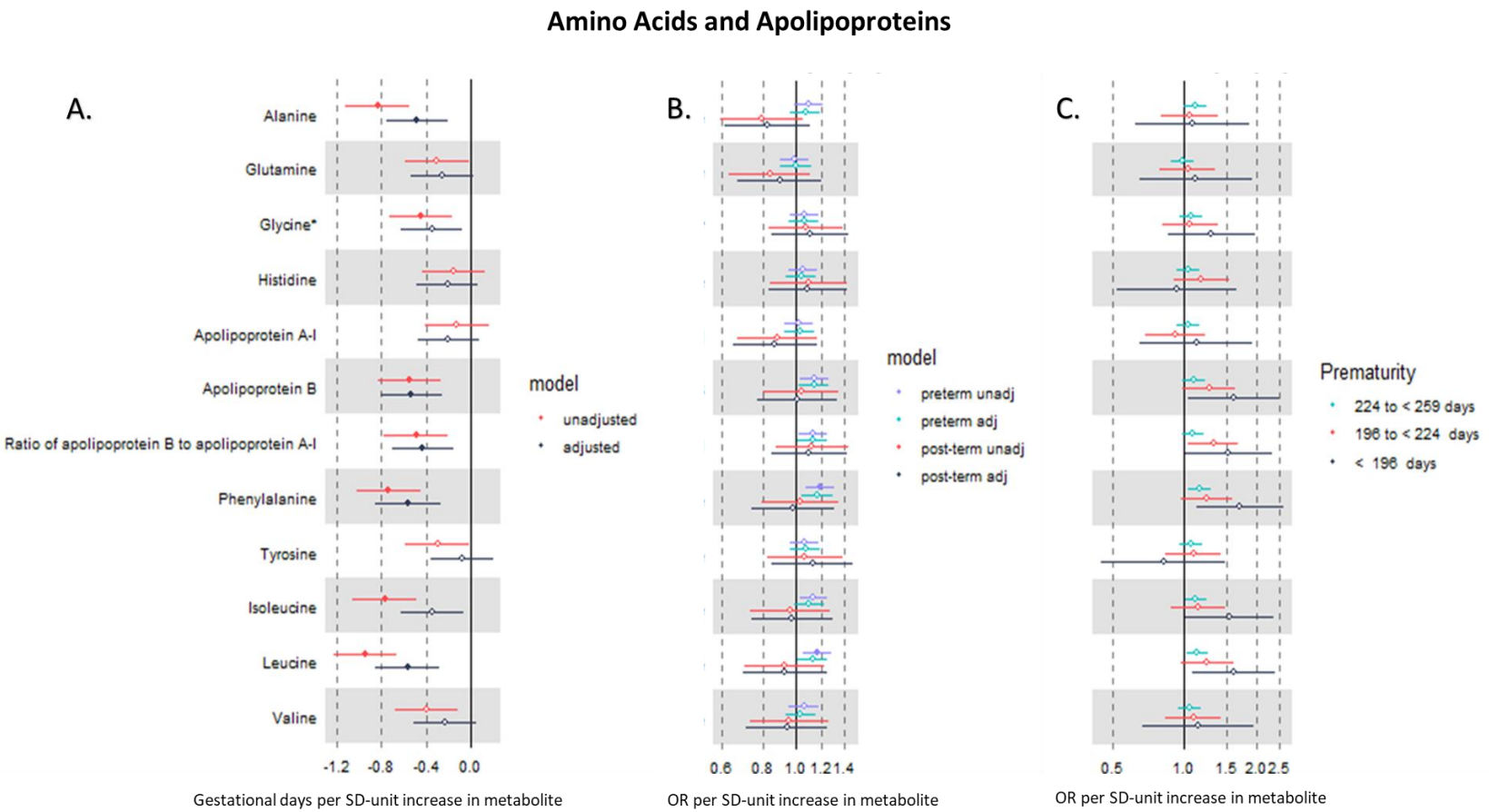

Figure 4.5: Associations of maternal gestational amino acids and apoplipoproteins concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher amino acid and apolipoprotein concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher amino acid and apolipoprotein concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher amino acid and apolipoprotein concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

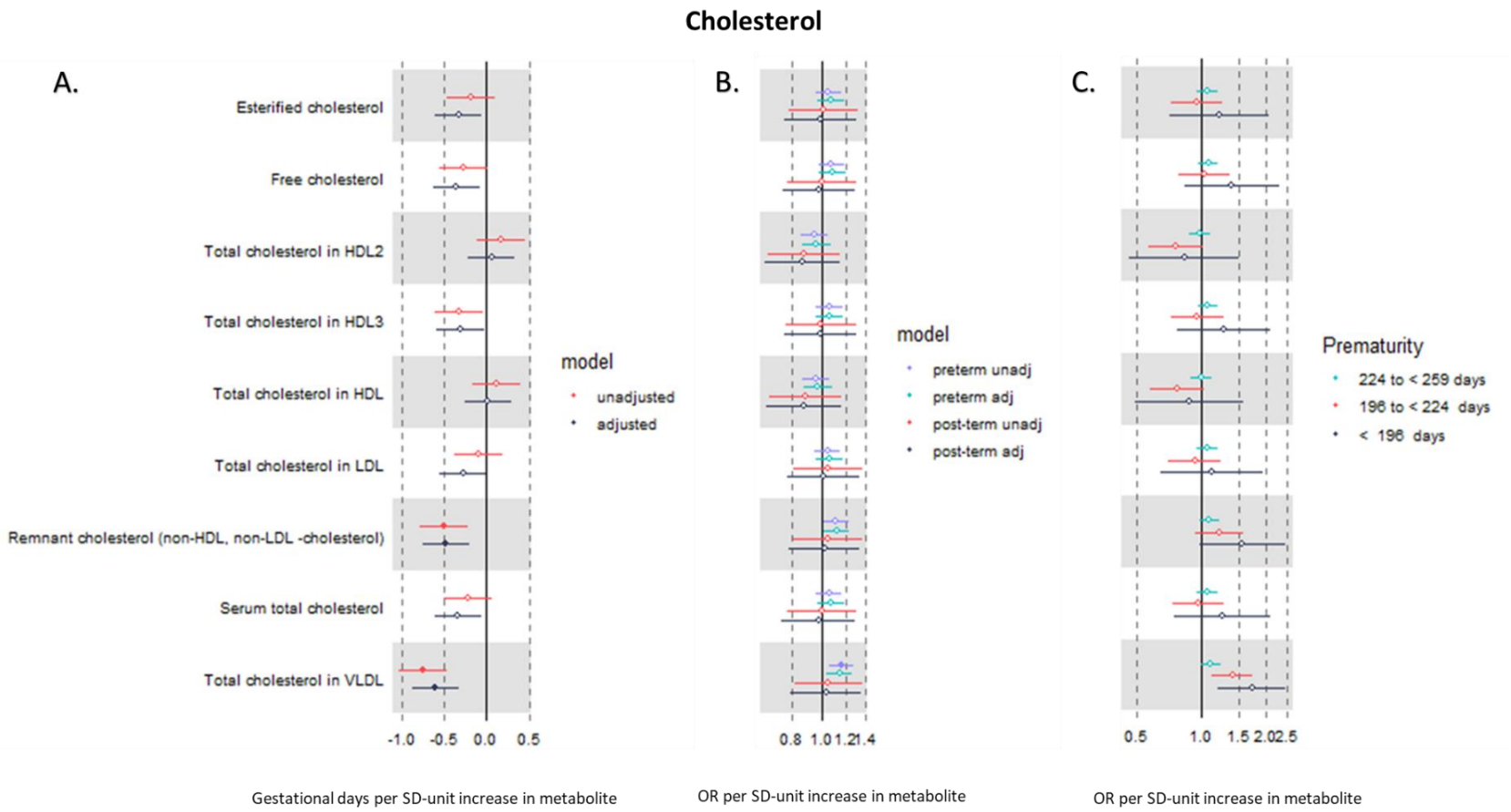

Figure 4.6: Associations of maternal gestational cholesterol metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher cholesterol concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher cholesterol concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher cholesterol concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

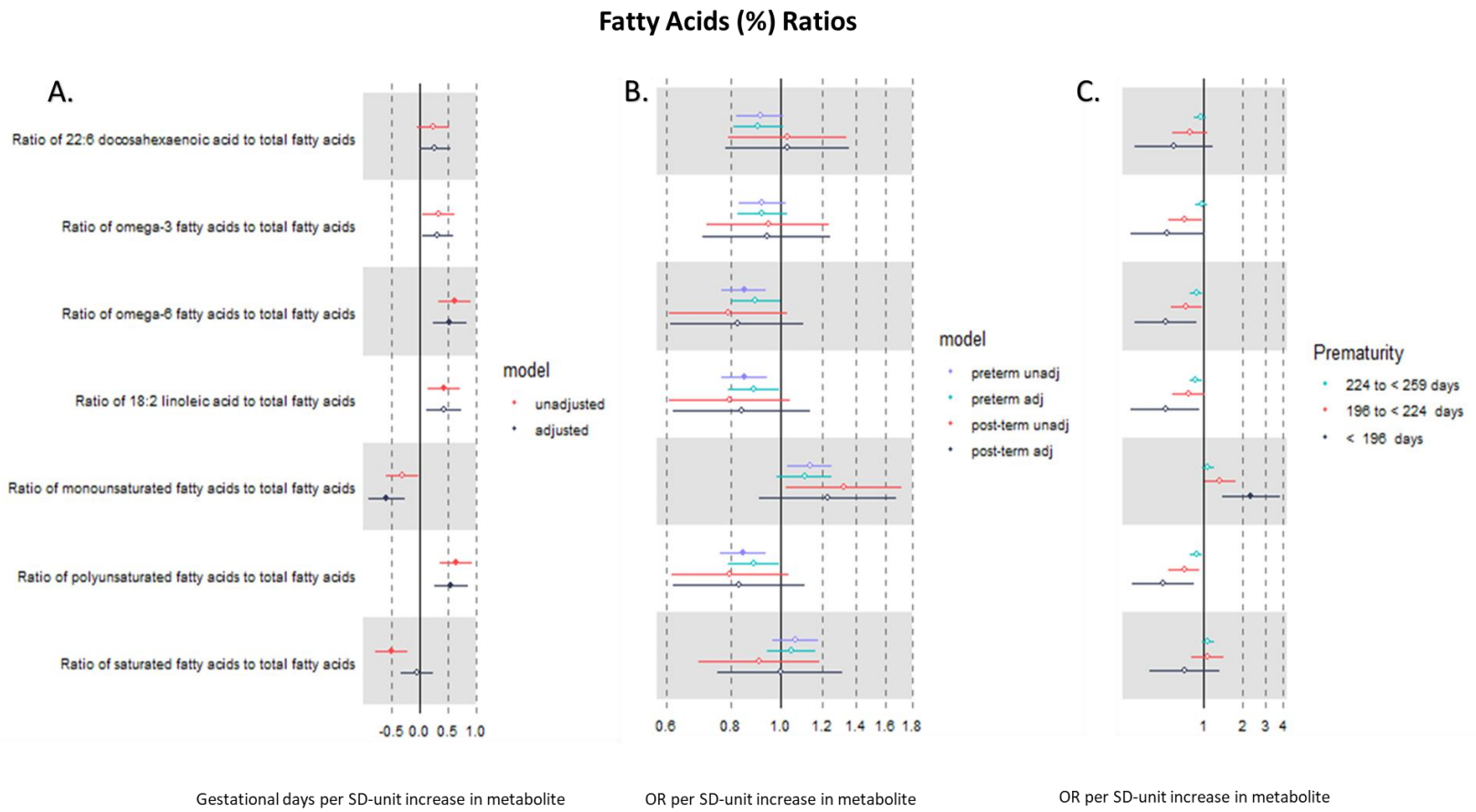

Figure 4.7: Associations of maternal gestational fatty acids (%) metabolites ratios (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher fatty acids (%) ratios concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher fatty acids (%) ratios concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher fatty acids (%) ratios concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

Figure 4.8: Associations of maternal gestational fatty acids metabolites concentrations and saturation measures (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher fatty acids and saturation measures concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher fatty acids and saturation measures concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher fatty acids and saturation measures concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

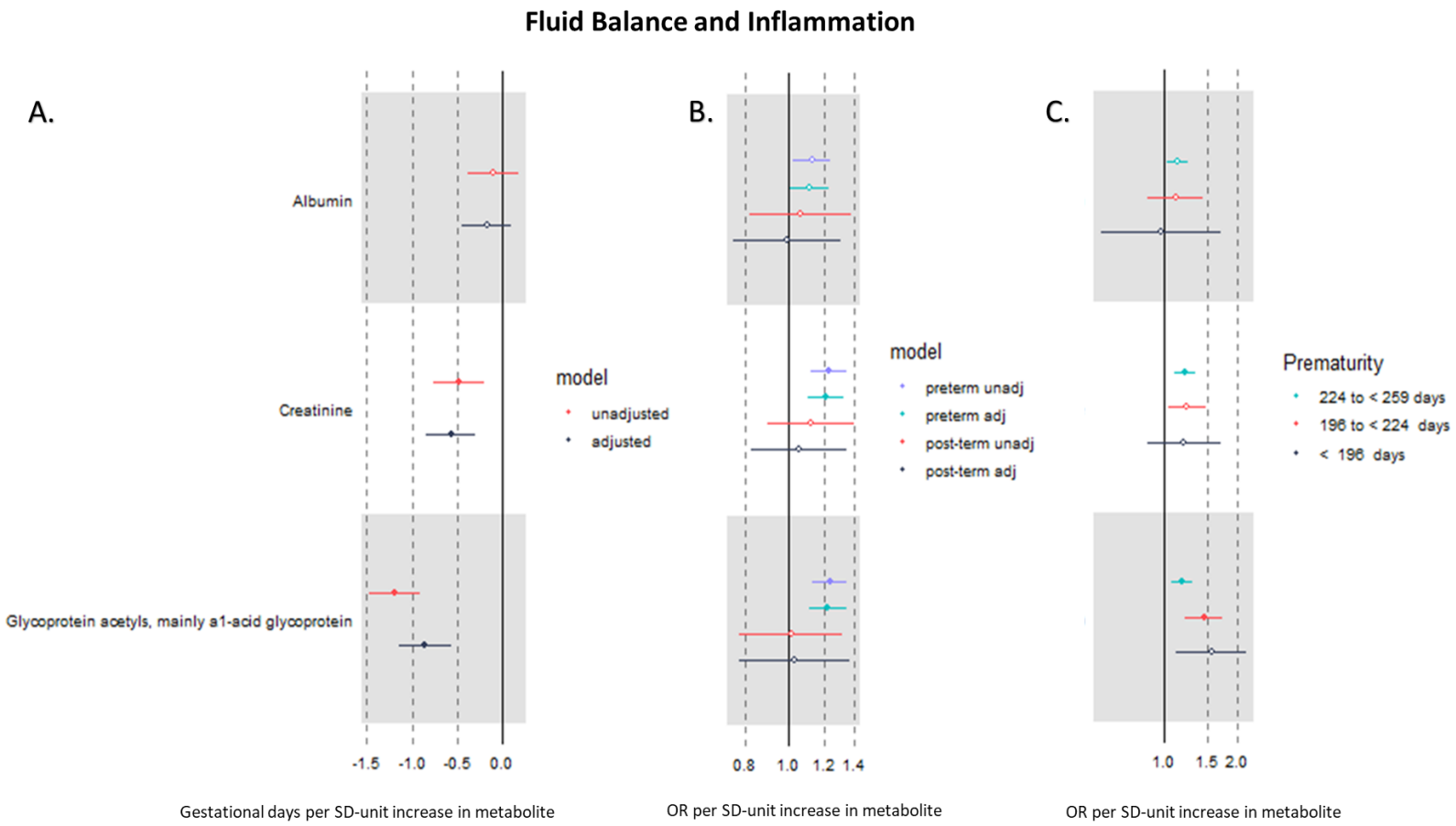

Figure 4.9: Associations of maternal gestational fluid balance and inflammation metabolite concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher fluid balance and inflammation markers concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher fluid balance and inflammation markers concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher fluid balance and inflammation markers concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

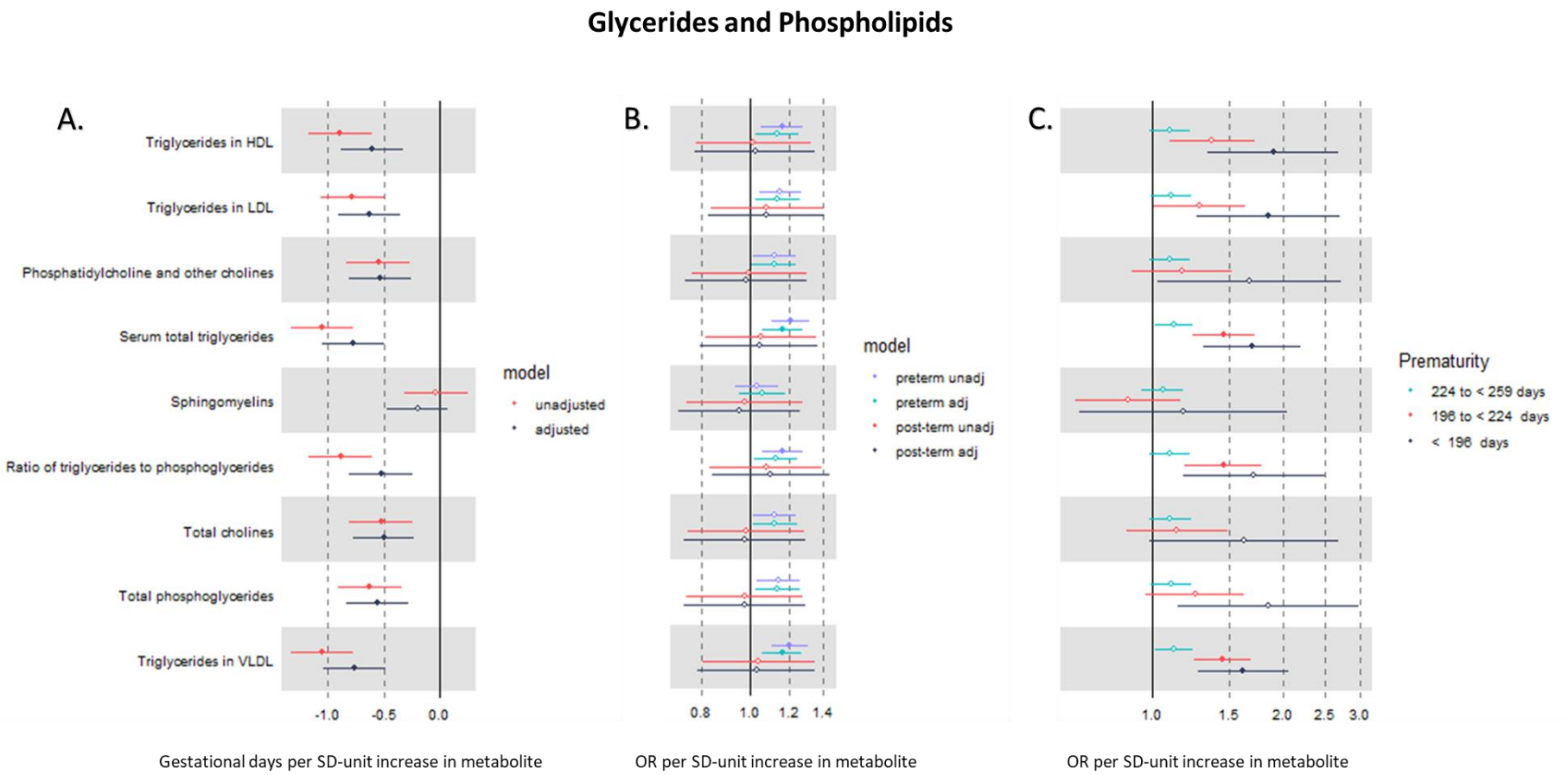

Figure 4.10: Associations of maternal gestational glycerides and phospholipids metabolite concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher glycerides and phospholipids concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher glycerides and phospholipids concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher glycerides and phospholipids concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

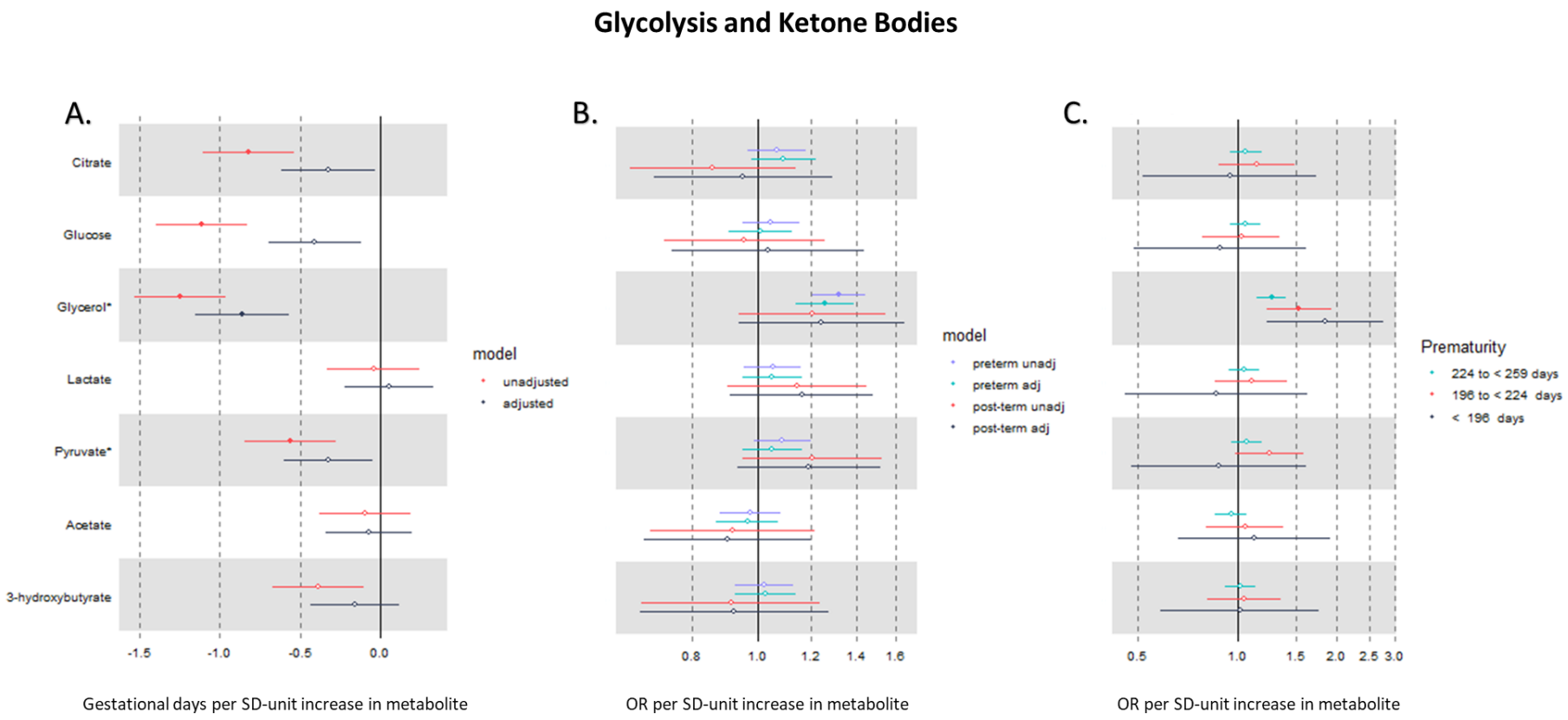

Figure 4.11: Associations of maternal gestational glycolysis and ketone bodies metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher glycolysis intermediates and ketone bodies concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher glycolysis intermediates and ketone bodies concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher glycolysis intermediates and ketone bodies concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

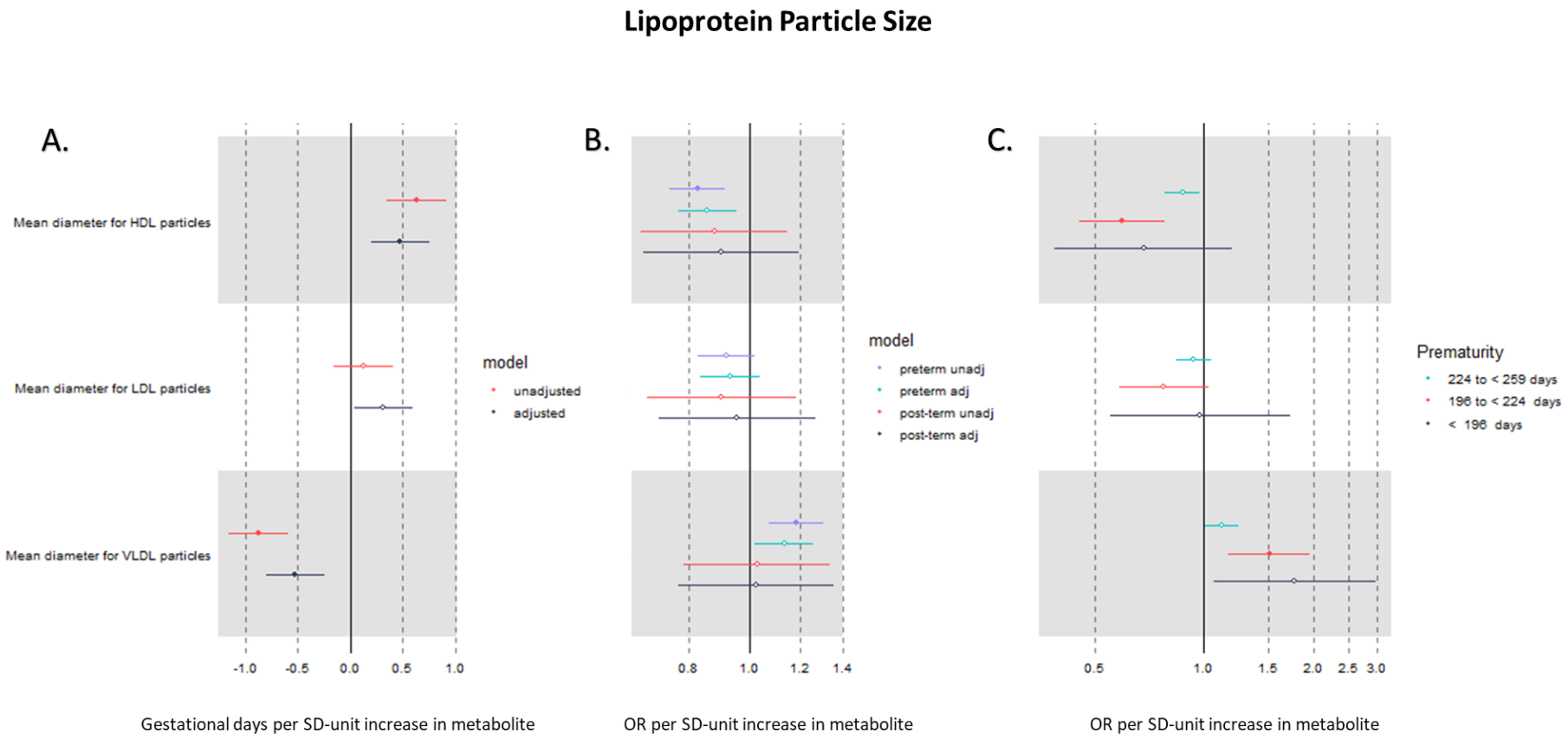

Figure 4.12: Associations of maternal gestational lipoprotein particle size (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher lipoprotein particle size , with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher lipoprotein particle size, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher lipoprotein particle size , with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

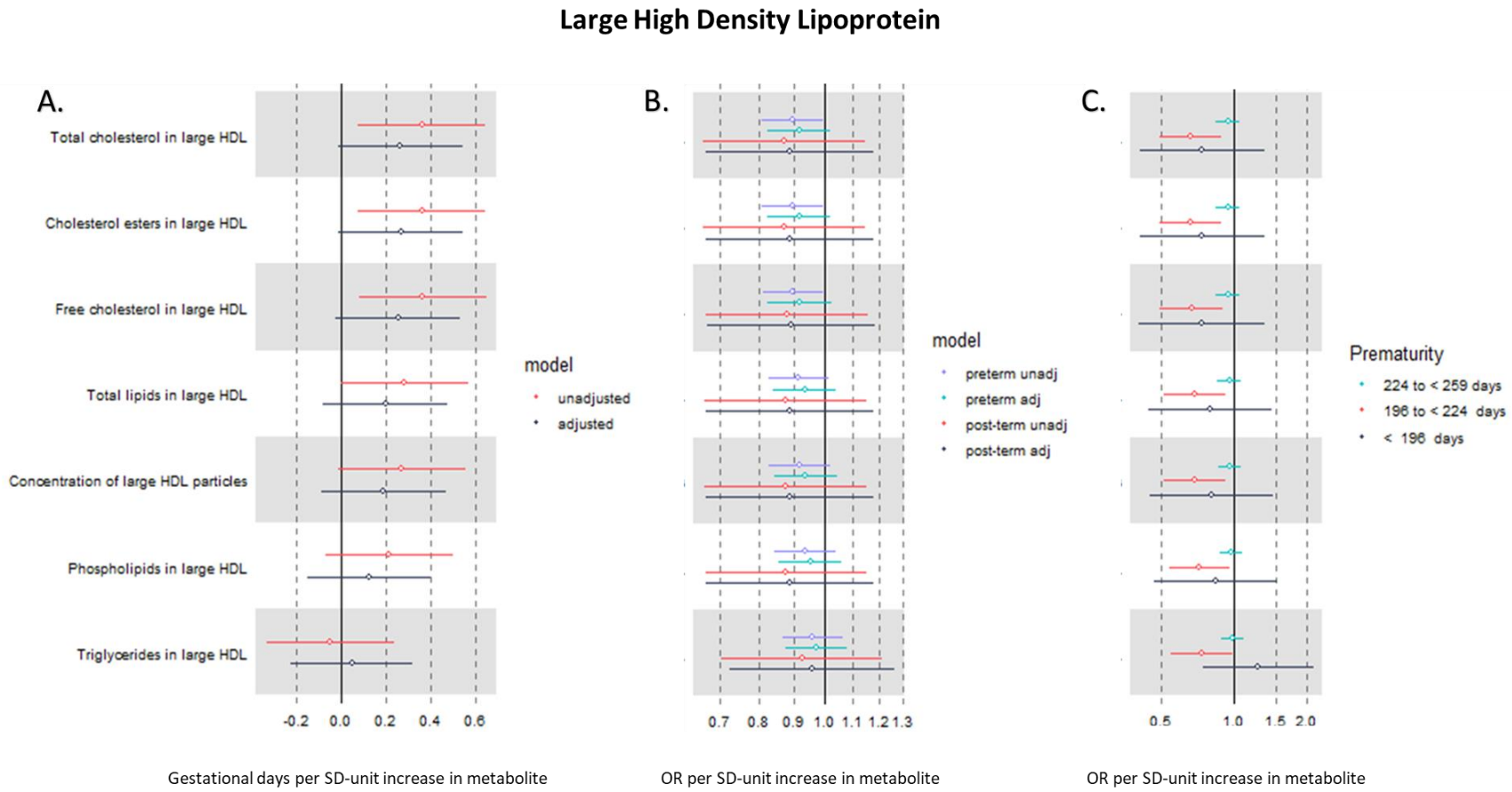

Figure 4.13: Associations of maternal gestational large high density lipoprotein metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher large high density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher large high density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher large high density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

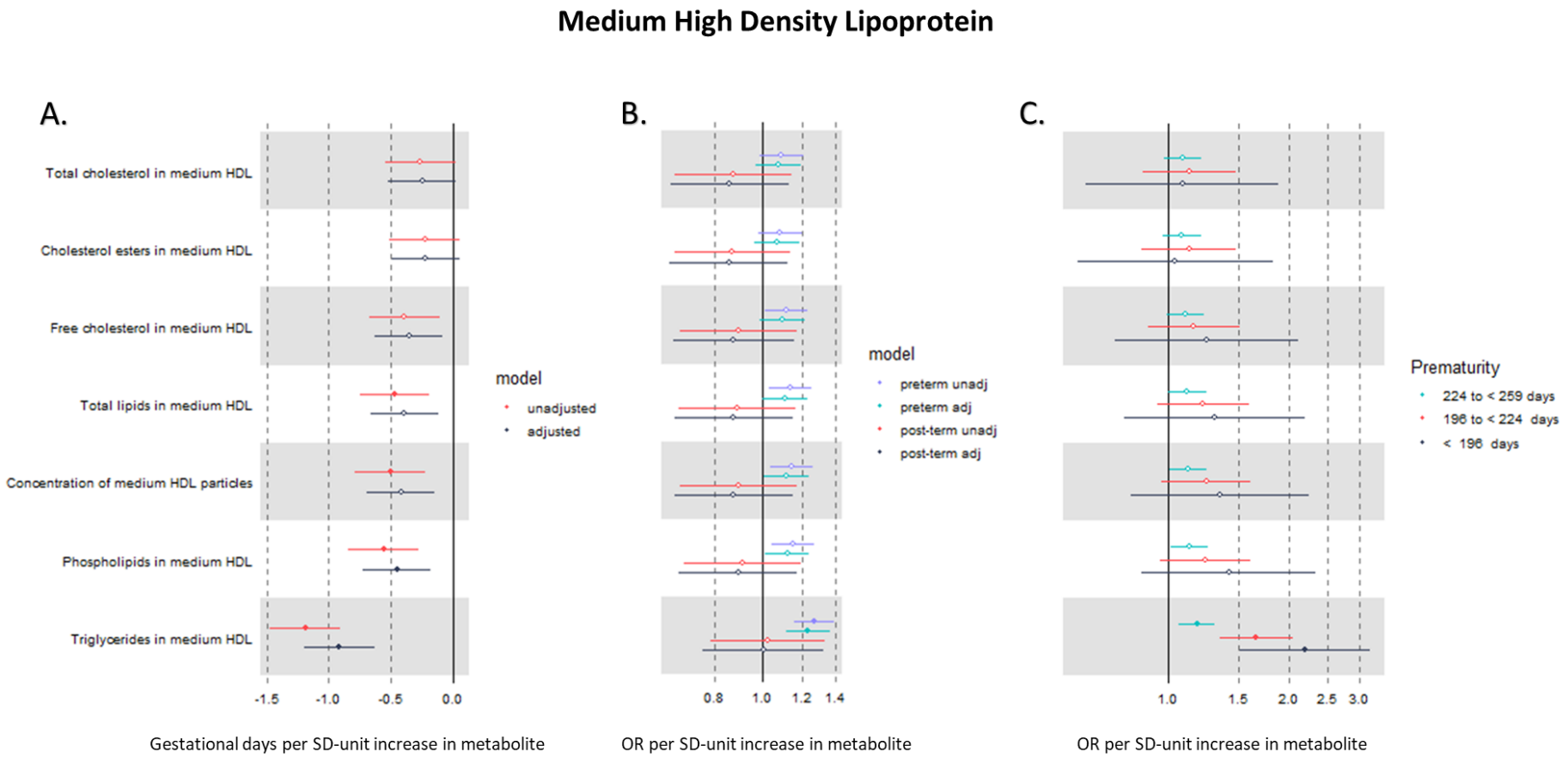

Figure 4.14: Associations of maternal gestational medium high density lipoprotein metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher medium high density lipoprotein traits concentration, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher medium high density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher medium high density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

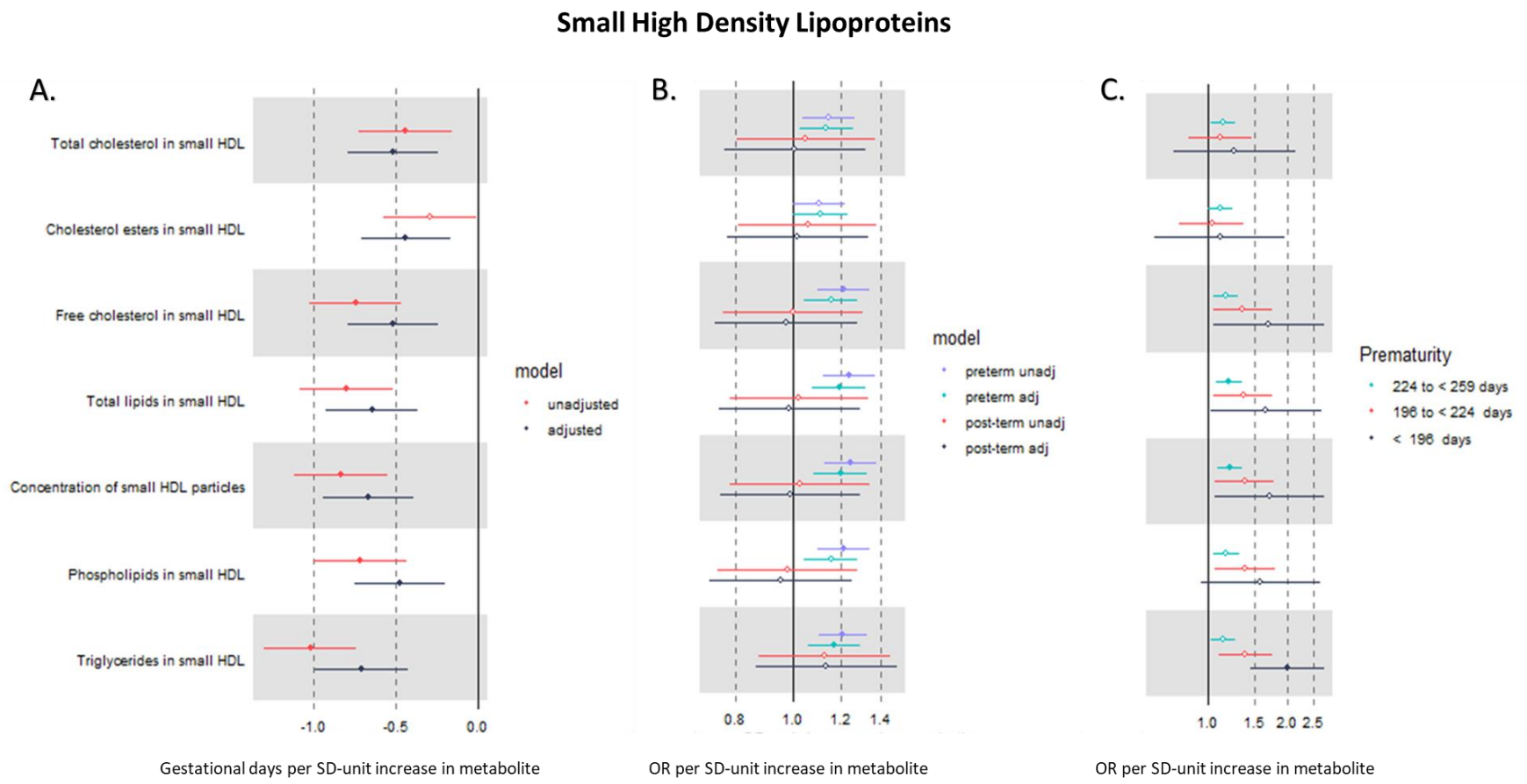

Figure 4.15: Associations of maternal gestational small high density lipoproteins metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher small high density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher small high density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher small high density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

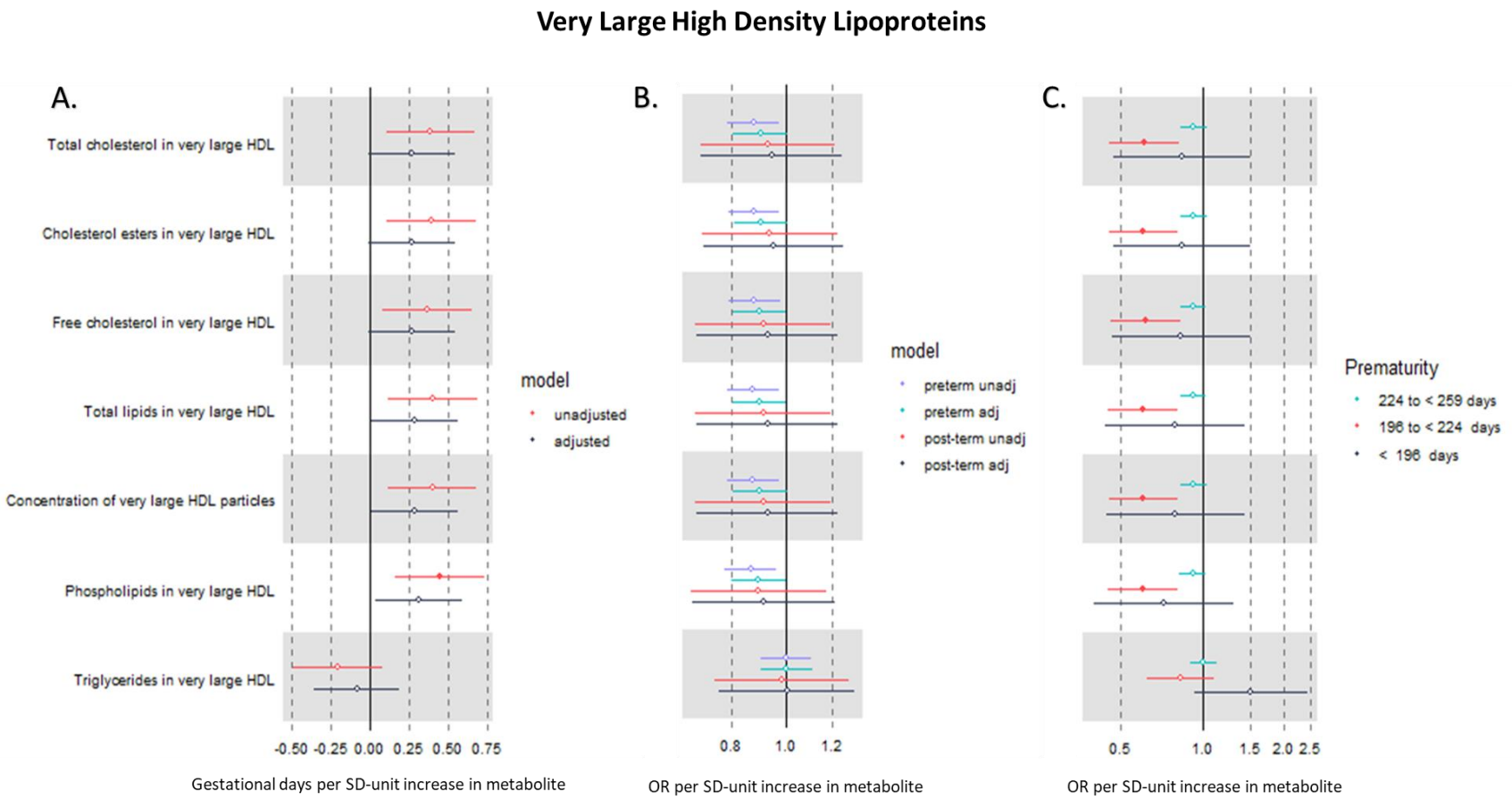

Figure 4.16: Associations of maternal gestational very large high density lipoprotein metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher very large high density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher very large high density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher very large high density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

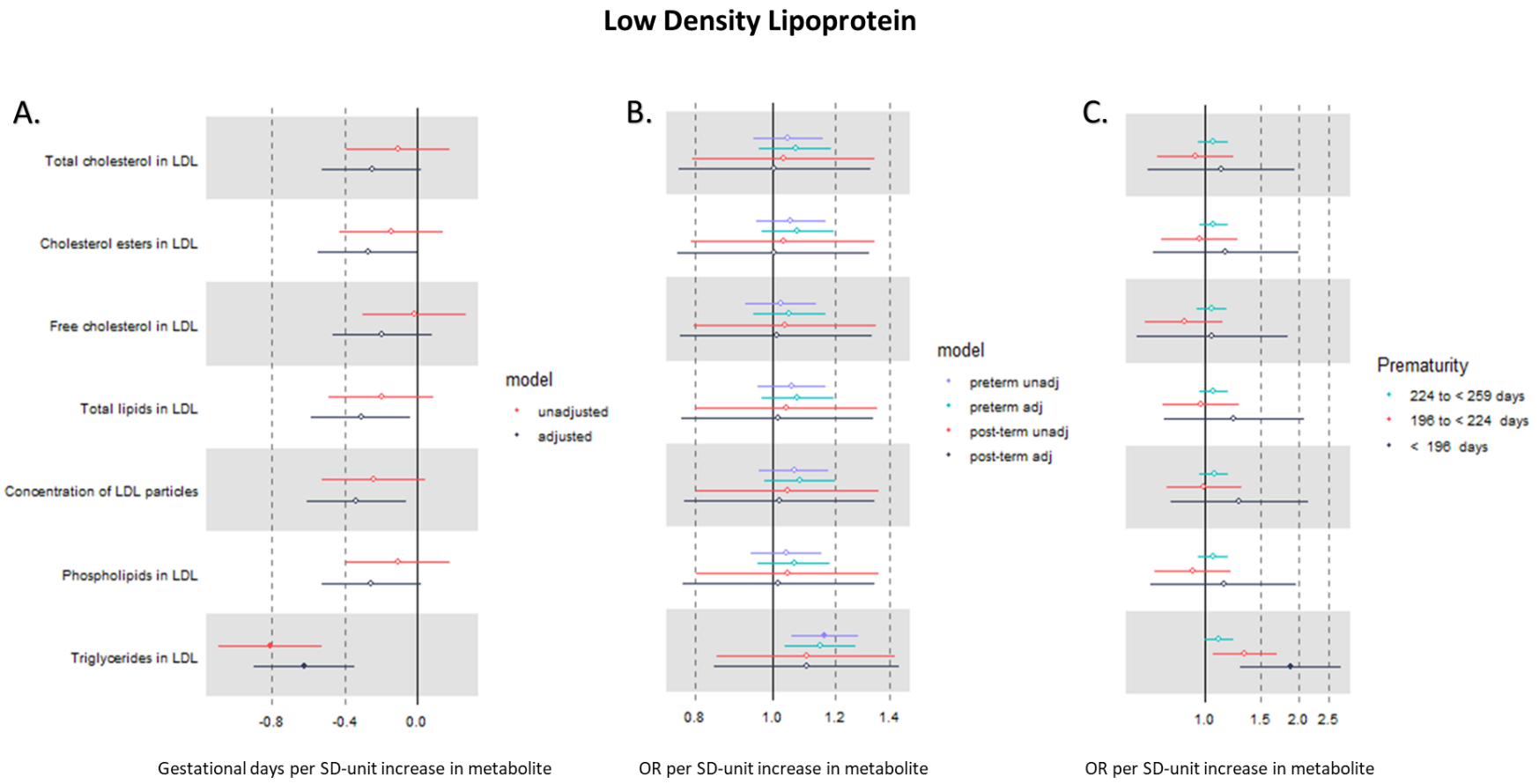

Figure 4.17: Associations of maternal gestational low density lipoprotein metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

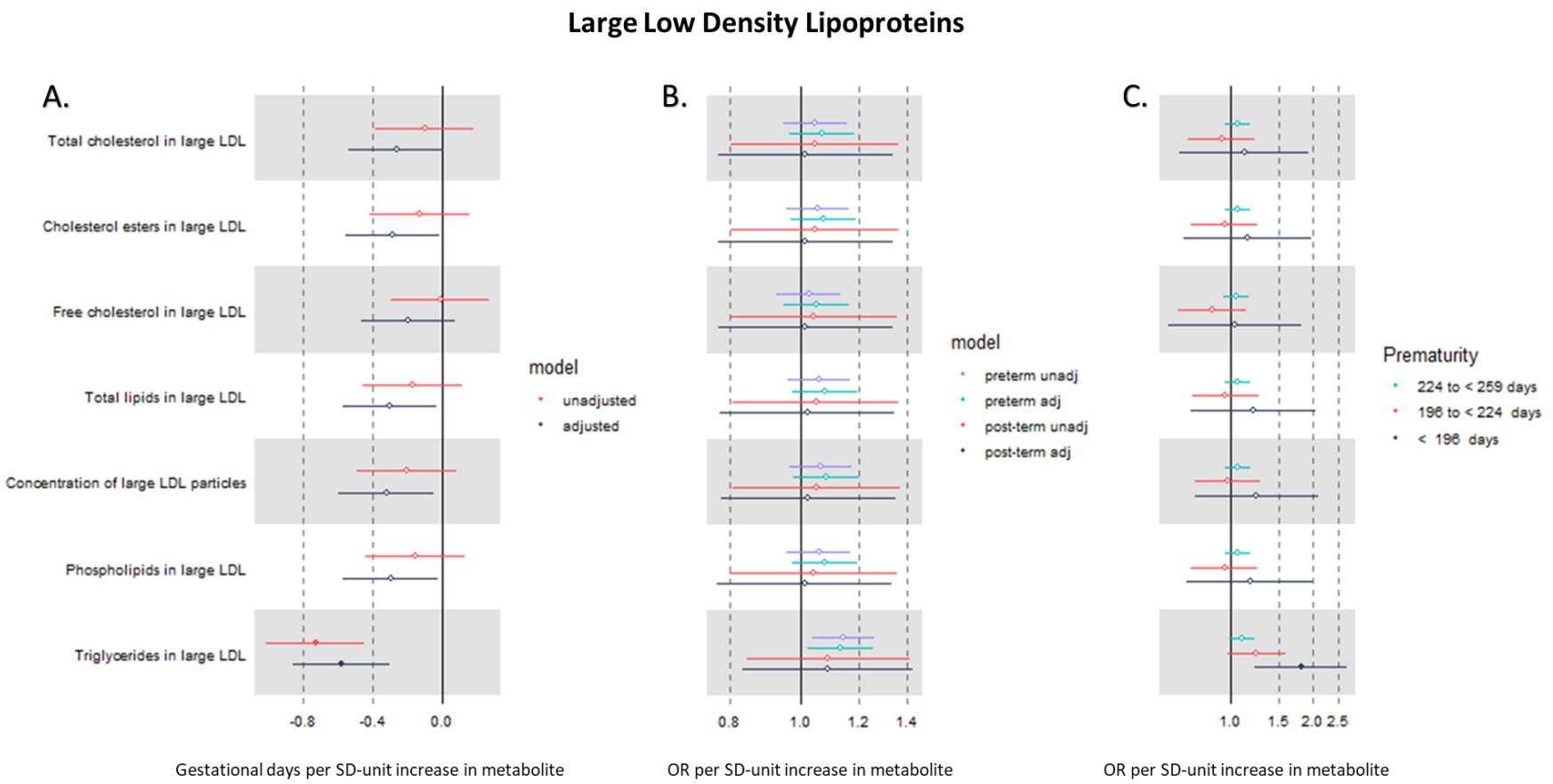

Figure 4.18: Associations of maternal gestational large low density lipoprotein metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher large low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher large low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher large low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

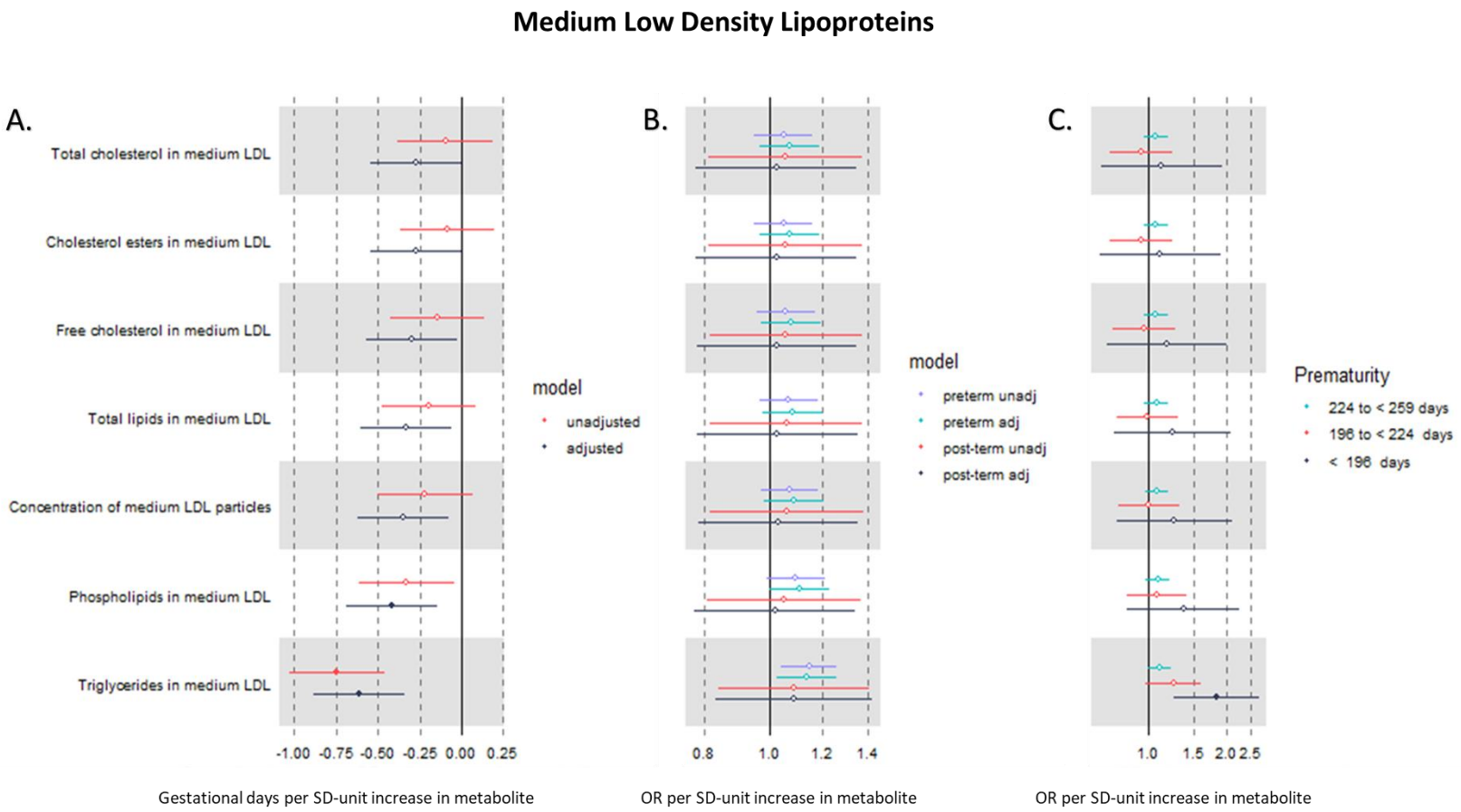

Figure 4.19: Associations of maternal gestational medium low density lipoprotein metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher medium low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher medium low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher medium low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

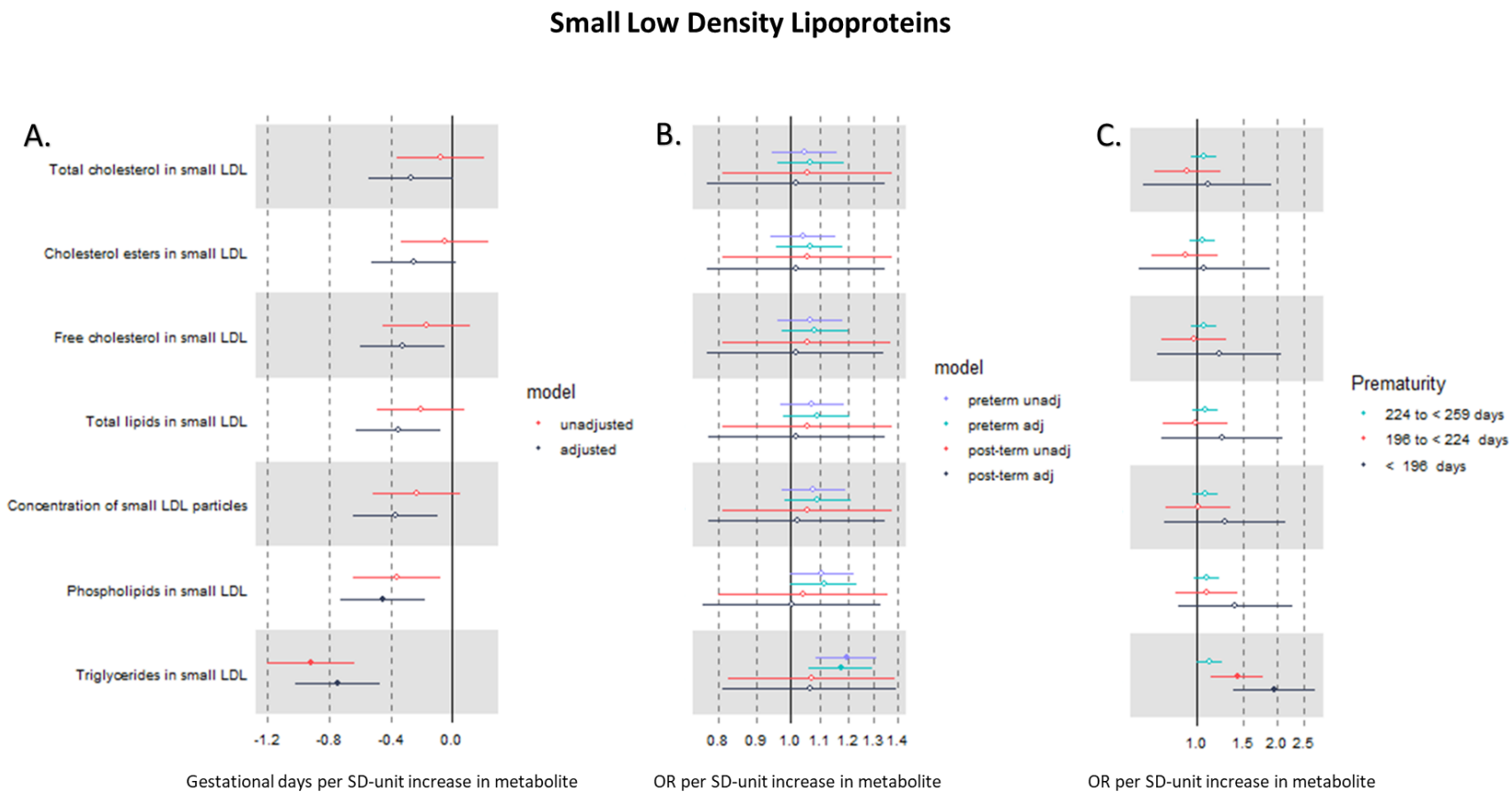

Figure 4.20: Associations of maternal gestational small low density lipoproteins metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher small low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher small low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher small low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

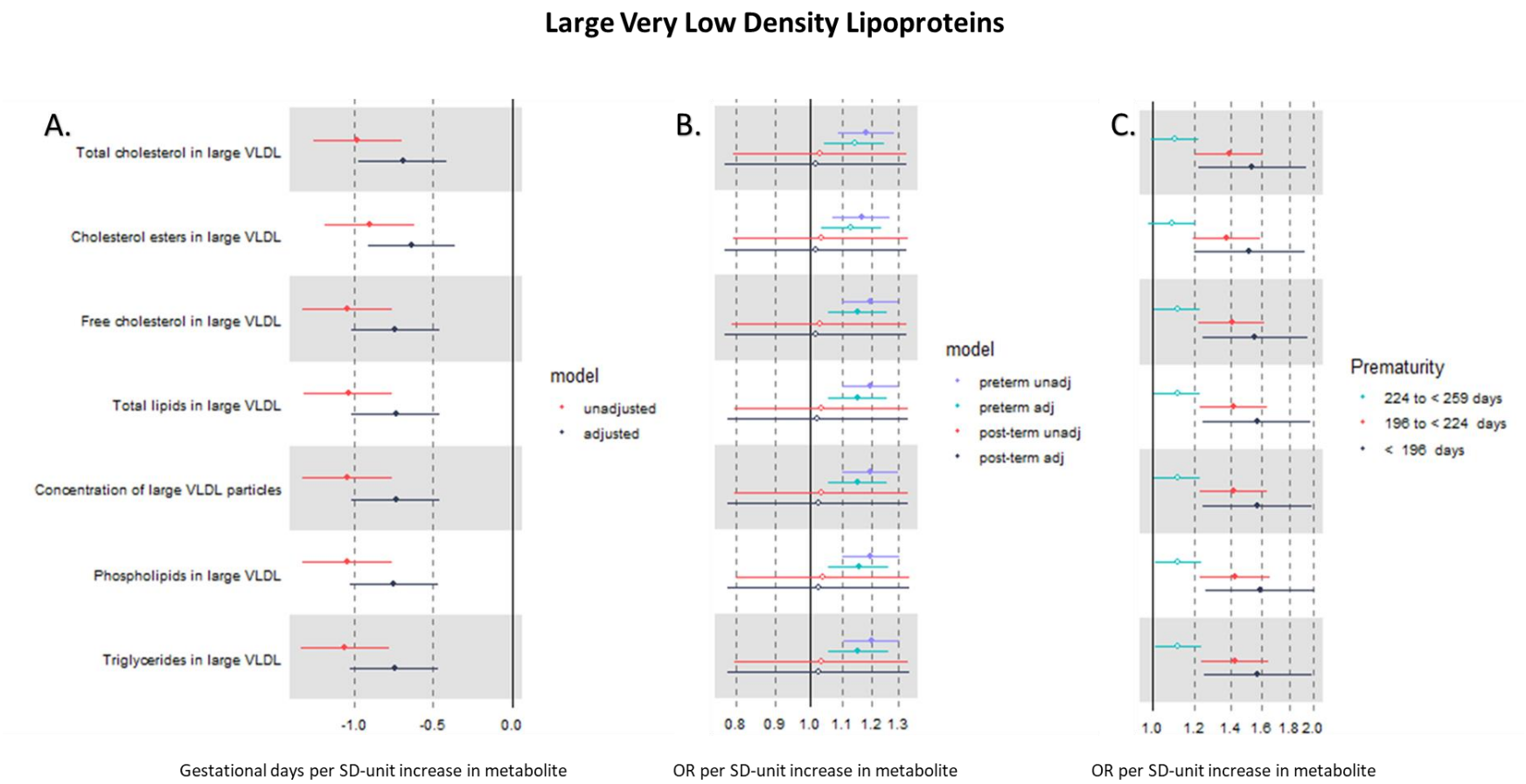

Figure 4.21: Associations of maternal gestational large very low density lipoproteins metabolite concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher large very low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher large very low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher large very low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

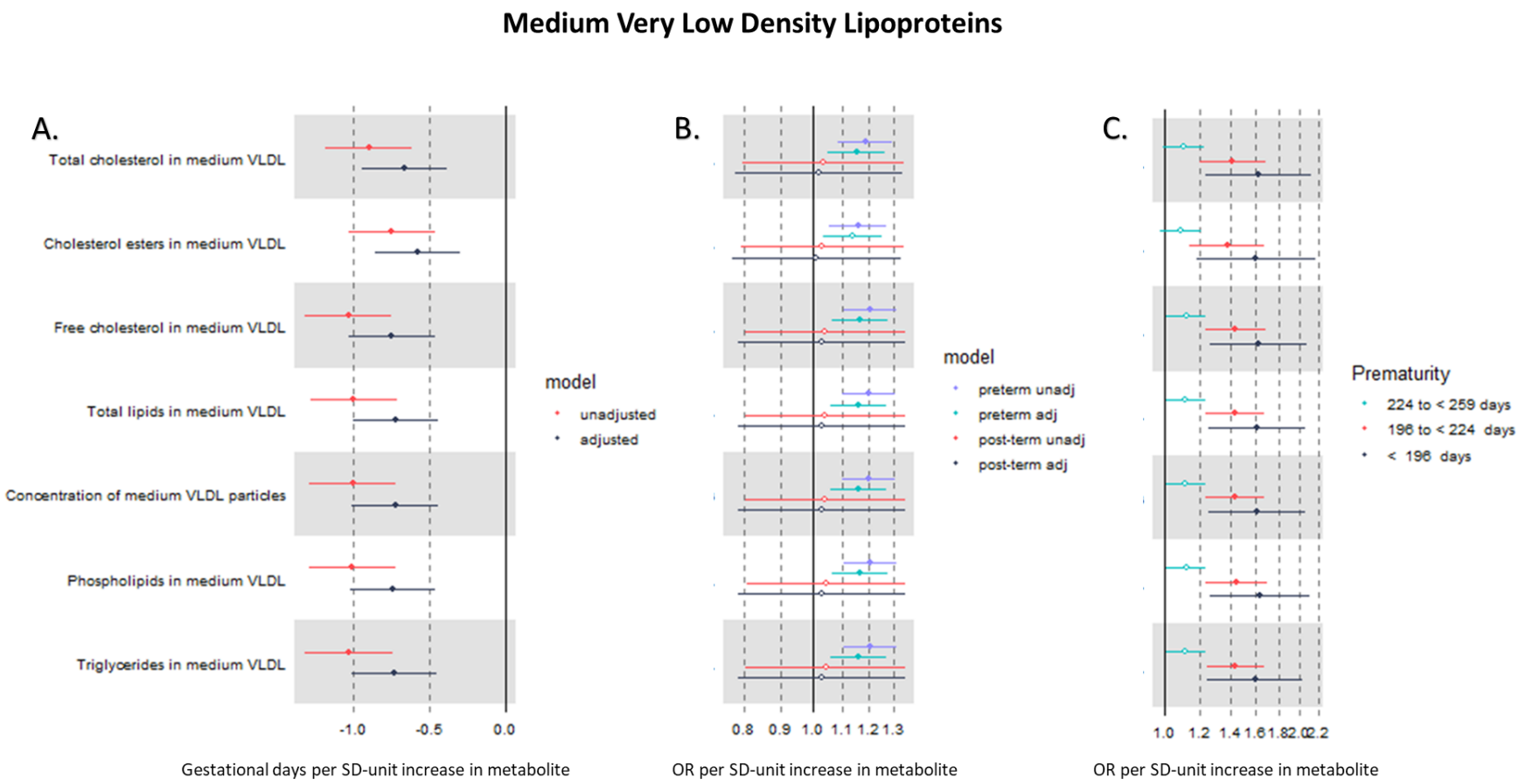

Figure 4.22: Associations of maternal gestational medium very low density lipoproteins metabolite concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher medium very low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher medium very low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher medium very low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

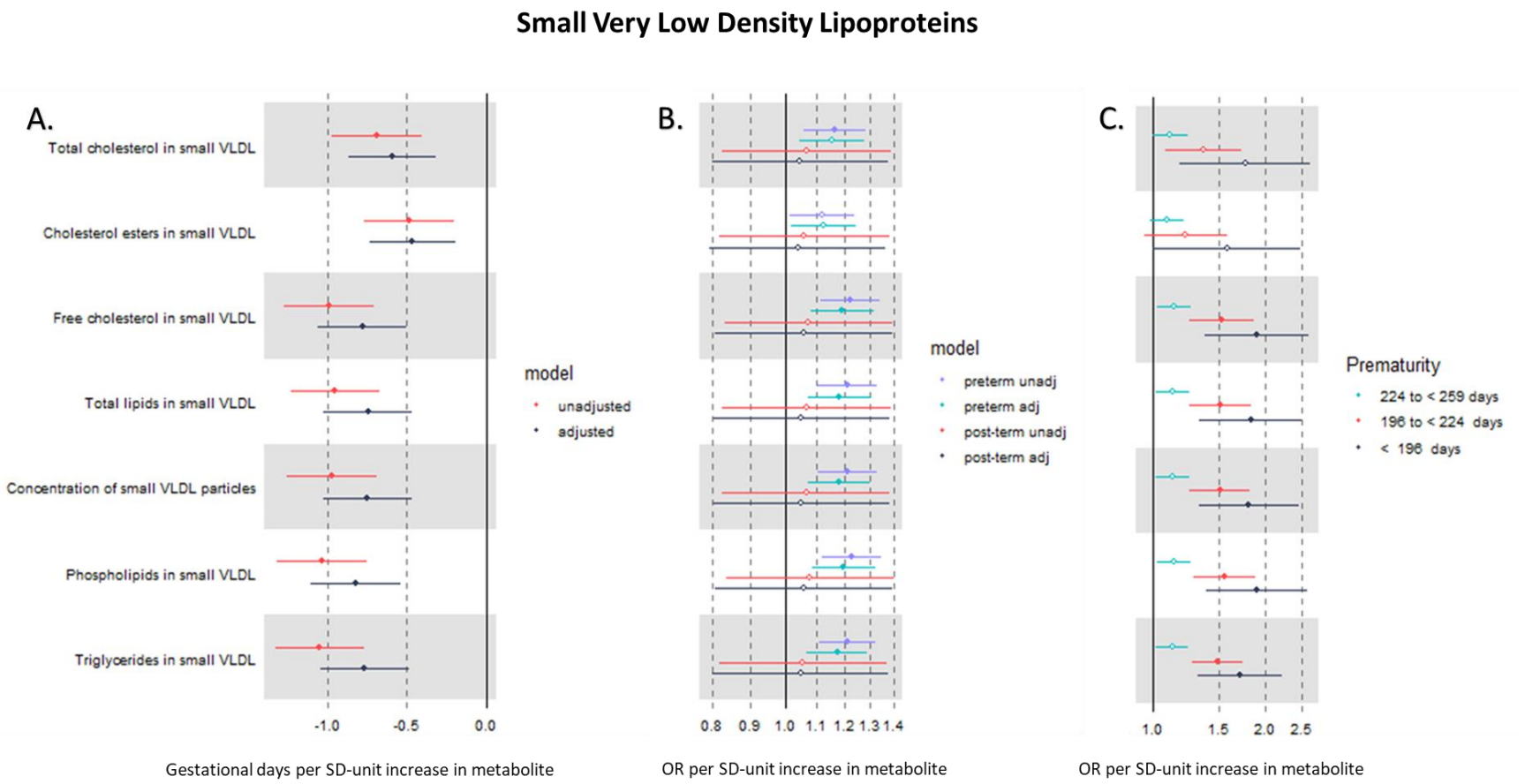

Figure 4.23: Associations of maternal gestational small very low density lipoproteins metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher small very low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher small very low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher small very low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

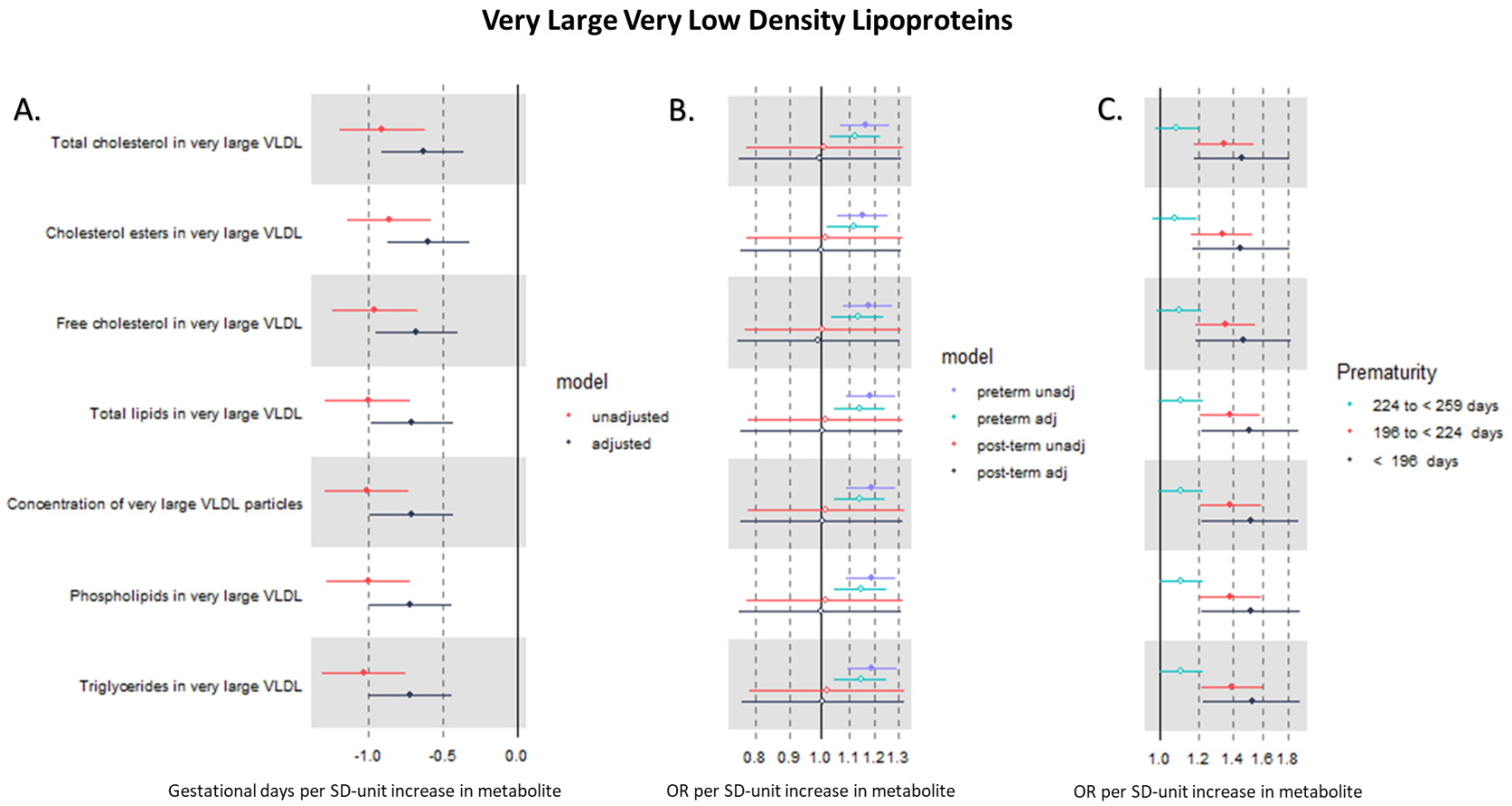

Figure 4.24: Associations of maternal gestational very large very low density lipoproteins metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher very large very low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher very large very low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher very large very low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

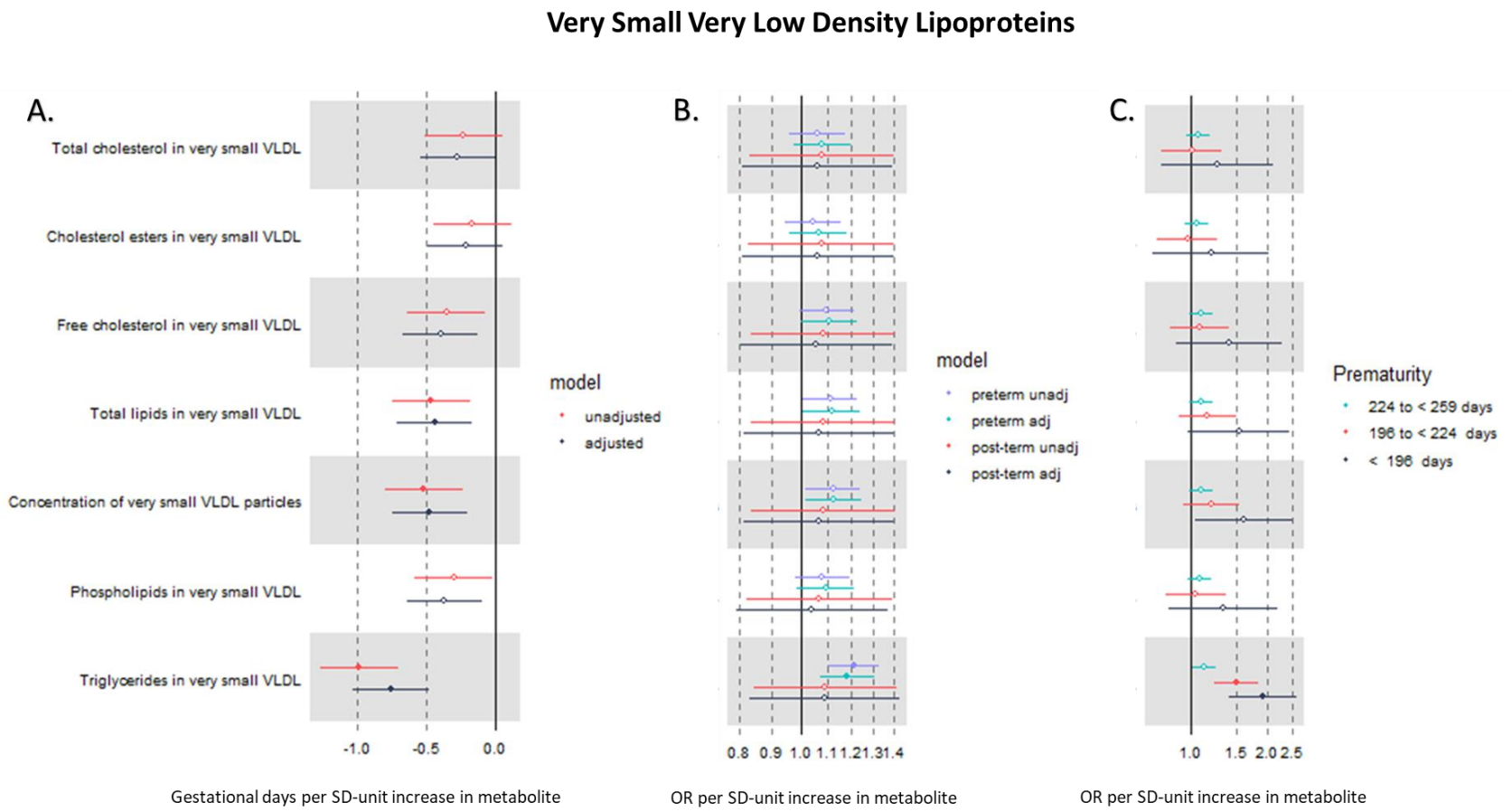

Figure 4.25: Associations of maternal gestational very small very low density lipoproteins metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher very small very low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher very small very low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher very small very low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

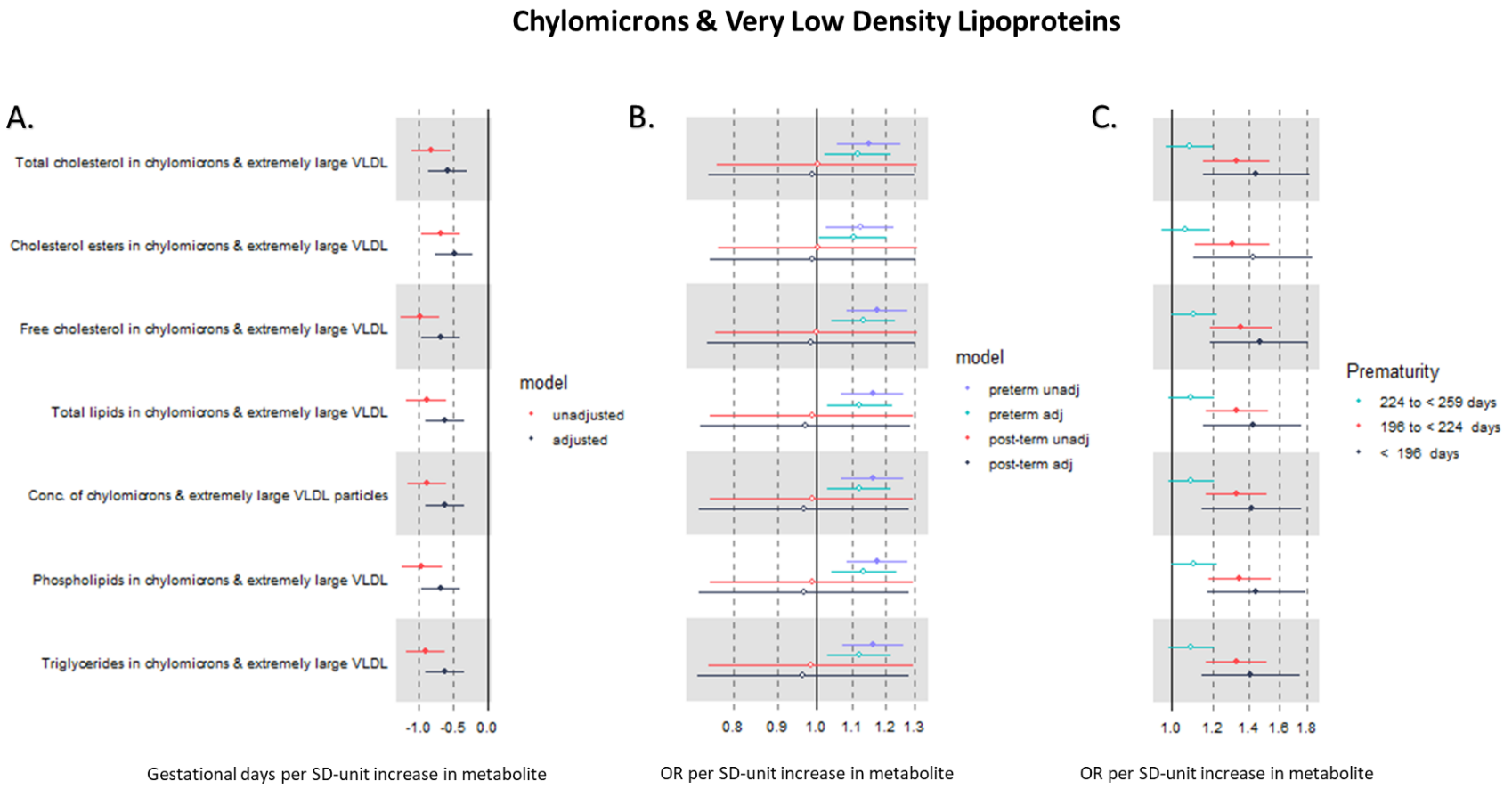

Figure 4.26: Associations of maternal gestational chylomicrons and very low density lipoproteins metabolites concentrations (measured at 26-28 completed weeks' gestation) with gestational age at delivery: Panel A shows results of adjusted (black) and unadjusted (red) difference in mean gestational age in days per 1SD higher chylomicrons & very low density lipoprotein traits concentrations, with their 95% confidence intervals (n=7440). Panel B shows results of adjusted and unadjusted odds ratios of preterm (<259 days or <37 completed weeks) compared to term (reference; 259 days to 294 days, or 37-42 completed weeks) and post-term (>294 days or >42 completed weeks) compared to term per 1SD higher chylomicrons & very low density lipoprotein traits concentration, with 95%CI [adjusted preterm results are light blue, unadjusted preterm are purple, adjusted postterm are black and unadjusted posterm are red] N = 392 for preterm, 6994 for term, and 54 for post-term. Panel C shows of unadjusted odds ratios of extremely preterm (<196 days, or <28 completed weeks) compared to term, very preterm (196 days to <224 days, or 28 to <32 completed weeks) compared to term, and moderate to late preterm (224 days to <259 days, or 32 weeks to <37 weeks) compared to term per 1SD higher chylomicrons & very low density lipoprotein traits concentration, with 95%CI [extremely preterm are black, very preterm are red, and moderate to late preterm are light blue]. N = 12 for extremely preterm results, 51 for very preterm results, and 329 for moderate to late preterm. Solid circles indicate significant at the p=0.0029 threshold, open circles indicate non-significant result.

4.7 Discussion

The results from this chapter show that there are associations between concentrations of numerous maternal circulating metabolites measured in the second trimester and gestational age at delivery. While it is not possible to say that any of the associations described here are causal, they do provide clues as to what may initiate spontaneous labour in humans and therefore indicate which metabolites should be prioritised in further investigations. These findings consistently point to triglycerides and numerous lipoprotein-related traits being associated with reduced gestational age at delivery, specifically VLDLs and HDL lipids, particle concentration and mean diameter, as well as other non-lipoprotein-related metabolites including glycoprotein acetyls (a1-acid glycoprotein), glycerol, creatinine, and amino acids such as phenylalanine, alanine and leucine. Although it could be argued that the observed differences in gestational age of not more than one day with a 1SD increase in concentration of metabolites is not clinically significant when taken in isolation, the persistent associations presented are useful in beginning to answer the aims of this study. Identification and investigation of these associations provide further evidence to putative factors involved in spontaneous parturition in humans.

It is interesting that for some of the metabolites measured increases in concentration were associated with altered odds of delivering preterm

compared with term, whereas there were no alterations in the odds of delivering post-term compared with term. This may be partly a result of low statistical power since few observations were characterised as post-term, however it should be emphasised that effect estimates were typically centred around the null value, and, therefore, it is likely that those who delivered at term had a similar metabolomic profile to those that delivered post-term. The sensitivity analysis involving splitting of the preterm group into extremely, very, and moderate and late preterm, while unadjusted and underpowered, indicates that there could be a dose-response association between these metabolites and the degree of prematurity.

4.7.1 Lipoproteins and triglycerides

Triglycerides and cholesterol are both insoluble in water and transported in the plasma within lipoproteins[274]. Triglycerides are comprised of three fatty acid molecules attached to a glycerol spine and are hydrolysed by lipases in the gut to form fatty acids and monoglycerides. Monoglycerides are re-esterified within enterocytes and incorporated into lipoproteins and chylomicrons[275]. Lipoproteins are complex particles containing a central core of triglycerides and cholesterol esters surrounded by phospholipids, free cholesterol, and apolipoproteins. There are seven classes of plasma lipoproteins, categorised according to size and lipid composition: very low density lipoprotein (VLDL),

intermediate density lipoprotein (IDL), low density lipoprotein (LDL), high density lipoprotein (HDL), chylomicron, chylomicron remnants, and Lipoprotein a (lp(a)). Chylomicrons are the largest and least dense lipoproteins, while HDL are the smallest and most dense[274, 276]. Chylomicron remnants, Lp(a), VLDL, IDL and LDL are reported to be pro-atherogenic, while HDL is reported to be anti-atherogenic; and increased plasma concentrations of cholesterol and lipoprotein are implicated in the formation of atherosclerosis and cardiac disease[274, 276]. While the effects of cardiac disease may not be initially seen to be directly relevant to answering the aims of this thesis, vascular impairment plays a role in pathological conditions of pregnancy, such as pre-eclampsia, intrauterine growth restriction and preterm birth[277], and therefore it is plausible that such interactions are important for gestational age at delivery.

Under normal conditions, hepatic triglyceride is released by the liver into the circulation via VLDL particles[275], and triglycerides are subsequently removed from VLDL by muscle and adipose tissues, resulting in the formation of IDLs which are enriched in cholesterol. LDLs are derived from both IDL and VLDL and hold most of circulating cholesterol. There is an association between concentrations of the smallest and most dense LDL particles and increased circulating triglycerides (hypertriglyceridemia), lower HDL concentrations, type

2 diabetes, obesity, metabolic syndrome and inflammatory and infectious states[274, 276].

Conversely, HDLs are important for reverse cholesterol transport, moving cholesterol from the peripheral tissues, including away from the walls of vessels, back to the liver for excretion, and thereby providing an anti-atherogenic effect[278,279]. HDL is a lipoprotein complex that is protein and cholesterol ester-rich, with reported inverse associations between HDL concentration and risk for cardiovascular disease[278]. HDL is anti-inflammatory, anti-apoptotic, anti-oxidant and anti-thrombotic[274]. As an anti-oxidant, HDL reduces the oxidative stress from oxidised LDL, and its role in promoting anticoagulation involves enhancement of the activity of protein S and protein C, and through inhibition of TNFα, neutrophil activation, adhesion and infiltration[278]; both of which could be important in pregnancy maintenance and gestational length. In the non-pregnant state, high plasma triglyceride concentrations result in HDL and LDL taking on more triglyceride from VLDL in exchange for cholesteryl esters. Hepatic lipase then catalyses the triglyceride-rich HDL, resulting in smaller HDL particles and thereby reducing the concentration of plasma HDL[278,277].

4.7.2 Lipoproteins and pregnancy

There are known changes in maternal lipoprotein metabolism during pregnancy, thought in part to be due to changes in insulin responsiveness. In normal early

pregnancy, there is heightened maternal insulin sensitivity and adipose tissue is stored, presumably in preparation for the increased demand by the fetus in later pregnancy and as a substrate for steroid hormone synthesis by the placenta[277-279]. Insulin sensitivity then decreases, with a shift towards insulin-resistance, as pregnancy progresses towards and into the third trimester[280], alongside a hypercoagulable, hyperlipidaemic, and increased inflammatory state[277-280]. It therefore appears that a certain balance is required here for ongoing pregnancy, and an imbalance, as suggested by the results of this study, this may lower the threshold for spontaneous labour.

VLDL holds most plasma triglycerides in both pregnant and non-pregnant women, and larger VLDLs contain more triglyceride. VLDL concentrations increase during normal pregnancy, likely secondary to the increased insulin resistance promoting release of fatty acids through lipolysis and increasing glucose availability for the fetus[278, 279], and increased VLDL particle size has been linked with recurrent preterm delivery[279]. In the non-pregnant state, HDL concentrations decrease when there is increased insulin resistance and increased triglycerides, for example in people who are obese or have type 2 diabetes[279]. Conversely, during pregnancy HDL concentrations increase across the first and second trimester[278] despite increasing triglyceride levels. It is suggested that the cause of the initial rise in HDL during pregnancy at around 10

weeks counters the oxidative stress resulting from establishment of the placental circulation (also around 10 weeks) with invasion of the trophoblast and release of the maternal spiral arteries[278]. The increased production of HDL during pregnancy, potentially driven by oestradiol, could be an adaptive and protective response to increased insulin resistance and increased triglyceride plasma concentrations during pregnancy, which could otherwise result in severe endothelial dysfunction and long-term damage[278]. During the third trimester HDL concentrations decrease[279].

In women who are obese, pregnancy is associated with both increased concentrations of triglycerides and VLDL, and decreased concentrations of HDL, when compared with pregnant women who are not obese. Previous researchers have concluded that this indicates there is not an adequate increase in HDL production by women who are obese, increasing the risk for endothelial dysfunction[278] and obesity-related conditions such as pre-eclampsia, preterm birth (spontaneous and iatrogenic), and stillbirth[278, 281]. Further, small LDL particle concentrations are increased in women who are obese and women with pre-eclampsia, both of which are associated with preterm birth[277].

In a retrospective cohort including 2.9 million pregnant women in the USA, 9162 women had dyslipidaemia, where there is increased LDL and VLDL with decreased HDL. Dyslipidaemia was associated with an increased risk for preterm

birth, even after adjustment for BMI, maternal age at delivery, ethnicity, hypertension, and education[282]. Another group modelled genetic risk scores for total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides in order to estimate genetic predisposition to dyslipidaemia in 164 women with pre-eclampsia and 110 controls, replicated in a US and European population. These genetic risk scores were not associated with the risk for pre-eclampsia[283], which is interesting as it suggests that simply having a genetic risk for dyslipidaemia does not in itself increase the risk for pre-eclampsia, although the null results may also be due to a lack of power. A further recent study, which used the same BiB NMR data investigated in this present study, used age-adjusted analysis to determine that within the BiB cohort gestational diabetes was positively associated with VLDL, VLDL cholesterol, phospholipids, most glycerides, and all metabolites related to glycolysis; and negatively associated with HDL and LDL, glutamine and fatty acid ratios. Pre-eclampsia had a positive association with VLDL lipoproteins, phospholipids, glycerides, metabolites of glycolysis, creatinine, and fatty acids; and was negatively associated with fatty acid ratios, linoleic, DHA, omega-3 and -6, SFA and PUFA. However, with adjustment for BMI the lipid associations, but not the amino acid associations, moved towards the null[236]. These results therefore support a link between lipids in pregnancy,

insulin resistance and obesity. In the present study these factors were adjusted for, and the association between a change in gestational age persisted.

In the present study, an increase in the mean diameter of HDL particles by 1SD was associated with an increased mean gestational age at delivery of half a day, and a 1SD increase in total cholesterol, cholesterol esters, and free cholesterol in large and very large HDLs was also associated with an increase in gestational age at delivery. This contrasts with the effect of a 1SD increase in the total cholesterol, cholesterol esters and free cholesterol in medium and small HDLs, which were associated with a reduction in gestational age at delivery, although the mean change was less than half a day. Moreover, an increase in triglycerides within any size of lipoprotein was consistently associated with a reduction in gestational length, albeit by a half to one day. Given that these results are controlled for pre-eclampsia, hypertension, pre-existing diabetes and gestational diabetes, this indicates that changes in the lipid profile may be important in the timing of spontaneous labour in humans, even in the absence of obvious pathology. While this may indicate that there is an underlying undetected insulin-resistance, it could also reflect that the trigger for normal labour in humans involves a mechanism related to vascular health, possibly at the maternal/placental interface. This could mean that for women with pre-existing dyslipidaemia, even at low levels, the pathways for spontaneous labour

are triggered earlier, including vascular pathology of the placenta that have previously been shown to be associated with PTB[117]. However, this is speculative, and further investigation would be required to investigate this hypothesis further.

4.7.3 Dyslipidaemia, inflammation and impaired vascular function

A potential link between dyslipidaemia and spontaneous labour could involve inflammation, as a shift towards an inflammatory environment may itself trigger labour. Elevated triglyceride concentrations in pregnancy are reportedly associated with smaller and more dense LDLs that are both pro-inflammatory and pro-atherogenic[39,278,277, 279]. Obesity in pregnancy was found to be associated with increased triglycerides and lower HDL lipoprotein concentrations and no change in LDL concentration, when compared with non-obese controls. Obesity was also associated with increased inflammatory markers such as Interleukin-6 and CRP, and endothelial-independent and endothelial-dependent vasodilatory responses were reduced in pregnant women who were obese when compared with lean [284].

One group investigated the effect of lipoprotein particle concentration and size, measured in pregnant women at 9 weeks gestation, on whether the pregnancies were delivered preterm. Lipoproteins were measured on an NMR metabolomics platform. The authors observed that women with PTB had lower VLDL particle

concentrations as well as increased systemic inflammation. The decreased VLDL particle concentration measurement was observed to be secondary to fewer small VLDL particles, which the authors suggested could reflect an impaired lipid response[277]. In the present study, associations between VLDL concentration and gestational age at delivery were reduced for all sizes of VLDL. While these associations were between half and one day decrease in mean gestational age per 1 SD increase in metabolite concentration for most sizes of VLDL, the associations with very small VLDL were less than half a day, and half of these were non-significant.

In the non-pregnant state, the observed dyslipidaemia and increased inflammatory changes of pregnancy described would be associated with impaired vascular function, production of reactive oxygen species (ROS), and potential vascular endothelial damage. However, during normal pregnancy vascular function is instead found to improve, with a positive association reported between triglyceride concentrations and flow-mediated dilatation of vessels during pregnancy[278]. HDL regulates endothelial nitric oxide (NO) synthase (eNOS) expression, and thereby promotes vasodilatation of blood vessels[278, 285]. Outside of pregnancy, dysfunction in NO production alongside endothelial dysfunction occurs prior to atherosclerosis, and during pregnancy this has been associated with vascular dysfunction and pre-eclampsia[285]. Further,

previous studies indicate that vascular dysfunction is a major factor in disorders of pregnancy such as preterm birth and stillbirth[277-279].

Within the prospective cohort Cardiovascular Risk in Young Finns study, 119 women (57 pregnant women matched with 62 controls) were assessed for lipid profile and brachial flow mediated dilatation (FMD), which is a measure of endothelial-dependent vasodilation. Brachial FMD is increased in normal pregnancy secondary to increased NO bioactivity. The authors found that serum LDL-cholesterol, HDL-cholesterol, triglycerides and total cholesterol were significantly increased during pregnancy when compared with non-pregnant controls, and that brachial FMD also increased, particularly at advanced gestation. There was a direct correlation between serum total cholesterol, triglycerides and FMD in the pregnant women, whereas there was a negative correlation in the non-pregnant women. The authors concluded that in healthy pregnant women increased gestational age was associated with improved endothelium dependent vasodilatation, despite the changing lipid profile[285].

In the present study, higher levels of HDL-related metabolites were associated with an increased gestational age at delivery and reduced odds of delivering preterm compared with term; whereas an increase in mean diameter for VLDL, which is associated with increased triglyceride concentrations, was associated with a shorter gestational length and increased odds for being delivered preterm.

While this fits with the previously described obesogenic pregnancy profile related with lipid dysfunction, the present study shows a persistent association even when controlled for maternal BMI, pre-eclampsia, and diabetes. On the background of the previous findings, and the other chapters in this thesis, this could potentially align with the idea that if HDL protects endothelial function, increased HDL levels could result in improved placentation and improved chances of the pregnancy continuing to term. Or, put another way, that those women with lower HDL levels in an otherwise healthy pregnancy have a lower threshold at which there may be vascular changes which may affect the placenta and lead to earlier labour. This could be related to inflammation. The inflammatory response that has been seen in preterm birth may therefore not always be related to pathogens but rather a low-grade inflammation driven by metabolic factors which may increase the threshold towards the point that triggers parturition. This would otherwise be later in people who don't have this high level of triglycerides already.

4.7.4 Creatinine

An increase in plasma creatinine was associated with a reduction in gestational age at delivery and increased the odds of delivering preterm in a dose-response manner. This could potentially fit with the dyslipidaemia picture, as increased creatinine concentration could indicate kidney involvement, where endothelial

dysfunction may have impaired renal function, resulting in increased creatinine concentrations. Again, this is despite having controlled for pre-eclampsia, BMI and mode of labour onset. However, this would need further investigation in future studies.

4.7.5 Alpha-1-acid glycoprotein

Following a search of the literature, there does not seem to be much research conducted into alpha-1-acid glycoprotein, an acute phase protein, and gestational age at delivery or preterm birth. In neonates, alpha-1-acid glycoprotein concentrations is higher in the cord blood of babies born vaginally than those born by elective caesarean section, with a correlation between gestational age at delivery and alpha-1-acid glycoprotein concentration. The researchers concluded that levels of alpha-1-acid glycoprotein are influenced by gestational age at delivery and mode of delivery[286] . Taken with the results of the present study, this could indicate that if levels of alpha-1-acid glycoprotein reflect a pro-inflammatory state, that this could be linked to earlier gestational age at delivery.

4.7.6 Comparison with results of Chapter 3

In Chapter 3, MS metabolomics were conducted on maternal plasma sampled at 28 weeks' gestation, 34 weeks' gestation, and in latent phase, and analysis conducted to determine if there were correlations with gestational age of

spontaneous labour. In BiB, metabolites were measured using NMR, which primarily detects abundant metabolites such as lipids. Studies in Chapters 3 and 4 have included different metabolites and analytical approaches. Therefore, while the results of the two studies cannot be directly compared, inferences can be made based on the overall patterns found in each of these studies, and it is interesting to see if the findings from both techniques support each other.

The over-representation analysis of the Chapter 3 correlation data can be viewed in section 3.5.19, and showed that: at 28 weeks' gestation, metabolites which were significantly correlated with gestational age at delivery were most over-represented in the glycerol phosphate shuttle, riboflavin metabolism, and glycerolipid metabolism pathways; and at 34 weeks, the most over-represented pathways were mitochondrial beta-oxidation of long chain saturated fatty acids, betaine metabolism, and glycerol phosphate shuttle pathways. The fact that several of the more over-represented pathways in Chapter 3 are related to lipid metabolism is consistent with results of this chapter, where many of the metabolites associated with gestational age at delivery were lipid-related.

There was also overlap between the two studies for amino acids, fluid balance and inflammation, and glyceride and phospholipids metabolite groups. None of the metabolites that associated with gestational age at delivery in the BiB cohort were significantly correlated with gestational age at delivery in Chapter 3,

however the direction of the non-significant correlations did match those of this Chapter. This lack of significance is likely a consequence of the smaller number of samples included in Chapter 3, as well as the MS metabolomics measuring different metabolites of the same compound. For example, N-acetylalanine, a metabolite of alanine, was significantly negatively correlated with gestational age at delivery when measured at 28 weeks and in latent phase in Chapter 3. This would match with the finding from the BiB cohort that increased alanine is associated with a lower gestational age at delivery. In summary, the analysis shows support between the results of the two chapters, although the differences in metabolomics technique and sample size means that they are not directly comparable.

4.8 Conclusions

While dyslipidaemia and vascular dysfunction may not seem to be immediately relevant to the aim of the present study, namely improving our understanding of the spontaneous trigger for labour in humans, the results in this chapter and the preceding chapters of this thesis begin to paint a picture that suggests that all pathologies of pregnancy may be interlinked, and that the link could be vascular physiopathology. Vascular dysfunction is associated with pre-eclampsia and preterm birth, and some of the changes found in myometrial tissue in this thesis also suggest differences in vascular proteins. Therefore, spontaneous

labour could be linked to a physiological vascular dysfunction, potentially at the placental/fetal interface, that usually occurs when the pregnancy reaches term. In the pathological setting this change occurs earlier, associated with shorter gestational length, pre-eclampsia and preterm labour.

In summary, the findings presented here support the hypothesis that the trigger for labour, particularly preterm spontaneous labour, could be associated with an inflammatory environment associated with dyslipidaemia and vascular dysfunction. Further, these results suggest potentially modifiable risk factors that could be prioritised for further investigation as fruitful targets to prevent preterm labour. Subsequently, the next steps will involve replicating results and using other approaches to strengthen causal inference on the relation of these maternal risk factors with gestational age at delivery.

4.9 Limitations of study

Limitations of this study include that given the NMR platform primarily measures lipids, the metabolites measured are all linked to each other and therefore the consistent findings of an association with gestational age may be because many of these metabolic measures are highly correlated. Currently BiB samples are being processed using MS metabolomics and therefore it will be interesting to repeat this study using the MS data, which represents more metabolic pathways. Further, although it has been attempted to adjust the analysis models for all

hypothesised key confounders, the results could still be biased by residual confounding due to unmeasured or inaccurately measured confounders.

A further important source of potential bias is the risk that incomplete data is not missing at random, which could mean that the selection of participants based on non-missing data (i.e. complete case analysis) in this study could introduce bias in the effect estimates. However, the similarity in the distribution of the key characteristics of the participants between the eligible and analysis samples is reassuring in this respect.

The BiB cohort represents a multi-ethnic group of differing parity. Although the results of this study were adjusted for ethnicity, it is possible that they would not be replicated in a population of very different ethnicity or cultural backgrounds. However, in two recent studies which investigated prediction models for pre-eclampsia[15] and fetal growth restriction at term[15], modelling using pregnancy metabolomics data among the Pregnancy Outcome Prediction (POP) study was replicated using the metabolomics data of the BiB cohort. This is despite differences in ethnicity and parity of the participants of the two cohorts: the POP study was conducted in Cambridge, UK, and by design recruited only nulliparous women, over 95% of whom were white. This validation for two different pregnancy outcomes across the two different cohorts suggests that such metabolomics associations are robust when

compared between different cohorts and indicates that the results presented in this chapter are generalisable to other populations.

CHAPTER 5. INVESTIGATING CHANGES IN PROTEIN PHOSPHORYLATION DURING MYOMETRIAL CONTRACTIONS

5.1 Background and rationale for study

Up to this point, the studies included in this thesis have investigated associations between maternal and cord blood metabolites and human labour. The two studies presented in this chapter move on to investigate the potential role of the myometrium in the process of parturition in humans. This is important as it is the myometrium of the uterus which coordinates contractions for expulsion and delivery of the baby and placenta, and therefore the metabolomic changes that have been shown to be associated with labour so far may affect myometrial function.

As discussed in Chapter 1, *in vitro* human myometrium intrinsically undergoes phasic contractions as a result of spontaneous cell membrane depolarisation. Slowly depolarising action potentials instigate sudden rapid entry of calcium ions (Ca^{2+}) into the myometrial cells upon opening of voltage-gated Ca^{2+} channels. These phasic contractions are driven by the reversible and opposing actions of myosin light chain kinase (MYLK) and myosin phosphatase (MYLP), coordination of which generates the force of myometrial contractions[54, 145, 287]. MYLK is a Ca^{2+}-calmodulin (Ca-CaM)-dependent enzyme that reversibly phosphorylates myosin light chains (MLC), and which reacts rapidly to the

changes in intracellular Ca^{2+} concentration. While MYLK activity is driven by Ca^{2+} concentration, regulatory phosphorylation of MYLP can occur that is not dependent of Ca^{2+} concentration[287]. Myometrial agonists such as oxytocin (OXT) exert their effects through binding to G protein-coupled receptors which increase the Ca^{2+} influx into the myocytes via the Gq/Phospholipase C pathway[287, 288]. Phosphorylation events of myometrial contractility have previously been investigated, including previous work by Hudson *et al.* (2017), which showed that a reversible phosphorylation of a regulatory subunit of MYLP (at Threonine 853) can occur during phasic myometrial contractions, and that this plays a role in the Ca^{2+}-sensitivity induced by oxytocin. Inhibition of MYLP would increase oxytocin-induced Ca^{2+}-sensitivity[287].

Failure to progress in labour, also described as arrest of descent or labour dystocia, is diagnosed clinically if a woman's cervix does not dilate at the expected rate of at least 2 cm every four hours for first labours. In subsequent labours this classification stands, and in addition failure to progress may be diagnosed if there is a slowing of the rate of dilatation[30]. Clinically, failure to progress is often explained as being secondary to cephalopelvic disproportion, where the fetus is deemed too large for the maternal pelvis, or because of malposition of the fetus[289]. However, this is not always the case, and it is hypothesised that for some women there may be an underlying issue regarding

functionality of the myometrium. If this hypothesis were true and the areas of these differences were elucidated, this could progress the quest for an improved understanding of the trigger for spontaneous labour in humans. It would also provide targets for drug development for IOL.

Previous researchers have performed genomic analysis of the myometrium of women who were diagnosed with failure to progress. For example, one group performed genomic analysis to compare myometrium taken from women who were at term and not in labour (elective caesarean section (ECS)) with women who had an emergency caesarean section for failure to progress. The researchers identified four hundred genes that were differentially expressed between these two groups. Following signalling pathway impact analysis, significant pathways were identified that were associated with failure to progress, including those involved in regulation of the actin cytoskeleton, cytokine-cytokine receptor interaction, complement and coagulation cascade, and focal adhesion. The authors also found increased protein expression of Interleukin-6, a pro-inflammatory cytokine, in the myometrium of women with failure to progress in labour. This group snap froze and processed the myometrial samples immediately following delivery, and therefore the results of this study may in part reflect the recent physiological stress of labour experienced by the emergency caesarean section group[290]. A similar study

identified differences in the gene expression between those who had an ECS (no labour) and those who had an emergency caesarean section (labour). This included pathways involved in responses to hypoxia, inflammation, apoptosis, stress, muscle contraction and chemokine signalling[289]. These findings indicate that there may be inherent differences in the myometrium of women who are diagnosed with failure to progress.

While genomic studies are very useful in determining which genes are being transcribed, they do not always reflect the proteins produced and the action of these proteins[291, 292]. Global phosphoproteomics allows investigation of the total proteome and phosphoproteome of cells and tissues, facilitating assessment of both the proteins present within a target tissue at a set time point and protein phosphorylation events. Post-translational phosphorylation of proteins is important as such modifications can alter protein function[293].

In the present study, global phosphoproteomics was used to investigate the phosphoproteome of myometrium sampled from women who had an ECS at term (not in labour) and the phosphoproteome of myometrium sampled from women who had an emergency caesarean section following failed induction of labour (IOL). Any differences between these two groups could reflect a myometrial phenotype that may be associated with failure to progress, and thereby identify those proteins which may be involved in the myometrial

pathway of spontaneous labour in humans. In addition, as discussed above, previous studies investigating the genomics of failed IOL have snap frozen myometrial samples at the time of delivery. It is therefore possible that any differences observed could be a direct result of the stress of labour experienced by the failed IOL group but not by the elective caesarean section group. In order to control for this, the experimental design for the present study included an equilibration period for all samples, intended to allow a wash-out of those proteins and phosphorylations which may have occurred as a direct result of the stress of the preceding labour in the failed IOL group. Following this equilibration period, samples from both groups were then subjected to the same experimental environments to better represent the inherent responses of the myometrium.

There were two parts to this study: Part 1 was continuation of work by Hudson *et al.* (2017) to investigate phoshophoproteomic differences between contracting myometrium and non-contracting myometrium sampled from women at ECS; and Part 2 investigated phosphoproteomic differences between myometrium sampled from women who had a failed IOL due to failure to progress, and those who had an ECS.

5.2 Ethical approval, participant consent and funding

Ethical approval for this study was granted by the North Somerset and South Bristol Research Ethics Committee, and funding was from the David Telling Charitable Trust (reference number 354). All women gave written informed consent.

5.3 Role of thesis researcher

Whereas for the other chapters, the author of this thesis (Katherine Birchenall) designed and performed all aspects of the studies, including recruitment, sample collection and statistical analysis. While this was the same for Part 2 of this chapter, this was not the case for Part 1.

For Part 1 of this study, the recruitment, experimentation and phosphoproteomic analysis had been performed prior to the start of this PhD, and the thesis author's role was in analysis of the data. For Part 2 of this chapter, the thesis author recruited and performed the experiments during the time of the PhD, processed the samples ready for phosphoproteomics, and analysed the data with support from Dr Heesom's group in the University of Bristol Proteomics Facility.

5.4 Aims

The aims of this chapter were:

1. To elucidate potential cellular pathways involved in the contraction and relaxation of the myometrium, and to investigate potential regulatory factors to guide future research.
2. To investigate whether myometrial phosphorylation patterns are different between women who have an elective caesarean section having previously laboured and reached full cervical dilatation in a previous pregnancy, and those who have an emergency caesarean section following failed induction of labour. Any differences may demonstrate essential signalling pathways or changes in myometrial protein phosphorylation that are required for effective coordination of myometrial contractions and timely vaginal delivery of the fetus.

If there are differences in phosphorylation patterns between the myometrium of women who have a failed induction of labour and women who have laboured to full dilatation, it would indicate that the proteins affected are functionally necessary for successful myometrial contractility. Such information could be used for development of better methods for inducing labour and would also add to our knowledge of the pathways

involved in labour, with adequate myometrial contractions an important end point.

5.5 Methodology

5.5.1 Myometrial organ bath

Myometrial samples were taken at the time of caesarean section following delivery of the baby and placenta. An approximately 1 cm by 1 cm by 1 cm strip of myometrium was sampled from the upper border of the lower uterine incision. This sample was obtained by the operating surgeon, and the tissue was then immediately washed in ice-cold isotonic saline and transported to the laboratory for immediate processing. With minimal handling of the myometrium, and while keeping the samples submerged in isotonic saline, the samples were cut into five strips of approximately 5 mm by 20 mm dimensions with a scalpel[287].

A four-chamber Myobath-II system (World Precision Instruments (WPI), Stevenage, UK) was used for the myometrial contractility experiments. Four of the cut myometrial strips were mounted using s-shaped hooks, and one was individually fixed under tension in each of the four 10ml chambers of the Myobath-II system (see Figure 5.1). A fifth strip was connected to the upper s-hook of the first bath to provide a pre-contracting control that was subjected to

the same solution exposures while not under tension and therefore did not contract[287].

The strips were equilibrated in oxygenated Krebs solution at 37 degrees Celsius for 45 minutes, with a buffer change every fifteen minutes (three changes). The composition of the Krebs solution used was: 1.25 mM Magnesium Sulphate ($MgS0_4$), 4.7 mM Potassium Chloride (KCL), 1.25 mM Calcium Chloride ($CaCl_2$), 2.5 mM Monosodium Phosphate (NaH_2PO_4), 130 mM Sodium Chloride (NaCl),

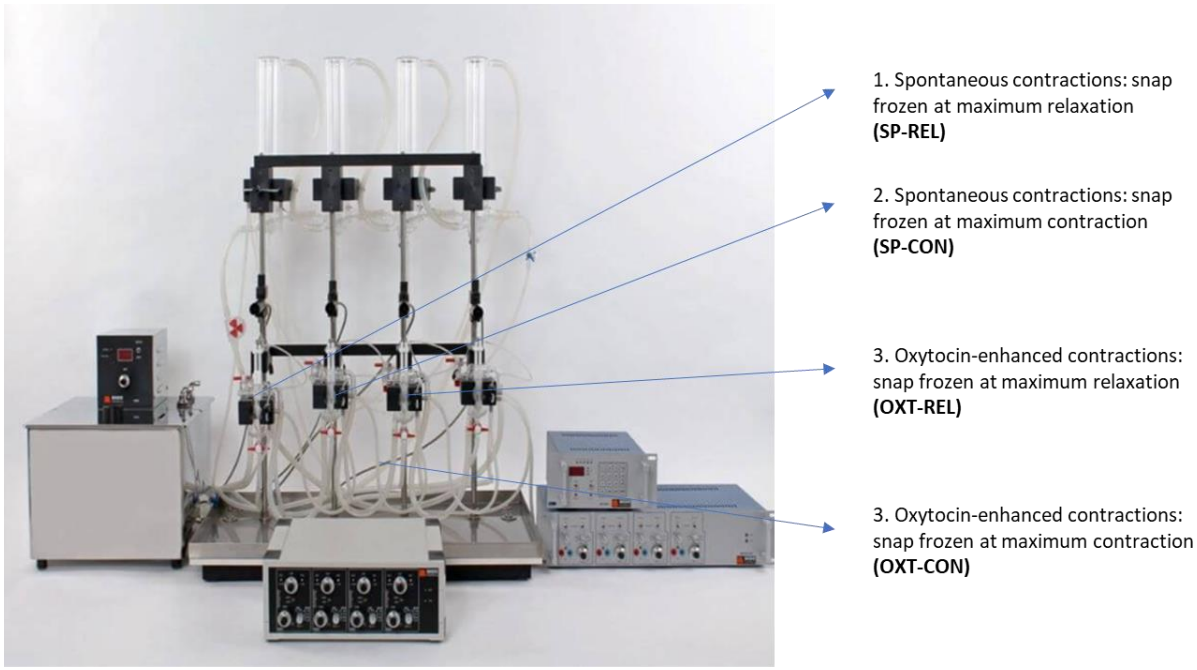

Figure 5.1: Myobath-II system with experimental conditions used in each of the four chambers used (World Precision Instruments)

25 mM Sodium Bicarbonate ($NaHCO_3$), 11.1 nM glucose and 10 mM Hepes at pH 7.6. This buffer solution had been optimised by Hudson *et al.* (2017)[145], and was therefore used for the work in this thesis so that the results could be more

directly comparable. The s-hooks attached at either end of the four myometrial strips were connected to force transducers, and this electronic data was collected using Lab-Trax data acquisition system which transformed the applied force from the myometrial strips into a proportional voltage (WPI, Stevenage, UK). Figure 5.2 shows an example output[287].

Through monitoring of the force transducer output on a monitor, the resting tension for each of the myometrial strips was set to 2g (as per Hudson *et al.* (2017)[145] methodology) through adjustment of the height of the upper s-hook (WPI, Stevenage, UK)[287]. The force transducer output was then monitored for each of the baths until spontaneous contractions were observed (occurring in most cases after approximately 60 minutes). According to the experiment conducted, the myometrial strips were either allowed to continue to contract spontaneously or the agonist oxytocin (OXT) (Merck, Darmstadt, Germany) was added directly to the bath[287]. For those experiments involving OXT, the strips were directly exposed to 10 nM OXT and contractions were observed until consistent (approximately 30 minutes).

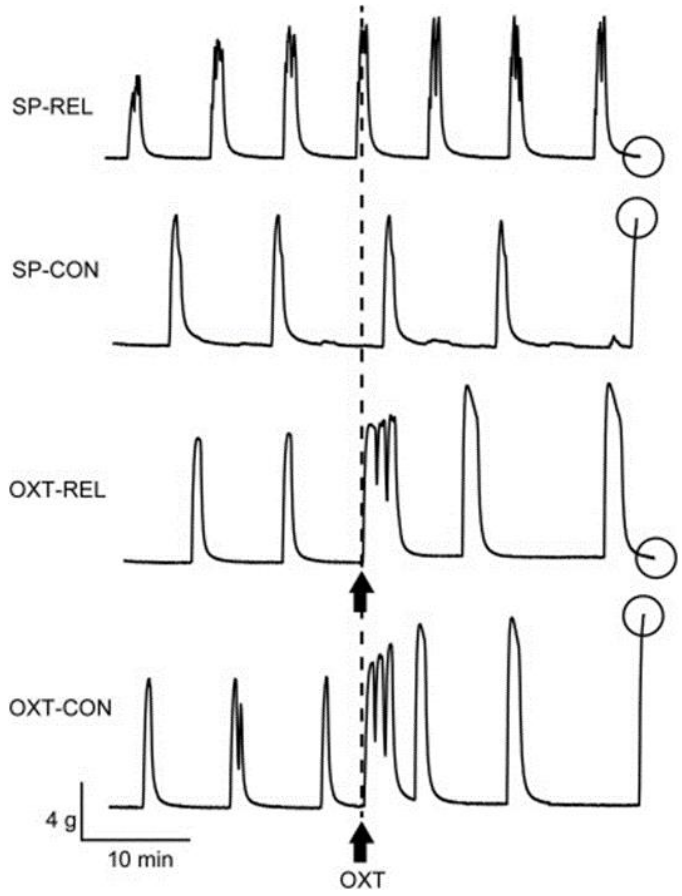

Figure 5.2: Example of electronic output of myometrial samples connected to force-transducers using Lab-Trax data acquisition system (World Precision Instruments) (figure adapted from Hudson & López-Bernal, 2012). Samples: SP-REL = spontaneous contractions with sample snap frozen at maximum relaxation; SP-CON = spontaneous contractions with sample snap frozen at maximum contraction; OXT-REL = oxytocin-enhanced contractions with sample snap frozen at maximum relaxation; and OXT-CON = oxytocin-enhanced contractions with sample snap frozen at maximum contraction.

Approximately 40 minutes after OXT was or was not added, the strips from all four baths were snap frozen and put into liquid nitrogen in quick succession. The snap freezing was performed by dipping two flat spatulas in liquid nitrogen and then holding the strips between the two spatulas. Each strip took approximately 5 seconds to raise from the organ bath, snap freeze, and place in liquid nitrogen. The following five conditions were used (see Figure 5.2):

1. PRE: no tension applied and considered pre-contraction.

2. SP-REL: spontaneous contraction (no OXT) snap frozen 20 seconds following the end of a phasic contraction.

3. SP-CON: spontaneous contraction (no OXT) snap frozen at peak phasic contraction.

4. OXT-REL: oxytocin-stimulated phasic contraction (with OXT) snap frozen 20 seconds following the end of a phasic contraction.

5. OXT-CON: oxytocin-stimulated contraction (with OXT) snap frozen at peak phasic contraction.

These tissues were then stored at -80 degrees Celsius until protein extraction was performed.

5.5.2 Tissue homogenisation

In preparation for proteomic analysis, the individual myometrial strips were homogenised in radioimmunoprecipitation assay (RIPA) buffer at approximately 100mg of wet tissue per millilitre of buffer, using a Polytron homogeniser, keeping everything on ice. The RIPA buffer used is made up of 150 mM NaCl, 50 mM Tris of pH 8.0, 1%NP-40 (a detergent to break down the cell membranes, including the nuclear membrane), 0.1% sodium dodecyl sulphate (SDS), 0.5% sodium deoxycholate, 1 x PhoSTOP, 1 x protease (cOmplete) (Roche Diagnostics

Limited, Burgess Hill, UK). The lysates were cleared through centrifugation for ten minutes at 16 000 g at 4 degrees Celsius. The BCA assay kit (Perbio Science UK, Cramlington, UK) was used to determine the protein concentrations. The protein concentrations were adjusted to 2mg per millilitre, and 100µg of protein was then used for the phosphoproteomic analysis[145].

5.5.3 Phosphoproteomics

Mass Spectrometry (MS) phosphoproteomic analysis was performed by Dr Kate Heesom's group in the University of Bristol Proteomics Facility, School of Medical Sciences (https://www.bristol.ac.uk/life-sciences/research/facilities/proteomics/). For the tandem mass tag (TMT) and phospho-peptide enrichment, 100µg of each sample was digested with 2.5 µg Trypsin at 37 degrees Celsius overnight, then labelled differentially with TMT four-plex reagents (according to the protocol suggested by the manufacturer, Thermo Fisher Scientific, Loughborough, UK). Each sample had a different isobaric tag. The labelled samples were pooled, allowed to evaporate to dryness, resuspended in buffer made up of 57% acetonitrile vol/vol (v/v), 26% lactic acid v/v, and 0.4% TFA v/v, and then underwent titanium dioxide ($Ti0_2$)-based phosphopeptides enrichment, again according to the manufacturer's instructions (Thermo Fisher Scientific, Loughborough, UK)[145, 294].

For the Nanoscale-Liquid Chromatography coupled to tandem Mass Spectrometry (Nano-LC MS), the enriched phosphopeptides were fractionated with a Dionex Ultimate 3000 nanoHPLC system (Thermo Scientific), in line with an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific). Peptides were diluted in 1% formic acid v/v and then transferred to an Acclaim PepMap C18 reverse phase analytical column (Dionex). A wash was then performed with 0.5% v/v acetonitrile in 0.1% v/v formic acid, following which the peptides were resolved on a 75um x 250 mm Acclaim PepMap C18 reverse phase analytical column (Dionex) with a flow rate of 300 µl per minute, across a 150 minute organic gradient of seven gradient segments of different percentage concentration of solvent B (aqueous 80% acetonitrile in 0.1% formic acid): 1 minute of 1-6% solvent B, 58 minutes of 6-15% solvent B, 58 minutes of 15-32% solvent B, 3 minutes of 32-40% solvent B, 1 minute of 40-90% solvent B, then held at 90% solvent B for 6 minutes, then finally reduced to 1% solvent B for 1 minute[294].

Following this step, ionisation of the peptides occurred using nanoelectrospray ionisation at 2.0 kV at a temperature of 250 degrees Celsius, with use of an emitter made of stainless steel and with an internal diameter of 30 µm (Thermo Scientific). An LTQ-Orbitrap Velos mass spectrometer, controlled by Xcalibur 2.1 Software (Thermo Scientific), was used to acquire the Tandem Mass Spectra.

This was operated in data-dependent acquisition mode. In the mass range m/z 300 to 1800, the Orbitrap was set to analyse at 60 000 resolution (at m/z 400), with subsequent selection of the top ten multiply charged ions within each duty cycle for MS/MS fragmentation using higher-energy collisional dissociation (HCD) with activation time of 0.1 millisecond, at a resolution of 7500, and normalised collision energy within the Orbitrap. Charge state filtering, where unassigned precursor ions were not selected for fragmentation, and dynamic exclusion were used[294].

5.5.4 Initial analysis

Proteome Discoverer software v2.1 (Thermo Scientific) was used to process and quantify the raw data files. A SEQUEST algorithm search was performed against the UniProt Human database (https://www.uniprot.org/) as well as a Common Contaminants database[295, 296]. Through this, it was noted whether the peptides matched with proteins from the human database or the contaminants database. Any peptides that were found exclusively in the contaminants database were excluded. For the total proteomics, the results were filtered using a 5% false discovery rate (FDR) cut-off and were then normalised on the total peptide amount for each sample. The results were then adjusted according to the pool samples so that results could be compared across the three different runs. When the phosphopeptides were analysed they were categorised as unique or not

unique. They were not unique if the phospho-peptide sequence matched to more than one master protein, and where it was not possible to determine which was the source master protein, these phosphopeptides were removed from the analysis (Figures 5.3 and 5.4).

The 5% FDR cut off was performed by Dr Heesom's laboratory as part of the proteomics processing. This involved searching each spectra generated by the MS process against a species protein database and also against the same database but for which all of the protein sequences are reversed (named a reverse decoy database). A score is given to each match of a spectra according to how closely the spectra match the that predicted for the given peptide sequence, so that a higher score indicates a better match. It would be expected that there would only be low scores for the decoy database as they were not real protein sequences. The distribution of these scores is then analysed for both databases, and a cut-off calculated where there is only a 5% chance that a peptide would match to the decoy database instead of the "real" database. All peptides below this cut off were then considered low-confidence peptides and excluded from the analysis.

For Part 1 of this study, the TMT tags allowed the abundance of individual peptides in each sample to be reported and the data were presented as the ratios for SP-REL/PRE (REL/PRE) (see Figure 5.2 for classification) in order to

identify potential proteins and phosphorylations which changed between the non-contracting and the contracting state in term not in labour myometrium. The programme R was primarily used to analyse the data. The total protein and phosphoprotein datasets were merged according to accession number, and the phosphoprotein REL/PRE ratios were normalised according to the REL/PRE ratios for the total proteins. The mean normalised REL/PRE value was then calculated for each of the measured phosphoproteins. Those total proteins and phosphoproteins which either doubled or halved in the myometrium from all three women were identified and put through String (String-db.org), Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/) and Reactome (https://reactome.org/) online software (described further below). This method was used rather than simply using the mean, as a consistent finding in all three samples was considered to be more clinically useful (Figure 5.3).

Part 2 aimed to compare the total protein and the phosphoproteome of myometrium taken from women who had an emergency caesarean section for failed IOL at term with the myometrium taken from women who had an ECS at term for each experimental condition (PRE, SP-REL, SP-CON, OXT-REL and OXT-CON). It was possible to perform more sophisticated statistical analysis for the results of Part 2 as the proteomics analysis was performed later (during the time

of the PhD). For this part of the study, all Peptide Spectral Matches (PSMs) of phosphopeptides with identical peptide sequences and predicted phosphorylation sites were summed to give a single abundance value for each unique phosphopeptide. These phosphopeptide abundance values were then normalised by the same normalisation factor as was applied to the total protein values. This meant that any change applied to the total protein was also applied to the phosphopeptides. These values were then scaled by the pool sample for each run in the same way as performed for the total proteins, so that abundances could be compared accurately across the experiments. At this point there were two key values: total protein abundance (normalised to total peptide for each protein and scaled according to the pool values for each protein) and phosphoprotein abundance (again, normalised to the total peptides and scaled according to the pool values). All the values for total protein abundance and phosphoprotein abundance for each of the samples (including all conditions) were $\log_2$-transformed as this achieved a distribution that was closer to normal to increase the power of the analysis. The next normalisation step was to subtract the $\log_2$ protein abundance from the $\log_2$ phosphoprotein abundance to give a phosphopeptides abundance relative to the total protein, i.e. a relative phosphopeptides abundance, allowing better comparison of real change in the phosphorylation of proteins, rather than such changes being eclipsed by

changes in total protein abundance. For each of the conditions (PRE, SP-REL, SP-CON, OXT-REL and OXT-CON) the mean log_2 phosphopeptide abundance was calculated for the failed IOL group and the elective caesarean section group, the log_2-fold change calculated and Welch's t-test performed. For the purpose of the analysis in this thesis, changes were considered significant if $p \leq 0.05$, and phosphorylation events which were significantly different between the failed induction of labour and the elective group were highlighted if the phosphorylation either doubled or halved (Figure 5.4).

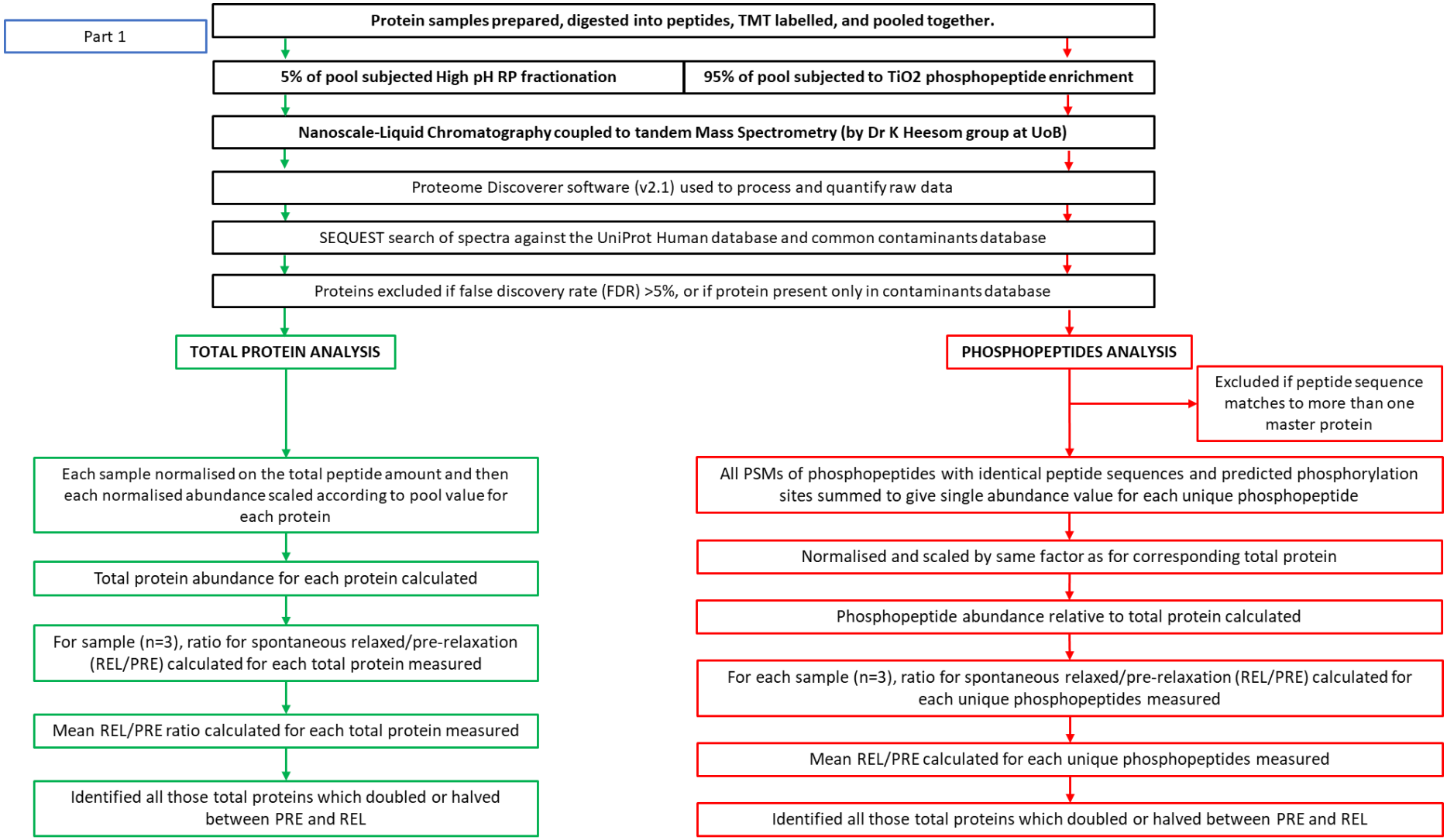

Figure 5.3: Flow diagram for methodology for analysis conducted for Part 1 (PSM = Peptide Spectral Matches)

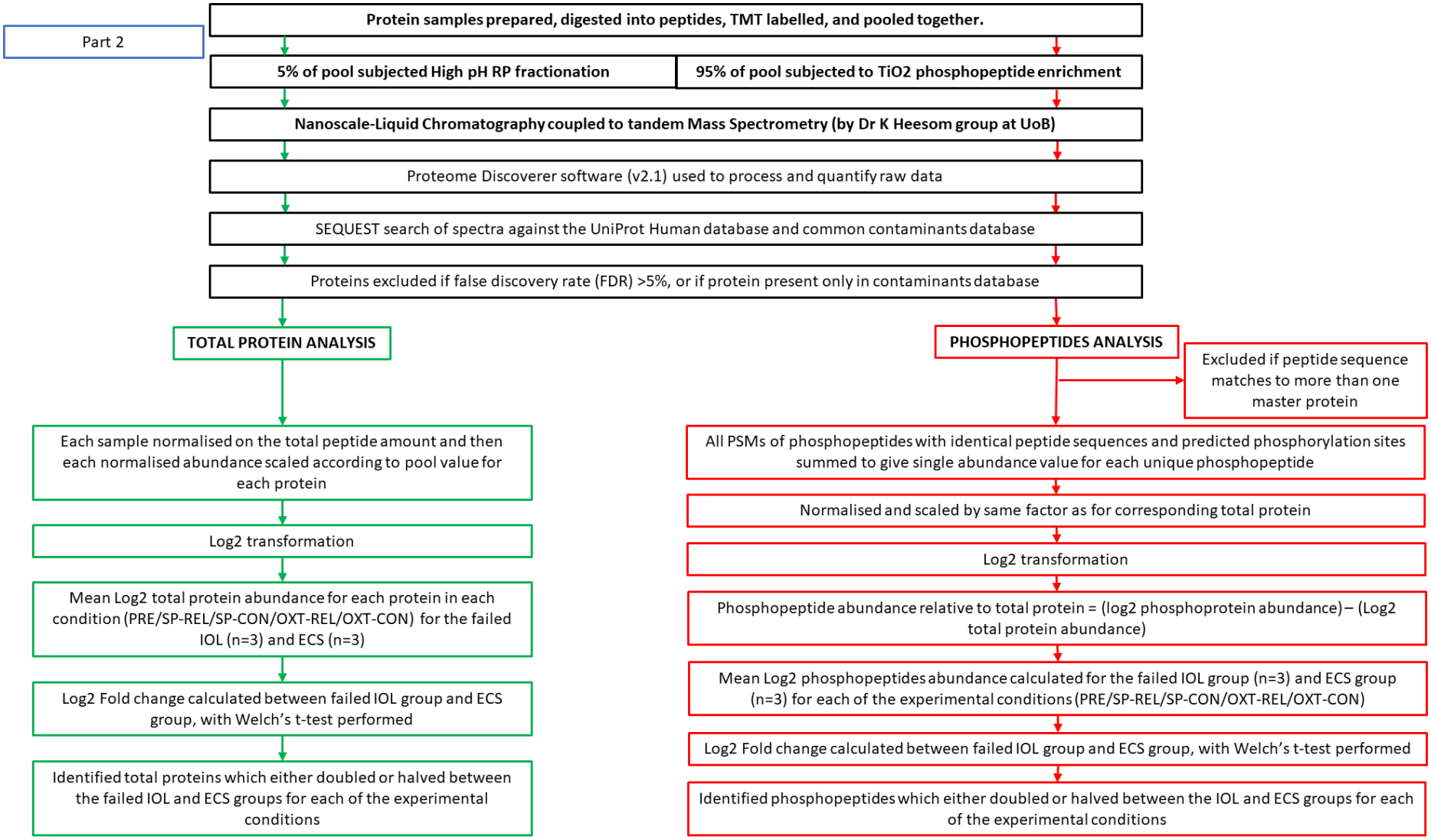

Figure 5.4: Flow diagram for methodology for analysis conducted for Part 2 (PSM = Peptide Spectral Matches)

5.5.5 Pathway analysis

The above methodology gave an overall summary of the proteins and phosphorylation events which occurred in the different conditions tested. Online software is available which aid identification of potential protein interactions within selected samples and perform analysis to determine if a specific set of proteins are over-represented among known pathways. Accession codes for all those proteins which either at least doubled or halved between compared conditions in Part 1 and Part 2 of this study were entered into the bioinformatic software programmes including String, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, and Reactome. String and Reactome allow performance of an "overrepresentation analysis", where those pathways which are enriched, or overrepresented, in the results of the study more than that which would be expected with chance are identified. For Reactome, a p-value is calculated comparing the proteins in the study sample with those that would be expected if randomly selected, as well as a false discovery rate (FDR), calculated using the Benjamini-Hochberg approach[297]. String analyses the list of proteins and calculates a p-value for Protein-Protein Interaction (PPI), which is a measure of whether the group of proteins has significantly more interactions than would be expected by chance (String-db.org).

5.6 Part 1 background and results

5.6.1 Background

As explained above, Part 1 of this study continued analysis of data produced by Hudson *et al.* (2017)[145]. The aim of this study was to conduct a global analysis of the changes in the myometrial phosphoproteome during spontaneous and OXT-induced contractions, and to potentially determine novel phosphorylation sites involved in contractility[145]. The initial analysis identified 22 phosphopeptides which either doubled or halved during spontaneous and/or OXT-augmented contractions. The largest change was seen in the phosphorylation of MYLK at serine 1760, which is within a site associated with decreased calmodulin binding and subsequent decreased kinase activity. This phosphorylation increased 9.83+/-3.27-fold ($p<0.05$) during spontaneous contraction (SP-CON) and 18.56+/-8.18-fold ($p<0.01$) during OXT-augmented contraction (OXT-CON), when compared with SP-REL[145]. The total protein changes from this study had not previously been analysed and were performed by the thesis author during this PhD.

5.6.2 Part 1 inclusion criteria

- Women undergoing an ECS at term for indications not relating to maternal or fetal disease, including fetal malposition, maternal request or previous caesarean section.

5.6.3 Part 1 exclusion criteria

- Emergency caesarean section
- Known maternal disease
- Maternal infection

5.6.4 Results

5.6.4.1 Part 1 participants' characteristics

The three women included all had an ECS between 37 and 41 completed weeks gestation. The women otherwise had low-risk pregnancies with no maternal or fetal concerns. Indications for ECS were: fetal malposition, previous caesarean section and maternal request.

5.6.4.2 Part 1 phosphoproteomics results

There were REL/PRE ratio data for 9174 proteins, and REL/PRE ratio information from at least one of the three samples for 4600 phosphorylation events.

5.6.4.3 Part 1 total proteins

Using the analysis method illustrated in Figure 5.3, 178 proteins at least halved between the pre-contracting phase (PRE) and the contraction phase (SP-REL) conditions (Table 5.1), and five at least doubled (Table 5.2). All those proteins which either at least doubled or halved in the spontaneously contracting phase compared with the pre-contracting phase (REL/PRE) in all three samples were entered into bioinformatic software programmes including Reactome, String, and KEGG, as described above[297].

Table 5.3 shows the 25 pathways which the Reactome software highlighted as being the most overrepresented. The entities found represent the number of entities found in the Part 1 dataset that are present in the named pathway, divided by the total number of known entities in that particular pathway. The entities p-value is the statistical test for over-representation, as described, and the entities FDR corrects the p-value test for multiple testing. The reactions found represent the number of pathways identified in the overall pathway for which there is at least one entity from the study dataset found. The reactions ratio is the ratio of reactions in the specific pathway which contain entities from the study dataset, divided by the total number of reactions within the Reactome database which contain entities from the study dataset[297].

Table 5.1: Accession number and protein description for total proteins where the REL/PRE ratio at least halved for all three women, in order of mean REL/PRE.

Protein description	Gene	Accession	REL/PRE for 565	REL/PRE for 592	REL/PRE for 604	Mean REL/PRE
Acid-sensing ion channel 5	ASIC5	Q9NY37	0.171	0.054	0.299	0.174675
Putative heat shock protein HSP 90-beta 4	HSP90AB4P	Q58FF6	0.194	0.066	0.277	0.179087
COL8A2 protein (Fragment)	COL8A2	Q4VAP9	0.160	0.101	0.312	0.190707
T-cell lymphoma invasion and metastasis 1 variant (Fragment)		Q59GK8	0.185	0.082	0.308	0.191501
Alternative protein SV2A	SV2A	L8E840	0.195	0.094	0.288	0.192258
Protein CXorf40B (Fragment)	CXorf40B	S4R3G8	0.134	0.194	0.250	0.192549
Heavy chain of factor I (Fragment)		Q6LAM1	0.195	0.136	0.266	0.198744
Leucine-rich repeat transmembrane neuronal protein 3	LRRTM3	Q86VH5	0.190	0.075	0.343	0.202634
Complement C2	C2	P06681	0.169	0.166	0.276	0.203823
MHC class I antigen (Fragment)	HLA-C	J9PWW9	0.193	0.097	0.322	0.204
Alpha-2-HS-glycoprotein	AHSG	P02765	0.186	0.119	0.308	0.204276
Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial (Fragment)	IDH3A	H0YLI6	0.199	0.106	0.313	0.206203
ETS domain-containing protein Elk-3 (Fragment)	ELK3	F8VZQ0	0.199	0.104	0.328	0.210359
Uncharacterized protein		B4E1Z4	0.192	0.160	0.294	0.215487
cDNA FLJ37293 fis, clone BRAMY2014813		Q8N1Y0	0.229	0.082	0.336	0.215557

Afamin	AFM	P43652	0.189	0.131	0.328	0.216285
Lymphocyte cytosolic protein 2 (Fragment)	LCP2	E5RKA2	0.211	0.101	0.350	0.220541
Ig kappa chain V-III region GOL OS=Homo sapiens PE=1 SV=1 - [KV307_HUMAN]		P04206	0.226	0.152	0.287	0.221674
cDNA FLJ53950, highly similar to Angiotensinogen		B4E1B3	0.183	0.153	0.337	0.224544
Serotransferrin (Fragment)	TF	C9JVG0	0.219	0.116	0.345	0.226664
Gasdermin-B	GSDML	B2CM73	0.172	0.115	0.402	0.229643
V4-1 protein (Fragment)	V4-1	Q5NV68	0.286	0.000	0.405	0.230223
Nuclear pore complex protein Nup98-Nup96 (Fragment)	NUP98	H0YDF4	0.247	0.174	0.281	0.233823
Complement factor I light chain	CFI	G3XAM2	0.187	0.174	0.343	0.234673
cDNA FLJ50624		B7Z5L2	0.277	0.119	0.313	0.236541
MRDS1 protein (Fragment)	MRDS1	Q8IZS4	0.251	0.096	0.366	0.237525
Transferrin variant (Fragment)		Q53H26	0.261	0.107	0.350	0.239237
Alpha-1-antichymotrypsin	SERPINA3	P01011	0.237	0.130	0.357	0.241487
Alpha-1-acid glycoprotein 2	ORM2	P19652	0.236	0.119	0.369	0.241575
Ceruloplasmin (Fragment)		Q1L857	0.236	0.130	0.363	0.242832
Myosin-reactive immunoglobulin light chain variable region (Fragment)		Q9UL83	0.309	0.153	0.270	0.243847
cDNA, FLJ94361, highly similar to Homo sapiens serine (or cysteine) proteinase inhibitor, clade		B2R9F2	0.216	0.138	0.380	0.244553

A(alpha-1 antiproteinase, antitrypsin), member 6 (SERPINA6), mRNA						
Sex hormone-binding globulin	SHBG	I3L145	0.211	0.180	0.348	0.246362
Alpha-1-antitrypsin	SERPINA1	P01009	0.222	0.142	0.377	0.246759
Thyroxine-binding globulin	SERPINA7	P05543	0.220	0.162	0.361	0.247522
Serum albumin	ALB	P02768	0.227	0.130	0.386	0.247628
Myosin-reactive immunoglobulin heavy chain variable region (Fragment) OS=Homo sapiens PE=2 SV=1 - [Q9UL88_HUMAN]		Q9UL88	0.306	0.137	0.311	0.251172
N-deacetylase/N-sulfotransferase (Heparan glucosaminyl) 1 variant (Fragment)		Q59GK2	0.253	0.200	0.305	0.252713
cDNA FLJ46572 fis, clone THYMU3041573		Q6ZR80	0.251	0.224	0.300	0.258675
cDNA FLJ53075, highly similar to Kininogen-1		B4DPP8	0.184	0.149	0.445	0.25953
Antithrombin-III	SERPINC1	P01008	0.189	0.196	0.404	0.262913
Peroxisomal proliferator-activated receptor A interacting complex 285	PRIC285	A7E2C9	0.259	0.146	0.399	0.267948
HSPC254 (Fragment)		Q9P0C7	0.240	0.187	0.379	0.268763
Hemopexin	HPX	P02790	0.232	0.180	0.398	0.269767
Apolipoprotein A-I	APOA1	P02647	0.277	0.186	0.364	0.275777
Torsin family 3, member A, isoform CRA_b	TOR3A	A0A024R943	0.269	0.160	0.403	0.277476
Vitamin D-binding protein	GC	P02774	0.264	0.190	0.379	0.277481
Alpha-1-acid glycoprotein 1	ORM1	P02763	0.268	0.163	0.403	0.277913

IGL@ protein	IGL@	Q6PIK1	0.287	0.198	0.349	0.278325
Ig kappa chain V-I region DEE		P01597	0.311	0.201	0.323	0.278384
C-reactive protein(1-205)	CRP	Q5VVP7	0.264	0.193	0.381	0.279079
Transthyretin	TTR	P02766	0.283	0.168	0.388	0.27967
Ig heavy chain V-III region TUR		P01779	0.243	0.212	0.385	0.279983
tRNA (guanine-N(7)-)-methyltransferase non-catalytic subunit WDR4	WDR4	P57081	0.237	0.159	0.450	0.282096
V3-4 protein (Fragment)	V3-4	Q5NV62	0.339	0.212	0.297	0.282735
Haptoglobin	HP	P00738	0.241	0.325	0.290	0.285602
Rheumatoid factor RF-ET6 (Fragment)		A2J1N5	0.463	0.106	0.294	0.287805
IgG H chain OS=Homo sapiens PE=2 SV=1 - [S6BGE0_HUMAN]		S6BGE0	0.287	0.174	0.409	0.289817
Putative upstream-binding factor 1-like protein 6	UBTFL6	P0CB48	0.266	0.197	0.410	0.291095
Claudin-10	CLDN10	P78369	0.267	0.188	0.421	0.291845
60S ribosomal protein L10-like	RPL10L	Q96L21	0.263	0.185	0.429	0.292394
Ig heavy chain variable region (Fragment)		A0A068LKQ0	0.293	0.200	0.391	0.294428
cDNA, FLJ93914, highly similar to Homo sapiens histidine-rich glycoprotein (HRG), mRNA		B2R8I2	0.215	0.264	0.407	0.295174
Ig kappa chain V-III region POM		P01624	0.364	0.105	0.420	0.296392
Ig kappa chain V-I region Roy		P01608	0.312	0.176	0.416	0.301331
Retinol binding protein 4, plasma, isoform CRA_b	RBP4	Q5VY30	0.236	0.219	0.454	0.303165

Zinc finger MYM-type protein 3	ZMYM3	A6NHN7	0.266	0.153	0.494	0.303994
Putative uncharacterized protein DKFZp686G11190	DKFZp686G11190	Q6MZQ6	0.277	0.159	0.484	0.306337
IgG L chain		S6BAR0	0.294	0.202	0.424	0.306421
Myosin-reactive immunoglobulin heavy chain variable region (Fragment)		Q9UL89	0.309	0.194	0.417	0.306845
NK1 transcription factor-related protein 2	NKX1-2	Q9UD57	0.311	0.204	0.417	0.310642
ATP synthase protein 8	ATP8	Q6RM58	0.275	0.304	0.362	0.313637
V1-22 protein (Fragment)	V1-22	Q5NV88	0.296	0.272	0.373	0.313656
Alpha-2-antiplasmin	SERPINF2	P08697	0.219	0.242	0.481	0.314162
Ig lambda chain V-II region BUR		P01708	0.303	0.199	0.441	0.314365
Myosin-reactive immunoglobulin light chain variable region (Fragment)		Q9UL70	0.357	0.257	0.330	0.314583
Ig gamma-2 chain C region	IGHG2	P01859	0.331	0.206	0.419	0.318381
Ig heavy chain variable region (Fragment)		A0A068LKQ5	0.330	0.218	0.414	0.320759
Immunoglobulin heavy chain variant (Fragment)		Q9NPP6	0.365	0.210	0.391	0.322146
Apolipoprotein A-II (Fragment)	APOA2	V9GYG9	0.275	0.303	0.406	0.328051
Heparin cofactor 2	SERPIND1	P05546	0.251	0.328	0.407	0.328895
Putative uncharacterized protein DKFZp686K18196 (Fragment)	DKFZp686K18196	Q6N092	0.302	0.236	0.451	0.329612
Ig kappa chain V-I region Wes		P01611	0.333	0.207	0.457	0.33268
VH3 protein (Fragment)	VH3	Q9Y509	0.341	0.239	0.420	0.333364

cDNA FLJ14473 fis, clone MAMMA1001080, highly similar to Homo sapiens SNC73 protein (SNC73) mRNA		Q96K68	0.331	0.225	0.445	0.333596
Coagulation factor XII-Mie		Q8IZZ5	0.286	0.320	0.398	0.334685
Protein IGKV2D-24 (Fragment)	IGKV2D-24	A0A075B6R9	0.322	0.248	0.436	0.335101
Kininogen 1, isoform CRA_b	KNG1	B4E1C2	0.241	0.354	0.414	0.336223
Complement component C6	C6	P13671	0.341	0.217	0.458	0.338469
Putative uncharacterized protein DKFZp686K04218 (Fragment)	DKFZp686K04218	Q7Z379	0.367	0.198	0.453	0.339185
Complement C3	C3	P01024	0.313	0.267	0.439	0.339726
Haptoglobin-related protein	HPR	P00739	0.345	0.330	0.348	0.3411
Myosin-reactive immunoglobulin light chain variable region (Fragment)		Q9UL78	0.331	0.292	0.404	0.342398
Bone marrow proteoglycan	PRG2	P13727	0.246	0.297	0.499	0.347319
cDNA FLJ51742, highly similar to Inter-alpha-trypsin inhibitor heavy chain H4		B7Z544	0.380	0.319	0.345	0.347931
DF protein	DF	Q6FHW3	0.271	0.440	0.334	0.348498
Alpha-2-macroglobulin	A2M	P01023	0.342	0.292	0.417	0.350212
Epididymis luminal protein 180 (Fragment)	HEL180	B6EDE2	0.401	0.196	0.455	0.350831
Ig kappa chain V-IV region STH (Fragment)		P83593	0.318	0.244	0.490	0.350888
Complement factor H-related protein 2	CFHR2	V9GYE7	0.343	0.306	0.406	0.351515
Coagulation factor XI	F11	D6RB32	0.270	0.285	0.500	0.351786

Rearranged VH4-34 V gene segment (Fragment)	VH4-34	Q7Z3Y6	0.343	0.243	0.477	0.354457
cDNA FLJ54981, highly similar to Asparaginyl-tRNA synthetase, cytoplasmic (EC 6.1.1.22)		B4DF91	0.343	0.259	0.469	0.357127
Protein IGKV3-11	IGKV3-11	A0A087WZW8	0.385	0.204	0.484	0.357979
Complement component C8 alpha chain	C8A	P07357	0.391	0.317	0.369	0.358861
Cryocrystalglobulin CC1 heavy chain variable region (Fragment)		B1N7B6	0.327	0.288	0.462	0.358917
Anti-H1N1 influenza HA kappa chain variable region (Fragment)		G3GAU4	0.311	0.284	0.484	0.359899
IgG H chain		S6BGD4	0.406	0.185	0.493	0.361408
Ig lambda-7 chain C region (Fragment)	IGLC7	A0A075B6L1	0.336	0.359	0.389	0.361505
Uncharacterized protein		Q7Z2U7	0.383	0.208	0.495	0.36181
cDNA FLJ78071, highly similar to Human MHC class III complement component C6 mRNA		A8K8Z4	0.422	0.248	0.421	0.363487
Ig kappa chain V-I region BAN		P04430	0.377	0.260	0.455	0.364185
Zinc-alpha-2-glycoprotein	AZGP1	P25311	0.350	0.260	0.491	0.367259
Immunoglobulin J chain	IGJ	P01591	0.387	0.327	0.395	0.369822
Complement C4A3 (Fragment)	C4A	V9H0D6	0.466	0.470	0.176	0.370672
Complement component 9, isoform CRA_a	C9	A0A024R035	0.387	0.273	0.457	0.372345
Tetranectin	CLEC3B	P05452	0.355	0.380	0.395	0.37671
Ig kappa chain V-I region Lay		P01605	0.377	0.294	0.461	0.377028
Ig mu heavy chain disease protein		P04220	0.328	0.437	0.371	0.378461

Ig mu chain C region	IGHM	A0A087WYJ9	0.334	0.383	0.421	0.37923
Ig kappa chain C region	IGKC	A0A087WYL9	0.362	0.373	0.405	0.379857
cDNA, FLJ95492, highly similar to Homo sapiens myosin regulatory light chain interacting protein(MIR), mRNA		B2RBG2	0.346	0.304	0.500	0.383065
Protein AMBP	AMBP	P02760	0.419	0.309	0.448	0.391841
Apolipoprotein L1 (Fragment)	APOL1	U5LKR8	0.315	0.416	0.453	0.394813
Molybdenum cofactor synthesis-step 1 protein B splice type I (Fragment)	MOCS1	Q9NP26	0.466	0.315	0.412	0.397583
cDNA, FLJ79457, highly similar to Insulin-like growth factor-binding proteincomplex acid labile chain		B0AZL7	0.299	0.439	0.458	0.398735
Carboxypeptidase N catalytic chain	CPN1	P15169	0.369	0.345	0.484	0.399288
Probable Xaa-Pro aminopeptidase 3	XPNPEP3	Q9NQH7	0.409	0.346	0.451	0.401726
Transcription factor AP-2-epsilon	TFAP2E	Q6VUC0	0.414	0.342	0.463	0.406294
Ig kappa chain V-I region EU		P01598	0.468	0.265	0.487	0.406536
CD5 antigen-like	CD5L	O43866	0.397	0.401	0.432	0.410149
Ig gamma-3 chain C region	IGHG3	A0A087WXL8	0.453	0.314	0.482	0.416541
Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1	INPP5D	Q92835	0.405	0.354	0.493	0.417309
Single chain Fv (Fragment)		Q9HCC1	0.378	0.428	0.465	0.423404
Calcium/calmodulin-dependent protein kinase type 1B	PNCK	Q6P2M8	0.441	0.372	0.470	0.427963

cDNA FLJ78207, highly similar to Human complement protein component C7 mRNA		A8K2T4	0.374	0.492	0.443	0.436489
Glutamate [NMDA] receptor subunit epsilon 2 variant (Fragment)		Q59HA9	0.489	0.407	0.470	0.455341
Carboxypeptidase B2	CPB2	A0A087WSY5	0.496	0.491	0.483	0.490011

Table 5.2: Accession number and protein description for total proteins where the REL/PRE ratio at least doubled for all three women, in order of mean REL/PRE.

Protein description	Gene	Accession	REL/PRE for 565	REL/PRE for 592	REL/PRE for 604	Mean REL/PRE
Mitogen-activated protein kinase 11	MAP3K11	E9PID4	3.521	4.269	3.022	3.604
Tristetraprolin (Fragment)	ZFP36	M0QZ04	4.009	3.966	2.334	3.437
Transcription factor jun-B	JUNB	P17275	4.744	5.589	2.397	4.243
C-C motif chemokine 2	CCL2	P13500	4.295	6.108	2.159	4.187
Cyclin-dependent kinase inhibitor 1	CDKN1A	P38936	7.001	10.025	2.616	6.547

Table 5.3: 25 most overrepresented total protein pathways according to Reactome analysis (ordered according to p-value size) where REL/PRE at least halved.

Pathway Name	Entities found	p-value for entities found	FDR for entities found	Reactions found	Reactions ratio
Scavenging of heme from plasma	20/99	1.11x10-16	6.66x10-15	12/12	9.61x10-4
Regulation of complement cascade	23/135	1.11x10-16	6.66x10-15	35/42	0.003
Complement cascade	24/146	1.11x10-16	6.66x10-15	57/71	0.006
Binding and uptake of ligands by scavenger receptors	20/129	2.22x10-16	9.99x10-15	17/33	0.003
Initial triggering of complement	17/111	4.75x10-14	1.71x10-12	14/21	0.002
Classical antibody-mediated complement activation	15/95	1.03x10-12	3.09x10-11	2/2	1.60x10-4
Creation of C4 and C2 activators	15/103	3.20x10-12	3.09x10-11	2/2	1.60x10-4
FCGR activation	14/101	3.32x10-11	7.31x10-10	6/6	4.8x10-4
CD22 mediated BCR regulation	12/70	8.26x10-11	1.65x10-9	¾	3.2x10-4

Role of phospholipids in phagocytosis	14/114	1.59x10-10	2.85x10-9	5/12	9.61x10-4
FCERI mediated Ca+2 mobilisation	14/117	2.21x10-10	3.54x10-9	5/11	8.81x10-4
FCERI mediated MAPK activation	14/119	2.75x10-10	4.12x10-9	9/20	0.002
Role of LAT2/NTAL/LAB on calcium mobilisation	13/102	4.73x10-10	6.62x10-9	2/7	5.61x10-4
Haemostasis	31/723	5.42x10-10	7.04x10-9	24/327	0.026
Antigen activates B cell receptor (BCR) leading to generation of second messengers	12/95	2.5x10-9	3x10-8	10/25	0.002
Regulation of actin dynamics for phagocytic cup formation	14/150	5.13x10-9	5.64x10-8	6/24	0.002
Fcgamma receptor (FCGR) dependent phagocytosis	14/175	3.44x10-8	3.44x10-7	17/42	0.003
Cell surface interactions at the vascular wall	16/246	5.87x10-8	5.87x10-7	3/64	0.005
FCERI mediated NF-kB activation	13/167	1.48x10-7	1.33x10-6	1/19	0.002

Fc epsilon receptor (FCERI) signaling	14/218	4.82x10-7	4.32x10-6	22/63	0.005
Platelet degranulation	11/128	5.40x10-7	4.32x10-6	3/11	8.81x10-4
Response to elevated platelet cytosolic Ca2+	11/133	7.82x10-7	6.26x10-6	3/14	0.001
Signaling by the B Cell Receptor (BCR)	12/176	1.79x10-6	1.25x10-5	13/43	0.003
Innate Immune System	33/1186	3.81x10-6	2.67x10-5	108/696	0.056
Post-translational protein phosphorylation	9/107	7.12x10-6	4.99x10-5	1/1	8.01x10-5

Table 5.4: Ten most overrepresented pathways according to Reactome analysis (ordered according to p-value size) where REL/PRE at least doubled.

Pathway Name	Entities found	p-value for entities found	FDR for entities found	Reactions found	Reactions ratio
Interleukin-4 and Interleukin-13 signaling	3/111	9.22x10-6	7.37x10-4	3/46	0.004
Cytokine Signalling in Immune system	4/954	2.3x10-4	0.009	5/699	0.056
Signaling by Interleukins	3/456	6.1x10-4	0.016	4/490	0.039
Transcriptional activation of cycle inhibitor p21	1/4	0.002	0.028	1/5	4x10-4
Transcriptional activation of p53 responsive genes	1/4	0.002	0.028	1/5	4x10-4
TFAP2 (AP-2) family regulates transcription of cell cycle factors	1/5	0.002	0.028	1/5	4x10-4
RUNX3 regulates CDKNIA transcription	1/7	0.003	0.032	2/6	4.8x10-4
TP53 Regulates transcription of genes involved in G1 cell cycle arrest	1/14	0.006	0.032	3/17	0.001

AKT phosphorylates targets in the cytosol	1/14	0.006	0.032	1/9	7.21x10-4
FOXO-mediated transcription of cell cycle genes	1/17	0.007	0.032	1/22	0.002

Figures 5.5A, 5.5B, and 5.6 show STRING diagrams (https://string-db.org/) summarising the total protein changes for those proteins which either doubled (Figure 5.5A), halved (Figure 5.5B) or either halved or doubled (Figure 5.6) in the REL compared to PRE ratio. There were significantly more protein-protein interactions (PPI) than would be randomly expected ($p<1.0x10-16$), and the diagrams indicate the potential interactions which may be occurring *in vivo* when in the contracting state. Analysis of the total proteins which at least halved in the spontaneously contracting state (REL/PRE) indicated changes of proteins involved in the complement and coagulation, with 11 of 74 proteins halving in the REL group compared with the PRE (Figure 5.7). These 11 proteins were: coagulation factor XI, antithrombin III, heparin cofactor II, α1-antitripsin, α2=antiplasmin, complement factor I, complement component 2, complement component 3, complement component 6 and complement component 8 subunit α.

For those proteins that at least doubled in all three samples, Reactome analysis indicated that the most overrepresented pathway was Interleukin-4 and Interleukin-13 signalling, followed by cytokine signalling in the immune system. Table 5.4 shows the 10 pathways which Reactome highlighted as being the most overrepresented according to the Reactome statistical analysis as described above. The greatest overall mean decreases were in Acid-sensing ion channel 5

(REL/PRE ratio = 0.175) and Putative Heat Shock protein beta4 (REL/PRE ratio = 0.179).

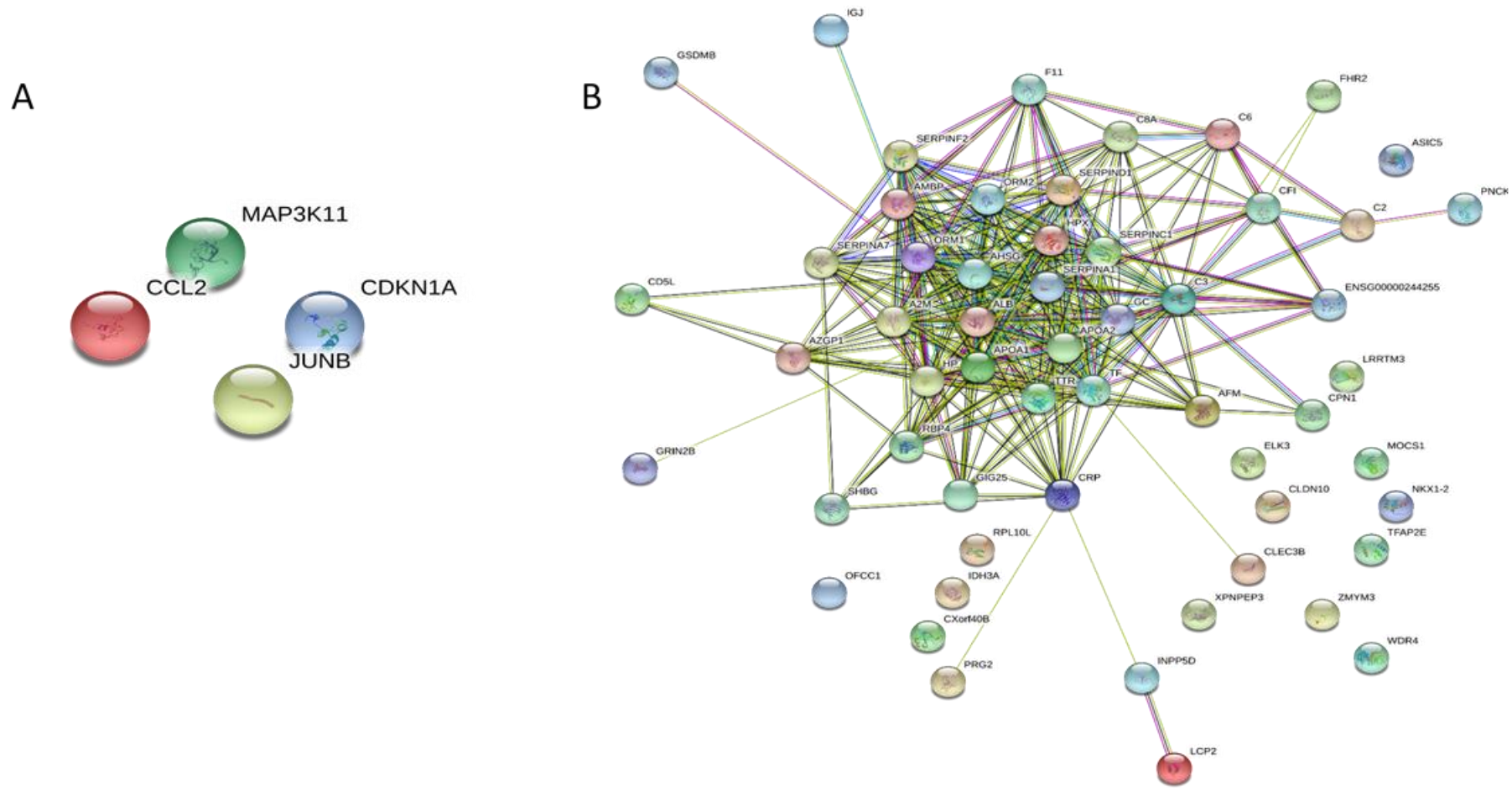

Figure 5.5: STRING diagram (https://string-db.org/) depicting protein-protein interactions between proteins differently expressed between the REL and PRE (REL/PRE) conditions. Proteins shown in A. have 2-fold higher expression in REL than in PRE. Proteins shown in B. have 2-fold higher expression in PRE than in REL (n=3 for both conditions). Both groups have significantly more protein-protein interaction (PPI) than expected (p<1.0-16).

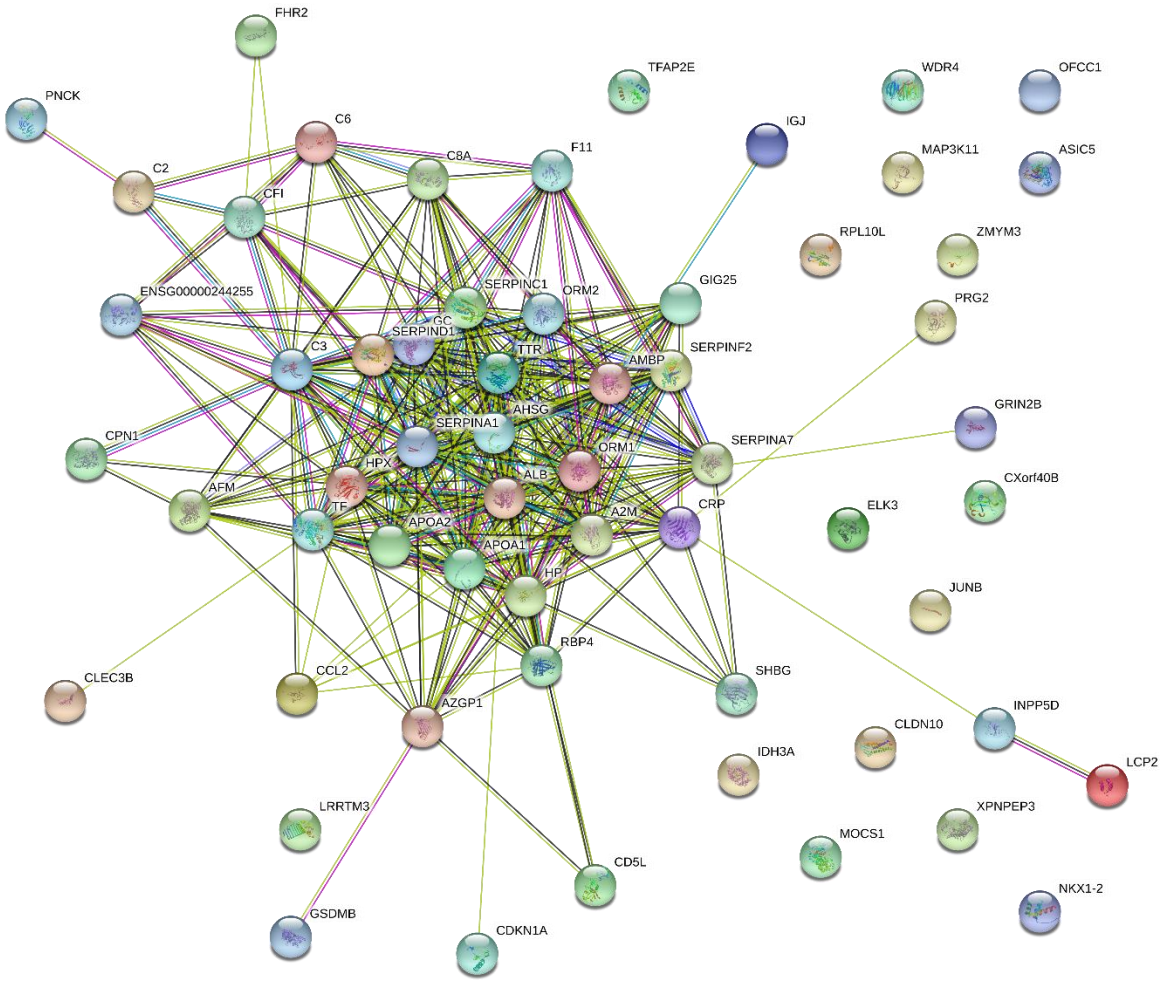

Figure 5.6: STRING diagram summarising the total protein data for those proteins which either at least doubled or halved in the REL condition compared with PRE (REL/PRE) for samples taken from all three women(https://string-db.org/) **. Both groups have significantly more protein-protein interaction (PPI) than expected (p<1.0-16).**

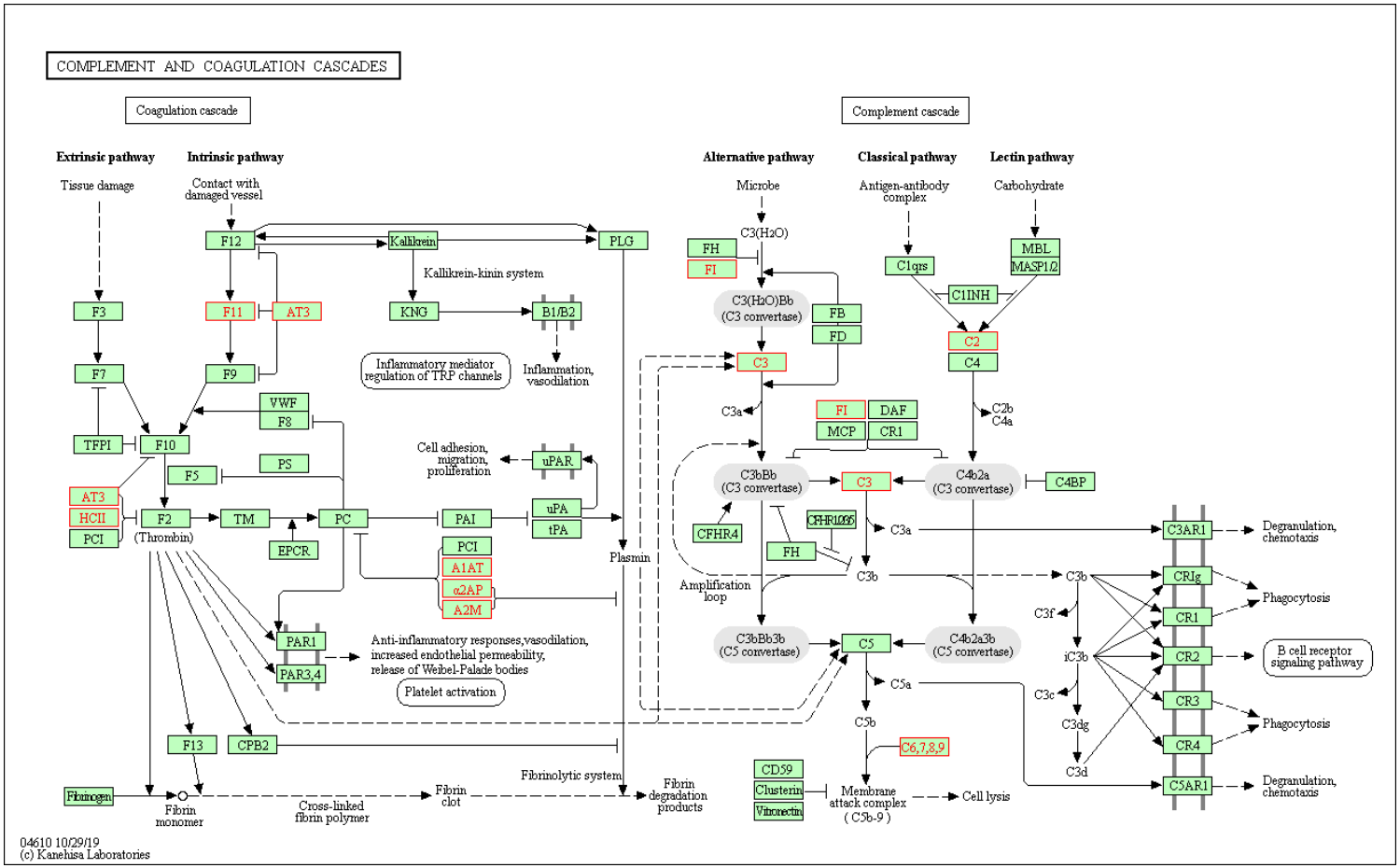

Figure 5.7: Proteins which at least halve between the pre-contracting state (PRE) and contracting state (SP-REL) highlighted in red within the Coagulation and Complement Cascade (produced using KEGG software, false discovery rate of p=4.21e-23. F11 = Coagulation Factor XI (Gene name (GN) = F11); AT3 = Antithrombin III (GN=SERPINC1); HCII = Heparin cofactor II (SERPIND1); A1AT = Alpha-1-antitrypsin (SERPINA1); α2AP = alpha-2-antiplasmin (GN=SERPINF2); A2M = Alpha-2-macroglobulin (GN=A2M); FI = Complement factor I (GN=CF1); C3 = Complement component 3 (GN = C3); C2 = Complement component 2 (GN=C2); C6 = Complement component 6 (GN = C6); C8A = Complement component 8 subunit α (GN=C8A). F2 =Thrombin. Can be viewed at: https://www.genome.jp/kegg-bin/show_pathway?hsa04610+A2M+C2+C3+C6+C8A+CFI+F11+SERPINA1+SERPINC1+SERPIND1+SERPINF2),

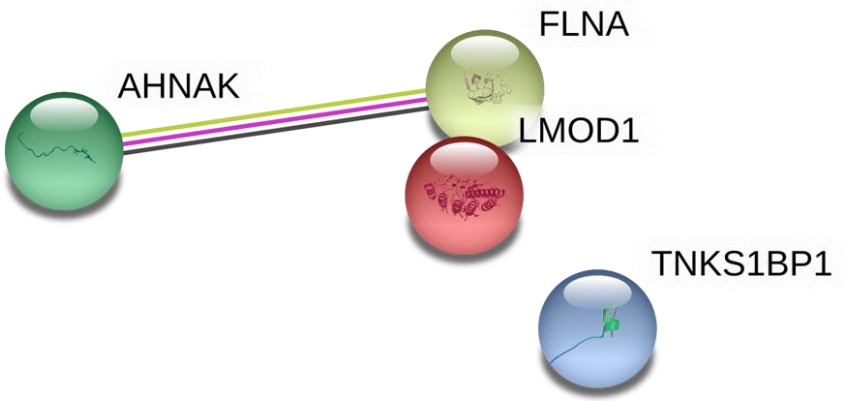

Figure 5.8: STRING diagram summarising the proteins for which there were phosphorylation events observed in all three samples and which either at least doubled or halved in the REL condition compared with PRE (REL/PRE) for samples taken from all three women (https://string-db.org/). Both groups have significantly more protein-protein interaction (PPI) than expected (p<1.0-16).

5.6.4.4 Part 1 phosphopeptides

There were many phosphorylation events observed. Table 5.5 shows the number of phosphopeptides measured for each woman and the number that mapped to one (i.e. unique) master protein according to the sequence. Two phosphopeptides at least halved between the pre-contracting condition and spontaneously contracting state (REL/PRE) in all three samples (Table 5.6), and three doubled (Table 5.7). Specifically, phosphorylation of neuroblast differentiation-associated protein AHNAK (AHNAK) at Serine 5552, and phosphorylation of 182 kDa tankyrase-1-binding protein at Serine 1297 were at least halved in the SP-REL condition compared with PRE; and phosphorylation of Filamin-A at Serine 968, phosphorylation of Leiomodin-1 at Serine 555, and phosphorylation of DYSL3 protein at Serine 518, Serine 522 and Threonine 514, all doubled in the REL condition compared with PRE. Of note, none of these phosphorylation sites were found to be significantly different between the failed IOL group and ELSCS group in part 2, although other phosphorylation events were significantly different on some of these same proteins.

Figure 5.8 is a STRING diagram (https://string-db.org/) summarising the proteins for which there were phosphorylation events observed in all three samples which either at least doubled or halved in the REL condition compared with PRE (REL/PRE) for samples taken from all three women. Again, there is significantly

more protein-protein interaction (PPI) than expected ($p<1.0\text{-}16$), indicating the potential interactions that may occur *in vivo*, and particularly highlighting a potential interaction between AHNAK and Filamin-A.

Table 5.5: number of phosphopeptides detected, number of which had a unique master protein, and number of related phosphoproteins.

Sample label	Number phosphopeptides detected	Number with unique master protein, and therefore used in analysis	Number of related phosphoproteins
565	56 299	3 172	1232
592	101 873	5 291	1 488
604	56 932	4997	1 648

Table 5.6: Phosphorylation events where REL/PRE ratio at least halved for all three women.

Protein description	Gene	Accession	Sequence detected	Site	REL/PRE for 565	REL/PRE for 592	REL/PRE for 604
Neuroblast differentiation-associated protein AHNAK	AHNAK	Q09666	gPAFNmAsPESDFGINLk	Serine 5552	0.201	0.175 0.276	0.456
182 kDa tankyrase-1-binding protein	TNKS1BP1	Q9C0C2	nmAPGAVcsPGESk	Serine 1297	0.474	0.159 0.280	0.487

Table 5.7: Phosphorylation events where REL/PRE at least doubled for all three women.

Protein description	Gene	Accession	Sequence	Site	565	592	604
Filamin-A	FLNA	P21333	sPFSVAVSPsLDLSk	Serine 968	3.114	2.094	2.097
Leiomodin-1	LMOD1	P29536	nSLsPATQR	Serine 555	3.473	3.704	2.455
					3.610	4.198	
						4.299	
						4.360	
DPYSL3 protein	DPYSL3	Q6DEN2	gGtPAGsARGsPTRPNPPVR	T 514	3.564	4.645	2.503
				Serine 518	4.246	5.147	
				Serine 522		12.251	

5.7 Part 2 background and results

5.7.1 Background

The rationale behind this part of the study was to determine if there are inherent differences between the proteomic profile of the myometrium of women who fail to progress following IOL and are delivered via emergency caesarean section and women who have an elective caesarean section. A better understanding of this could result in better selection of women for recommendation for induction of labour, potentially an improved or tailored forms of induction, and also it could indicate which myometrial processes and pathways are important for labour and vaginal delivery. The majority of the experimental stages for Part 2 were performed during this PhD by the thesis author, including recruitment, processing of myometrial samples, and Myobath II experiments. However, three of the six Myobath II experiments were performed with support from Dr Hudson. All protein extraction for phosphoproteomics and all analysis of data was performed solely by the thesis author, with support from Dr Heesom's group.

5.7.2 Inclusion criteria for the failed IOL group

- Induction of labour for non-fetal/maternal concerns
- Non-smoker
- No maternal infection
- Term pregnancy (>37 weeks' gestation)

5.7.3 Inclusion criteria ECS group

- Elective caesarean section for non-fetal/maternal concerns
- Non-smoker
- Term pregnancy (>37 weeks' gestation)

5.7.4 Exclusion criteria

- Pre-term pregnancy
- Maternal infection
- Maternal or fetal disease

5.7.5 Part 2 results

5.7.5.1 Participants' characteristics

There were three women in each group. Table 5.8 shows the participant demographics for Part 2, with three women in each group. Of note, all the women in the induction of labour group were induced for post-term, all were in their first pregnancy and all have subsequently had another pregnancy for which they had an ECS. All the ECS group had previously had one pregnancy and all three had laboured to fully dilated: two had a normal vaginal delivery and one had a trial of forceps but converted to caesarean section as malposition of baby and fetal bradycardia. This is important as shows that the myometrium in the ECS group had all previously laboured to full dilatation and did not have failure to progress. The indication for ECS were placenta praevia, maternal request,

and previous third-degree perineal tear. The gestational age at delivery was different between the two groups, and this is likely because ECS are routinely conducted between 39 and 40 completed weeks' gestation, whereas all of the failed IOL group were induced for post-dates, which is routinely offered from 40 weeks and 12 days gestation at St Michael's Hospital. The IOL procedure for all three women in the IOL group was with vaginal prostaglandins followed by artificial rupture of membranes and intravenous infusion of oxytocin, as per local protocols. All three women had an emergency caesarean section in labour: one women failed to progress beyond 3 cm dilatation and there was fetal distress; one failed to progress beyond 6 cm ; and the other failed to progress beyond 8 cm dilatation.

Table 5.8: Maternal demographics for Part 2.

Demographics	Failed IOL group	ECS group	p-value
Age at Recruitment (years)	**30**[a] (26-34)	**36.7**[a] (34-40)	**NS**
BMI	**24.6**[a] (20-32.7)	**26.2**[a] (20-29.2)	**NS**
PMH	**Nil**	**NIL**	**NA**
Gravida	**1**[b] (1-3)	**3**[b] (2-4)	**NS**
Parity	**0**[b] (0)	**1**[b] (1)	
Alcohol during pregnancy	**NIL**	**NIL**	**NA**
Ethnicity	**ALL WE**	**ALL WE**	**NA**
Smoking during pregnancy	**NIL**	**2 NIL** **1 3/day**	**NS**
Indication for induction	**Post-term**	**NA**	**NA**
Indication for elective CS	**NA**	**1 PP** **1 MR** **1 PTDT**	**NA**
Uterotonic given at delivery	**3 Carbetocin**	**3 Carbetocin**	**NA**
Gestational age at delivery	**293.3**[b] (292-295)	**268.7**[b] (258-272)	**0.006**
Maternal complications	**NIL**	**NIL**	**NA**
Birthweight	**3.7**[a] (2.44-4.18)	**3.4**[a] (3.3-3.6)	**NS**
Baby sex	**3 M**	**1 F** **2 M**	**NA**

[a]Mean ; [b]Median; BMI=Body Mass Index; PMH=Past Medical History; CS=Caesarean Section; WE=White European; PP = Placenta Praevia; MR=Maternal Request; PTDT=Previous Third Degree Tear; F=Female; M=Male

5.7.5.2 Part 2 total proteins

The phosphoproteomics results are presented below. Unfortunately, three samples were inadequate for processing in the ECS group, and for each of the

women one of the conditions did not have a sample. One woman had no sample for PRE, one no sample for SP-CON and one no sample for OXT-CON.

In the pre-contracting (PRE) group: the mean concentration of 68 proteins at least doubled in the failed IOL group (n=3) compared with the ECS group (n=3), and 13 of these were significant ($p \leq 0.05$) (Table 5.9); and the mean concentration of 76 of the total proteins at least halved in the failed IOL group, and 19 of these were significant ($p \leq 0.05$) (Table 5.10). Figure 5.9 presents these results in a volcano plot.

In the spontaneously contracting peak relaxation (SP-REL) group: the mean concentration of 89 proteins at least doubled in the failed IOL group (n=3) compared with the ECS group (n=3), and 33 of these were significant at the $p<0.05$ threshold (see Table 5.11); the mean concentration of 84 proteins at least halved in the failed IOL group, and 23 of these were significant (see Table 5.12). Figure 5.10 presents these results in a volcano plot.

In the spontaneously contracting peak contraction (SP-CON) group: the mean concentration of 100 proteins at least doubled in the failed IOL group (n=3) compared with the ECS group (n=2), and 29 of these were significant (see Table 5.13); while the mean concentration of 57 at least halved in the failed IOL group, and 16 of these were significant (see Table 5.14). Figure 5.11 presents these results in a volcano plot.

In the OXT-stimulated contracting sample taken at maximal relaxation (OXT-REL) group: the mean concentration of 122 proteins at least doubled in the failed IOL group (n=3) compared with the ECS group (n=3), and 35 of these were significant (see Table 5.15); the mean concentration of 124 proteins at least halved in the failed IOL group, and 14 of these were significant (see Table 5.16). Figure 5.12 presents these results in a volcano plot.

In the OXT-stimulated contracting sample taken at maximum contraction (OXT-CON) group: the mean concentration of 84 proteins at least doubled in the failed IOL group (n=3) compared with the ECS group (n=2), and 18 of these were significant (see Table 5.17); and the mean concentration of 170 proteins at least halved in the failed IOL group, and 20 of these were significant (see Table 5.18). Figure 5.13 presents these results in a volcano plot.

The most significant (where $p \leq 0.05$) increases in total protein in the failed IOL group were seen in the relaxed state, with or without OXT stimulation (SP-REL and OXT-REL). Table 5.19 shows the total proteins for which the mean concentration at least doubled in the failed IOL group in one or more conditions. Matrix Gla protein and Lysl oxidase homolog 2 significantly at least doubled in all five conditions; Transforming growth factor-beta-induced protein ig-h3, TNC variant protein and Prostacyclin synthase significantly at least doubled in all five conditions other than PRE; and Polypeptide N-acetyl-galactosaminyl-transferase

significantly at least doubled for the failed IOL group in all five conditions other than OXT-CON.

The most significant ($p \leq 0.05$) decreases in mean concentration of total protein were observed for the failed IOL group in the OXT-stimulated conditions, both relaxed (OXT-REL) and contracted (OXT-CON), however not all were significant (14/124 and 20/170, respectively). If it is surmised that the myometrium in the failed IOL group did not result in adequate coordination of contractions to cause effective cervical dilatation, these results could be interpreted as the myometrium taken from this group was less responsive to stimulation by OXT than the myometrium from the elective CS group. Therefore, these proteins may be important for the pathway involved in effective myometrial function in induced labour and potentially spontaneous labour. Table 5.20 lists the total proteins for which the mean concentration at least halved in the failed IOL group in at least two of the five conditions. Membrane metallo-endopeptidase and Atrial natriuretic peptide-converting enzyme were significantly at least halved in the failed IOL group in all conditions other than OXT-REL; Nuclear receptor subfamily 2, isoform CRA_a was significantly at least halved in the failed IOL group in all conditions other than OXT-CON; and Peptidyl-prolyl cis-trans isomerase-like 3 was significantly at least halved in the failed IOL group in all conditions other than PRE. Fatty acid-binding protein, Aldehyde oxidase,

Activating signal co-integrator 1 complex subunit 2, Endosialin, and Epididymis secretory protein Li 45 all significantly at least halved in the failed IOL group in three out of five of the conditions compared with the ECS group. MHC class I antigen, CDC42 effector protein (Rho GTPas binding) 4 isoform CRA_a, Selenium-binding protein 1, Scavenger receptor class A member 5 and Tubulin polymerization-promoting protein family member 3 were all significantly at least halved in two of the conditions.

Table 5.9: Total proteins which significantly at least doubled in the failed IOL group under the PRE condition (significant at $p \leq 0.05$).

Protein	Log2-FC	p-value
Keratin, type I cytoskeletal 10	1.030	0.021
Protein S100-A9	1.047	0.019
Immunoglobulin heavy variable 3-35 (non-functional) (Fragment)	1.081	0.027
Quinone oxidoreductase	1.090	0.040
GCT-A3 light chain variable region (Fragment)	1.151	0.036
MHC class I antigen	1.190	0.048
Uncharacterized protein	1.212	0.007
Polypeptide N-acetylgalactosaminyltransferase	1.322	0.021
Connective tissue growth factor	1.338	0.011
Lysyl oxidase homolog 2	1.422	0.006
Phospholipid transfer protein, isoform CRA_c	1.926	0.004
Prostaglandin G/H synthase 1	2.522	0.019
Matrix Gla protein	2.684	0.021

Table 5.10: Total proteins which significantly at least halved in the failed IOL group under the PRE condition (significant at $p \leq 0.05$).

Protein Description	Log2-fold change	p-value
Membrane metallo-endopeptidase	-2.010	0.018
EF hand domain family, member D1 variant (Fragment)	-1.915	0.014
Atrial natriuretic peptide-converting enzyme	-1.555	0.028
Calcitonin receptor-like, isoform CRA_a	-1.432	0.022
Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-5	-1.300	0.013
Epididymis secretory protein Li 45	-1.293	0.029
Gamma-glutamyltransferase 5	-1.278	0.045
Aminopeptidase	-1.265	0.042
Nuclear receptor subfamily 2, group F, member 2, isoform CRA_a	-1.207	0.015
Guanine nucleotide-binding protein subunit gamma	-1.202	0.041
cDNA FLJ55809	-1.124	0.012
Activating signal cointegrator 1 complex subunit 2	-1.091	0.022
MHC class I antigen (Fragment)	-1.090	0.027
MHC Class I antigen (Fragment)	-1.080	0.040
Acyl-CoA synthetase short-chain family member 3, mitochondrial	-1.075	0.002
Choline/ethanolamine kinase	-1.032	0.018
Protein ABHD11	-1.029	0.041
Podocalyxin	-1.017	0.015
Histone H1x	-1.006	0.016

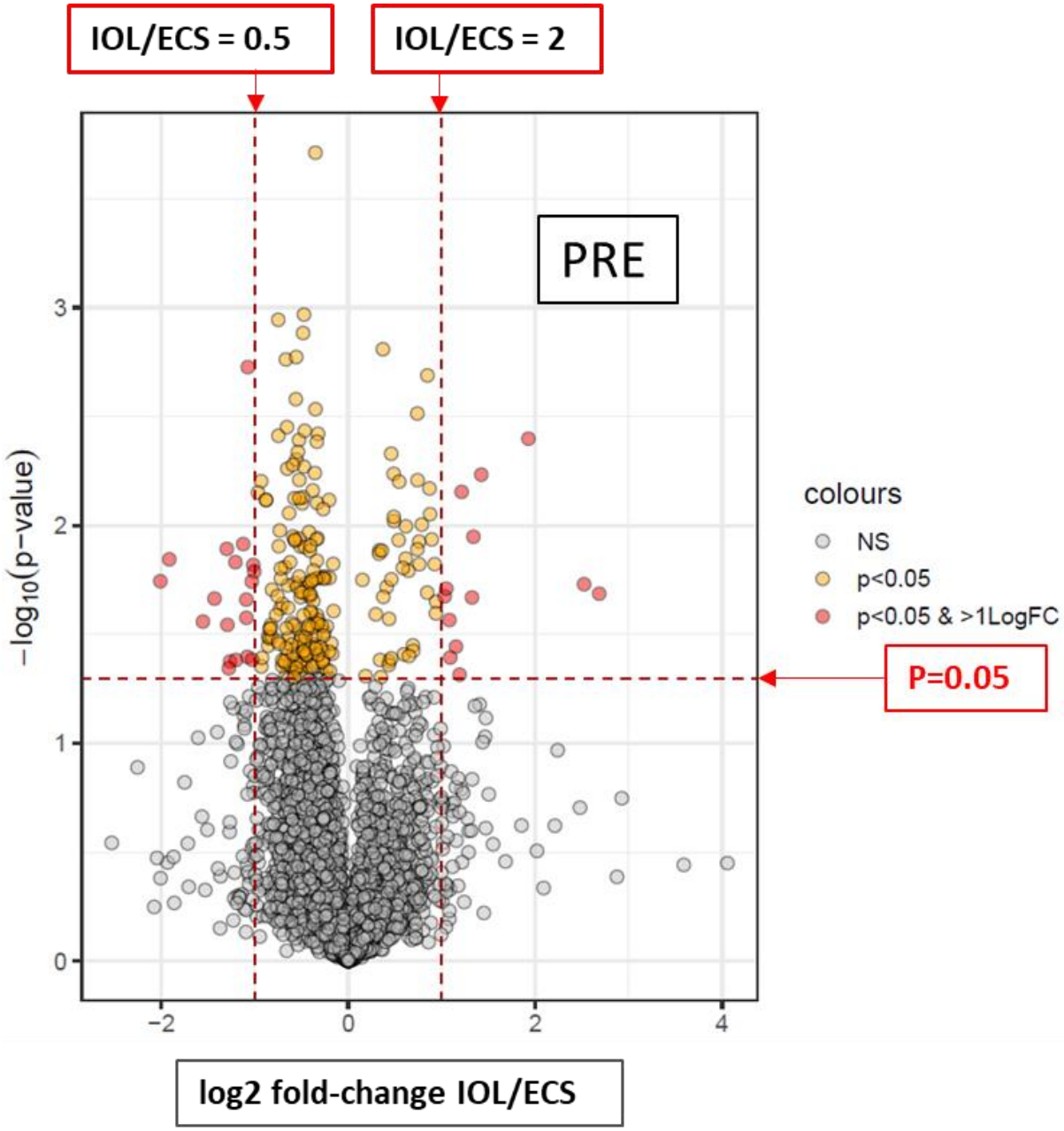

Figure 5.9: Volcano plot comparing change in log2-fold change in total protein between failed IOL and ECS groups in the pre-contracting condition. Volcano plot illustrating the mean log2-fold change in total protein between the failed IOL group (n=3) and the ECS group (n=3) (IOL/elcs) (x-axis) plotted against the –log10(p-value) (y-axis) in the pre-contracting condition. Horizontal dashed red line indicates p=0.05, above which differences between the failed IOL group and ECS group are significant; two vertical red dashed lines indicate halving and doubling between the failed IOL and ECS groups. Dot colour scheme: Black = non-significant and neither halved nor doubles; grey = either halved or doubled but not significant; orange = significant difference but neither halved nor doubles; red = significant difference and either halved or doubled.

Table 5.11: Total proteins which at least doubled in the failed IOL group and were significant at p<0.05 threshold in the SP-REL condition.

Protein	Log2-FC	p-value
Follistatin-related protein 1	1.007	0.002
Tropomyosin alpha-4 chain (Fragment)	1.044	0.002
Chromosome 1 open reading frame 24, isoform CRA_a	1.085	0.004
Lactadherin	1.130	0.029
Solute carrier family 25 member 4 isoform 1 (Fragment)	1.147	0.048
Myosin-reactive immunoglobulin heavy chain variable region(Fragment)	1.182	0.028
Collagen alpha-1(XII) chain	1.183	0.039
Insulin-like growth factor-binding protein 7	1.209	0.016
Fibulin-1	1.245	0.016
Inter-alpha (Globulin) inhibitor H2, isoform CRA_a	1.269	0.002
Fibulin-1	1.273	0.008
Ubiquitin-like protein ISG15	1.303	0.017
Prostacyclin synthase (Fragment)	1.316	0.027
Inter-alpha-trypsin inhibitor heavy chain H1	1.347	0.017
Phospholipid transfer protein, isoform CRA_c	1.350	0.037
Interferon-induced protein with tetratricopeptide repeats 1	1.351	0.018
OX-2 membrane glycoprotein	1.419	0.017
Amine oxidase	1.598	0.013

Lysyl oxidase homolog 2	1.676	0.003
cDNA FLJ90373 fis, clone NT2RP2004606, highly similar to Metalloproteinase inhibitor 1	1.760	0.028
Polypeptide N-acetylgalactosaminyltransferase	1.862	0.035
Uncharacterized protein DKFZp686F13142	1.910	0.005
Connective tissue growth factor	1.931	0.027
Calmodulin regulator protein PCP4	2.009	0.039
Pentraxin-related protein PTX3	2.082	0.007
Matrix Gla protein	2.509	0.025
Thrombospondin 1, isoform CRA_a	2.556	0.023
TNC variant protein (Fragment)	2.559	0.007
Transforming growth factor-beta-induced protein ig-h3	2.636	0.012
Serpin peptidase inhibitor, clade E (Nexin, plasminogen activator inhibitor type 1), member 1, isoform CRA_b	3.002	0.003
Serpin peptidase inhibitor, clade E (Nexin, plasminogen activator inhibitor type 1), member 2, isoform CRA_b	3.029	0.003
cDNA FLJ78437, highly similar to Homo sapiens cartilage oligomeric matrix protein (COMP), mRNA	3.080	0.039
Thrombospondin-4	3.196	0.012

Table 5.12: Total proteins which at least halved in the failed IOL group and were significant at p<0.05 threshold in the SP-REL condition.

Protein description	Log2-FC	p-value
Membrane metallo-endopeptidase	-2.205	0.034
Tubulin polymerization-promoting protein family member 3	-1.926	0.018
MHC class I antigen	-1.834	0.016
HCG1773630	-1.811	0.040
Fatty acid-binding protein, epidermal	-1.717	0.019
Aldehyde oxidase	-1.663	0.024
Endosialin	-1.648	0.008
Selenium-binding protein 1	-1.562	0.002
Myomegalin	-1.560	0.043
Scavenger receptor class A member 5	-1.507	0.027
ASPA protein (Fragment)	-1.389	0.037
Peptidyl-prolyl cis-trans isomerase-like 3	-1.382	0.024
Nuclear receptor subfamily 2, group F, member 2, isoform CRA_a	-1.375	0.019
Insulin-like growth factor-binding protein 5	-1.214	0.023
Epididymis secretory protein Li 45	-1.147	0.013
cDNA, FLJ95861, highly similar to Homo sapiens Meis1, myeloid ecotropic viral integration site 1homolog (mouse) (MEIS1), mRNA	-1.144	0.004
Pleckstrin homology domain-containing family A member 5	-1.127	0.036
3-hydroxybutyrate dehydrogenase, type 2, isoform CRA_b	-1.114	0.003
StAR-related lipid transfer protein 5	-1.042	0.008
Cadherin 11, type 2, OB-cadherin (Osteoblast)	-1.033	0.003
Parathymosin	-1.022	0.038
Atrial natriuretic peptide-converting enzyme	-1.010	0.025
cDNA, FLJ92910, highly similar to Homo sapiens programmed cell death 4 (neoplastic transformation inhibitor) (PDCD4), transcript variant 1, mRNA	-1.004	0.023

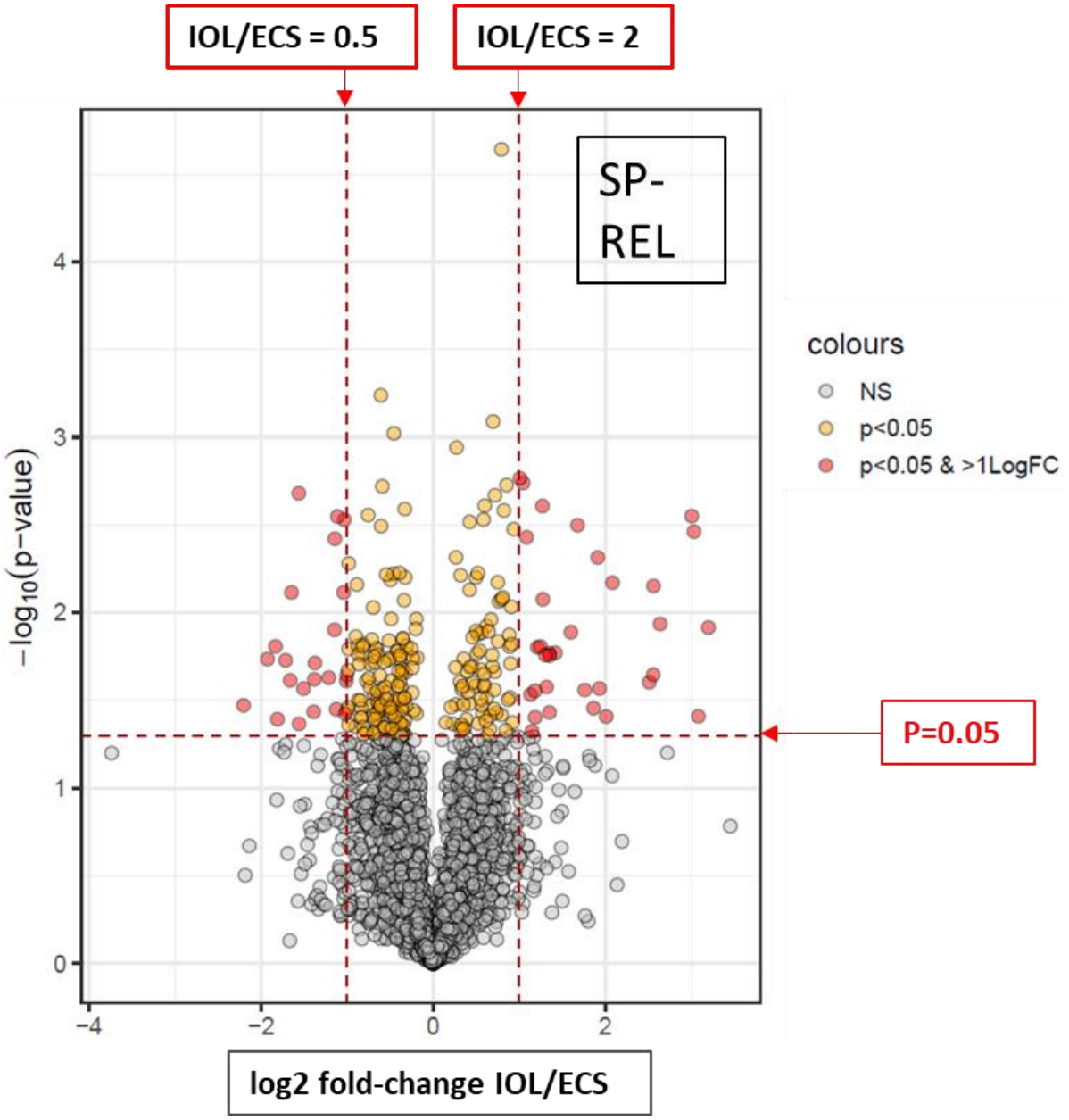

Figure 5.10: Volcano plot illustrating the log2-fold change in total protein between the failed IOL and ECS group in the spontaneously contracting group at peak relaxation. Volcano plot illustrating the mean log2-fold change in total protein between the failed IOL group (n=3) and the ECS group (n=3) (IOL/elcs) (x-axis) plotted against the –log10(p-value) (y-axis) in the spontaneously contracting group snap frozen at peak relaxation. Horizontal dashed red line indicates p=0.05, above which differences between the failed IOL group and ECS group are significant; two vertical red dashed lines indicate halving and doubling between the failed IOL and ECS groups. Dot colour scheme: Black = non-significant and neither halved nor doubles; grey = either halved or doubled but not significant; orange = significant difference but neither halved nor doubles; red = significant difference and either halved or doubled.

Table 5.13: Total proteins which at least doubled in the failed IOL group and were significant at p<0.05 threshold in the SP-CON condition.

Protein	Log2-FC	P-value
Actin alpha cardiac muscle 1 (Fragment)	1.003	0.034
Rho GTPase-activating protein 45	1.031	0.032
Inter-alpha (Globulin) inhibitor H2, isoform CRA_a	1.039	0.048
Lambda-chain (AA -20 to 215)	1.064	0.046
Solute carrier family 25 member 4 isoform 1 (Fragment)	1.092	0.037
NADH:ubiquinone oxidoreductase MLRQ subunit homolog, isoform CRA_a	1.136	0.008
Prostacyclin synthase (Fragment)	1.176	0.015
Protein S100-A12	1.188	0.025
Latent-transforming growth factor beta-binding protein 2	1.189	0.041
Connective tissue growth factor	1.238	0.020
Lysozyme C	1.279	0.012
Collagen alpha-1(XII) chain	1.298	0.034
[Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 1, mitochondrial	1.299	0.023
Protein S100-A8	1.370	0.006
Myosin-reactive immunoglobulin light chain variable region (Fragment)	1.378	0.011
ASPN protein	1.401	0.036
Polypeptide N-acetylgalactosaminyltransferase	1.490	0.007
Lysyl oxidase homolog 2	1.554	0.020
Serpin peptidase inhibitor, clade E (Nexin, plasminogen activator inhibitor type 1), member 2, isoform CRA_b	1.568	0.022
Protein S100-A9	1.613	0.011
Calmodulin regulator protein PCP4	1.622	0.028
Serpin peptidase inhibitor, clade E (Nexin, plasminogen activator inhibitor type 1), member 1, isoform CRA_b	1.730	0.021
Myosin-reactive immunoglobulin heavy chain variable region (Fragment)	1.780	0.008
Thrombospondin 1, isoform CRA_a	1.870	0.024
Biglycan (Fragment)	2.075	0.034
TNC variant protein (Fragment)	2.128	0.032

Transforming growth factor-beta-induced protein ig-h3	2.199	0.024
Matrix Gla protein	2.350	0.026
Thrombospondin-4	2.796	0.042

Table 5.14: Total proteins which at least halved in the failed IOL group and were significant at p<0.05 threshold in the SP-CON condition.

Protein description	Log2-FC	p-value
MHC class I antigen (Fragment)	-2.576	0.049
Membrane metallo-endopeptidase	-2.080	0.035
MHC class I antigen	-2.078	0.006
Ephrin-B1	-1.838	0.022
Peptidyl-prolyl cis-trans isomerase-like 3	-1.609	0.012
Four and a half LIM domains protein 5	-1.564	0.018
Epididymis luminal protein 57	-1.554	0.038
Epididymis secretory protein Li 45	-1.467	0.003
NAD(P)H dehydrogenase [quinone] 1	-1.360	0.045
Scavenger receptor class A member 5	-1.298	0.027
Fatty acid-binding protein, epidermal	-1.285	0.047
Aldehyde oxidase	-1.212	0.013
Selenium-binding protein 1	-1.109	0.020
Atrial natriuretic peptide-converting enzyme	-1.090	0.007
SAM and SH3 domain-containing protein 1	-1.088	0.028
Nuclear receptor subfamily 2, group F, member 2, isoform CRA_a	-1.072	0.040

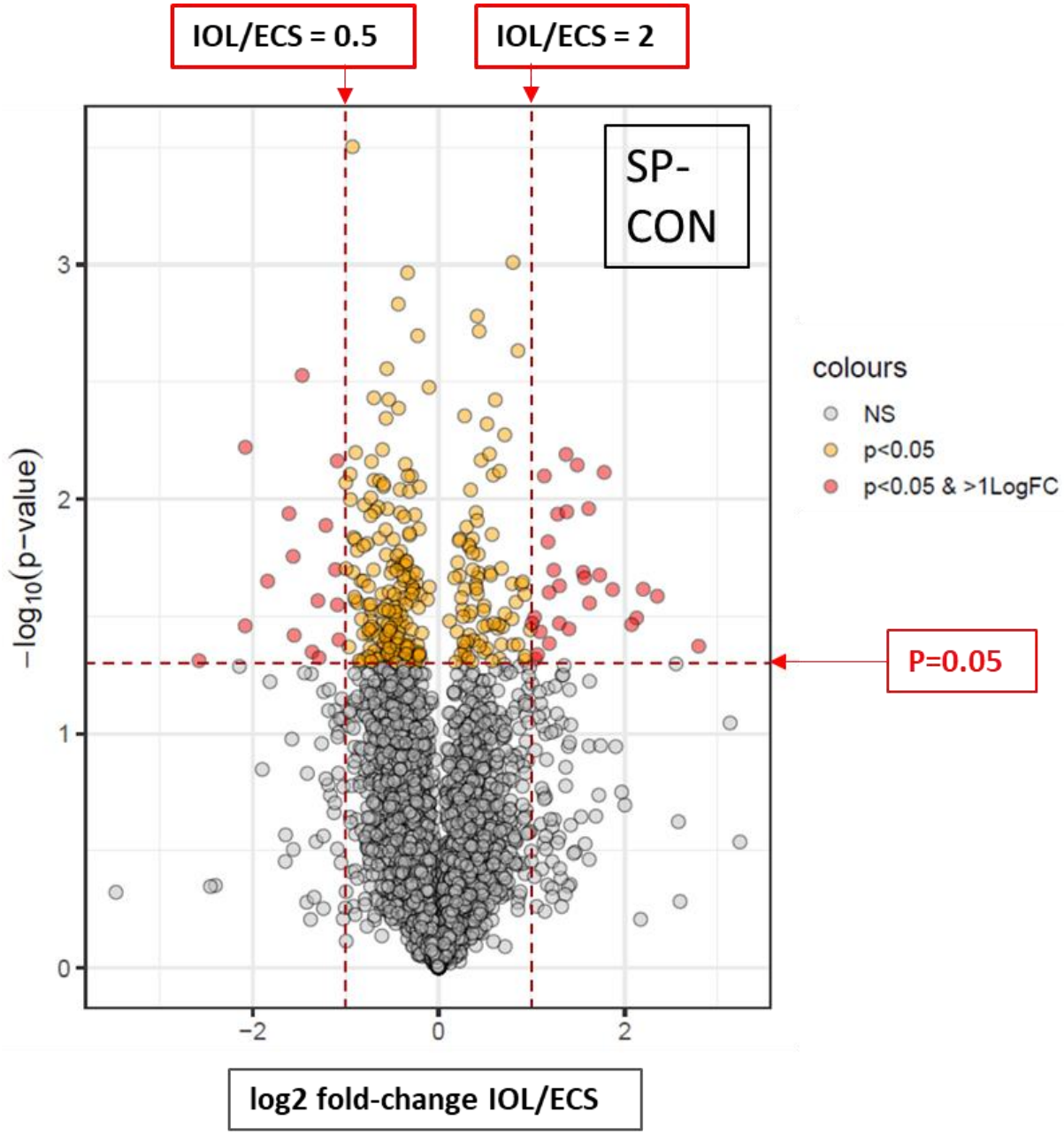

Figure 5.11: Volcano plot of comparing log2-fold change in total protein between the failed IOL group and ECS group in the spontaneously contracting group at peak contraction. Volcano plot illustrating the mean log2-fold change in total protein between the failed IOL group (n=3) and the ECS group (n=2) (IOL/elcs) (x-axis) plotted against the –log10(p-value) (y-axis) in the spontaneously contracting group snap frozen at peak contraction. Horizontal dashed red line indicates p=0.05, above which differences between the failed IOL group and ECS group are significant; two vertical red dashed lines indicate halving and doubling between the failed IOL and ECS groups. Dot colour scheme: Black = non-significant and neither halved nor doubles; grey = either halved or doubled but not significant; orange = significant difference but neither halved nor doubles; red = significant difference and either halved or doubled.

Table 5.15: Total proteins which at least doubled in the failed IOL group and were significant at p<0.05 threshold in the OXT-REL condition.

Protein	Log2-FC	p-value
Monocarboxylate transporter 2	1.002	0.006
Follistatin-related protein 1	1.044	0.034
Nuclear envelope protein okuribin	1.057	0.034
Tropomyosin alpha-4 chain (Fragment)	1.091	0.017
Thrombospondin-3	1.116	0.024
Probable phospholipid-transporting ATPase IIA	1.122	0.026
Secreted protein, acidic, cysteine-rich (Osteonectin), isoform CRA_a	1.132	0.024
cDNA FLJ46731 fis, clone TRACH3019621, highly similar to UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminephosphotransferase (EC 2.7.8.15)	1.140	0.040
Anaphase-promoting complex subunit 5	1.149	0.043
Microfibril-associated glycoprotein 4	1.166	0.016
Fibroleukin	1.171	0.013
Protein CYR61	1.184	0.049
OX-2 membrane glycoprotein	1.186	0.030
Cysteine-rich motor neuron 1 protein	1.214	0.003
Chromosome 1 open reading frame 24, isoform CRA_a	1.307	0.037
Neurofilament light polypeptide isoform 2 (Fragment)	1.329	0.033
Prostacyclin synthase (Fragment)	1.368	0.002
Alcohol dehydrogenase 1B	1.389	0.033
Filamin-A (Fragment)	1.414	0.024
Inter-alpha-trypsin inhibitor heavy chain H1	1.420	0.019
Inter-alpha (Globulin) inhibitor H2, isoform CRA_a	1.425	0.011
Lysyl oxidase homolog 2	1.435	0.034
Polypeptide N-acetylgalactosaminyltransferase	1.440	0.003
cDNA FLJ90373 fis, clone NT2RP2004606, highly similar to Metalloproteinase inhibitor 1	1.851	0.003
Diphosphoinositol polyphosphate phosphohydrolase 3-alpha	2.005	0.038
Thrombospondin 1, isoform CRA_a	2.060	0.028

TNC variant protein (Fragment)	2.091	0.021
Pentraxin-related protein PTX3	2.112	0.019
Transforming growth factor-beta-induced protein ig-h3	2.148	0.042
Cytochrome b5 type B (Fragment)	2.211	0.010
Serpin peptidase inhibitor, clade E (Nexin, plasminogen activator inhibitor type 1), member 1, isoform CRA_b	2.577	0.013
Matrix Gla protein	2.739	0.016
Thrombospondin-1	2.752	0.034
Thrombospondin-4	3.192	0.018
cDNA FLJ78437, highly similar to Homo sapiens cartilage oligomeric matrix protein (COMP), mRNA	3.280	0.002

Table 5.16: Total proteins which at least halved in the failed IOL group and were significant at p<0.05 threshold in the OXT-REL condition.

Protein description	Log2-FC	p-value
MHC class I antigen (Fragment)	-5.499	0.013
Platelet membrane glycoprotein Ib beta	-1.823	0.018
Peptidyl-prolyl cis-trans isomerase-like 3	-1.707	0.011
SEC14-like 2 (S. cerevisiae), isoform CRA_b	-1.616	0.022
Fatty acid-binding protein, epidermal	-1.467	0.038
CDC42 effector protein (Rho GTPase binding) 4, isoform CRA_a	-1.451	0.021
Tubulin polymerization-promoting protein family member 3	-1.397	0.024
Endosialin	-1.295	0.000
Nuclear receptor subfamily 2, group F, member 2, isoform CRA_a	-1.214	0.004
Cytosolic 5'-nucleotidase 3A	-1.189	0.008
6-phosphogluconate dehydrogenase, decarboxylating	-1.187	0.003
Activating signal cointegrator 1 complex subunit 2	-1.108	0.026
cGMP-dependent 3',5'-cyclic phosphodiesterase	-1.093	0.041
cDNA, FLJ92910, highly similar to Homo sapiens programmed cell death 4 (neoplastic transformation inhibitor) (PDCD4), transcript variant 1, mRNA	-1.089	0.011

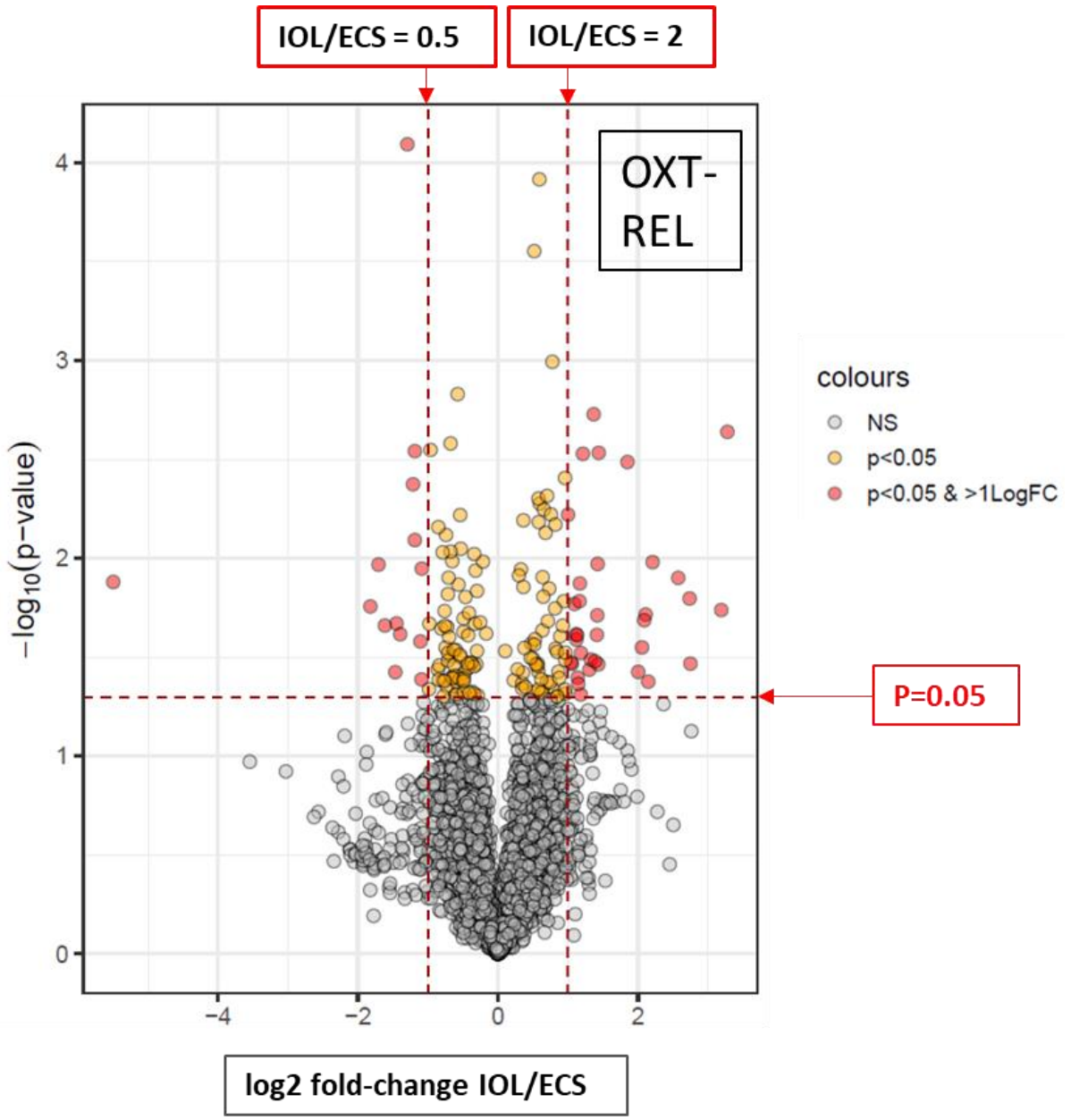

Figure 5.12: Volcano plot of change in total protein between the failed IOL and ECS groups in the oxytocin-stimulated group at peak relaxation. Volcano plot illustrating the mean log2-fold change in total protein between the failed IOL group (n=3) and the ECS group (n=3) (IOL/elcs) (x-axis) plotted against the –log10(p-value) (y-axis) in the oxytocin-stimulated group snap frozen at peak relaxation. Horizontal dashed red line indicates p=0.05, above which differences between the failed IOL group and ECS group are significant; two vertical red dashed lines indicate halving and doubling between the failed IOL and ECS groups. Dot colour scheme: Black = non-significant and neither halved nor doubles; grey = either halved or doubled but not significant; orange = significant difference but neither halved nor doubles; red = significant difference and either halved or doubled.

Table 5.17: Total proteins which at least doubled in the failed IOL group and were significant at p<0.05 threshold in the OXT-CON condition.

Protein	Log2-FC	p-value
ATP synthase protein 8	1.067	0.023
Fibulin-1	1.075	0.016
Aldehyde dehydrogenase X, mitochondrial	1.089	0.032
Metallothionein-2	1.111	0.034
Keratin 16	1.192	0.025
Zinc transporter 7	1.199	0.039
HBV PreS1-transactivated protein 1	1.253	0.024
Lysyl oxidase homolog 2	1.282	0.045
Thrombospondin-3	1.297	0.019
Prostacyclin synthase (Fragment)	1.358	0.039
Septin 6 isoform E	1.389	0.029
Cyclin-dependent kinase 2-associated protein	1.520	0.002
Protein unc-13 homolog B	1.695	0.013
Pentraxin-related protein PTX3	1.770	0.020
Matrix Gla protein	2.119	0.027
TNC variant protein (Fragment)	2.348	0.025
Transforming growth factor-beta-induced protein ig-h3	2.526	0.034
MHC class I antigen (Fragment)	3.447	0.037

Table 5.18: Total proteins which at least halved in the failed IOL group and were significant at p≤0.05 threshold in the OXT-CON condition.

Protein description	Log2-FC	p-value
MHC class I antigen	-3.402	0.039
MHC class I antigen	-2.311	0.026
Membrane metallo-endopeptidase	-2.056	0.047
cDNA FLJ55606, highly similar to Alpha-2-HS-glycoprotein	-1.614	0.025
Pregnancy-specific beta-1-glycoprotein 1	-1.564	0.025
Peptidyl-prolyl cis-trans isomerase-like 3	-1.454	0.009
Transferrin variant (Fragment)	-1.399	0.003
Aldehyde oxidase	-1.338	0.015
Serum albumin	-1.308	0.043
F-box/WD repeat-containing protein 5	-1.285	0.048
Atrial natriuretic peptide-converting enzyme	-1.231	0.024
CDC42 effector protein (Rho GTPase binding) 4, isoform CRA_a	-1.194	0.016
Beta-2-microglobulin	-1.182	0.042
Endosialin	-1.177	0.003
Alpha-1-antitrypsin	-1.159	0.043
Pirin	-1.138	0.002
Acid sphingomyelinase-like phosphodiesterase 3a	-1.138	0.016
cDNA FLJ53691, highly similar to Serotransferrin	-1.128	0.035
Testicular secretory protein Li 41	-1.050	0.044
Activating signal cointegrator 1 complex subunit 2	-1.002	0.002

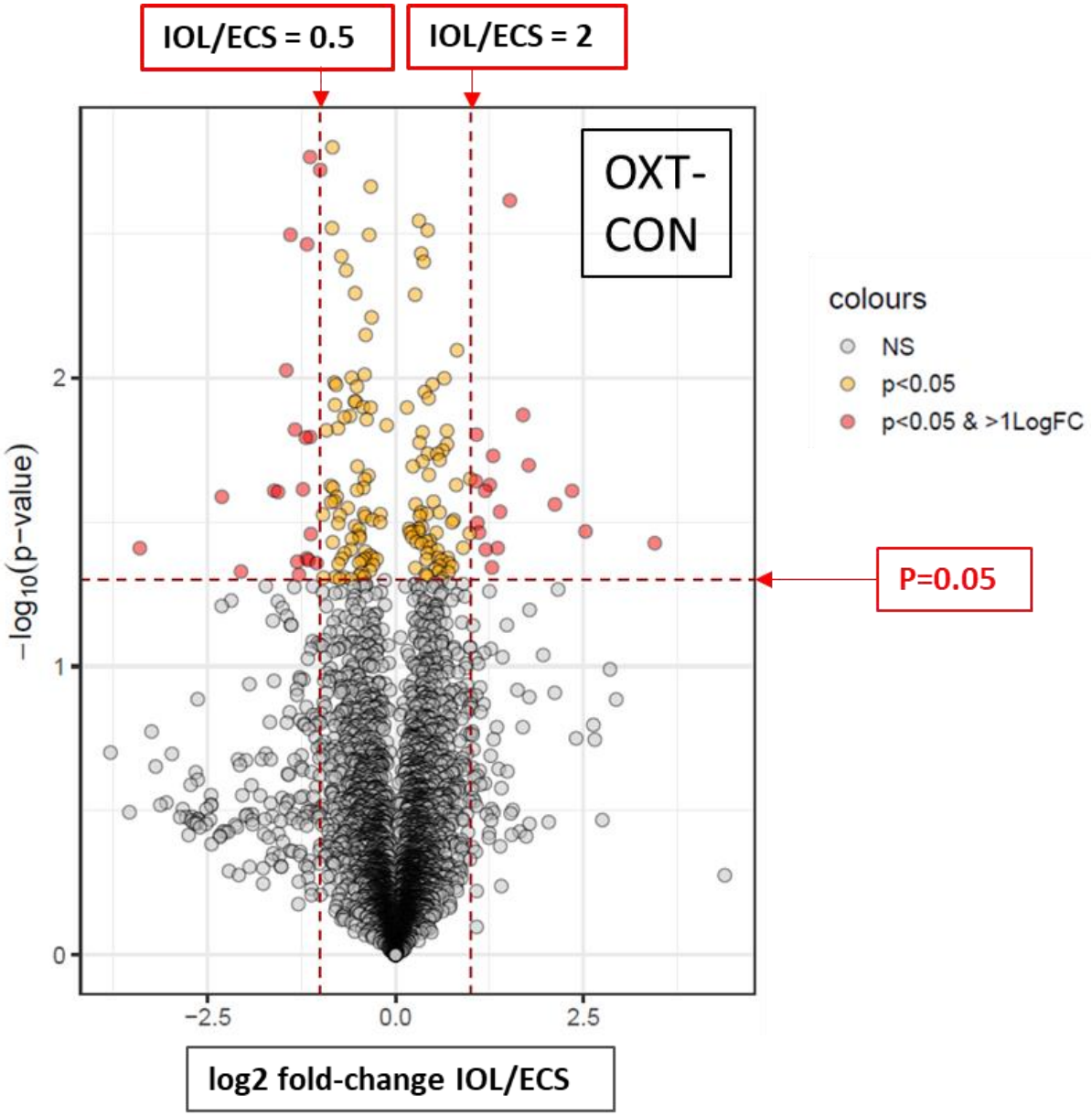

Figure 5.13: Volcano plot of change in total protein between the failed IOL and ECS group in the oxytocin-stimulated group at peak contraction. Volcano plot illustrating the mean log2-fold change in total protein between the failed IOL group (n=3) and the ECS group (n=2) (IOL/elcs) (x-axis) plotted against the –log10(p-value) (y-axis) in the oxytocin-stimulated group snap frozen at peak contraction. Horizontal dashed red line indicates p=0.05, above which differences between the failed IOL group and ECS group are significant; two vertical red dashed lines indicate halving and doubling between the failed IOL and ECS groups. Dot colour scheme: Black = non-significant and neither halved nor doubles; grey = either halved or doubled but not significant; orange = significant difference but neither halved nor doubles; red = significant difference and either halved or doubled.

Table 5.19: Proteins which significantly (p<0.05) at least double in the failed IOL group for at least two of the conditions.

Protein name	Gene name	Accession	Total REL-SP	Total REL-OT	Total CON-SP	Total CON-OT	PRE
Matrix Gla protein	MGP	P08493	✓	✓	✓	✓	✓
Lysyl oxidase homolog 2	LOXL2	Q9Y4K0	✓	✓	✓	✓	✓
Polypeptide N-acetylgalactosaminyltransferase		B3KNV8	✓	✓	✓		✓
Transforming growth factor-beta-induced protein ig-h3	TGFBI	Q15582	✓	✓	✓	✓	
TNC variant protein (Fragment)	TNC variant protein	Q4LE33	✓	✓	✓	✓	
Prostacyclin synthase (Fragment)	PGIS	Q6LEN0	✓	✓	✓	✓	
Serpin peptidase inhibitor, clade E (Nexin, plasminogen activator inhibitor type 1), member 1, isoform CRA_b	SERPINE1	A0A024QYT5	✓	✓	✓		
Thrombospondin 1, isoform CRA_a	THBS1	A0A024R9Q1	✓	✓	✓		
Inter-alpha (Globulin) inhibitor H2, isoform CRA_a	ITIH2	D3DRR6	✓	✓	✓		
Pentraxin-related protein PTX3	PTX3	P26022	✓	✓		✓	
Thrombospondin-4	THBS4	P35443	✓	✓	✓		
Connective tissue growth factor	CTGF	Q5M8T4	✓		✓		✓
Serpin peptidase inhibitor, clade E (Nexin, plasminogen activator inhibitor type 1), member 2, isoform CRA_b	SERPINE2	A0A024R498	✓		✓		
Chromosome 1 open reading frame 24, isoform CRA_a	C1orf24	A0A024R978	✓	✓			
Solute carrier family 25 member 4 isoform 1 (Fragment)	SLC25A4	A0A0S2Z3H3	✓		✓		
cDNA FLJ78437, highly similar to Homo sapiens cartilage oligomeric matrix protein (COMP), mRNA		A8K3I0	✓	✓			
cDNA FLJ90373 fis, clone NT2RP2004606, highly similar to Metalloproteinase inhibitor 1		B3KQF4	✓	✓			
Phospholipid transfer protein, isoform CRA_c	PLTP	B3KUE5	✓				✓
Fibulin-1 OS=Homo sapiens		B4DUV1	✓			✓	
Tropomyosin alpha-4 chain (Fragment)	TPM4	K7ELP0	✓	✓			
Protein S100-A9	S100A9	P06702			✓		✓
Inter-alpha-trypsin inhibitor heavy chain H1	ITIH1	P19827	✓	✓			

OX-2 membrane glycoprotein	CD200	P41217	✓	✓			
Calmodulin regulator protein PCP4	PCP4	P48539	✓		✓		
Thrombospondin-3	THBS3	P49746		✓		✓	
Follistatin-related protein 1	FSTL1	Q12841	✓	✓			
Collagen alpha-1(XII) chain	COL12A1	Q99715	✓		✓		
Myosin-reactive immunoglobulin heavy chain variable region (Fragment)		Q9UL90	✓		✓		
Total number (/26)			24	19	17	8	6

Table 5.20: Proteins which significantly (p<0.05) at least halved in the failed IOL group for at least two of the conditions.

Protein name	Gene name	Accession	Total REL-SP	Total REL-OT	Total CON-SP	Total CON-OT	PRE
Membrane metallo-endopeptidase	MME	B7ZKY6	✓		✓	✓	✓
Nuclear receptor subfamily 2, group F, member 2, isoform CRA_a	NR2F2	F1D8R0	✓	✓	✓		✓
Peptidyl-prolyl cis-trans isomerase-like 3	PPIL3	Q9H2H8	✓	✓	✓	✓	
Atrial natriuretic peptide-converting enzyme	CORIN	Q9Y5Q5	✓		✓	✓	✓
Fatty acid-binding protein, epidermal	FABP5	Q01469	✓	✓	✓		
Aldehyde oxidase	AOX1	Q06278	✓		✓	✓	
Activating signal cointegrator 1 complex subunit 2	ASCC2	Q9H1I8		✓		✓	✓
Endosialin	CD248	Q9HCU0	✓	✓		✓	
Epididymis secretory protein Li 45	HEL-S-45	V9HWG3	✓		✓		✓
MHC class I antigen	HLA-B	A0A173ADG8	✓		✓		
CDC42 effector protein (Rho GTPase binding) 4, isoform CRA_a	CDC42EP4	B2R6D8		✓		✓	
cDNA, FLJ92910, highly similar to Homo sapiens programmed cell death 4 (neoplastic transformation inhibitor) (PDCD4), transcript variant 1, mRNA		B2R6E2	✓	✓			
Selenium-binding protein 1	SELENBP1	Q13228	✓		✓		
Scavenger receptor class A member 5	SCARA5	Q6ZMJ2	✓		✓		
Tubulin polymerization-promoting protein family member 3	TPPP3	Q9BW30	✓	✓			
Total			11	8	10	7	5

5.7.5.3 Study 2 phosphoproteomics

In the pre-contracting (PRE) group: 8 phosphopeptides at least doubled in the failed IOL group (n=3) compared with the ECS group (n=2) (see Table 5.21), none of which were significant; and 7 phosphopeptides at least halved in the failed IOL group compared with the ECS group (see Table 5.22), and one of these was significant.

In the SP-REL group: 4 phosphopeptides at least doubled in the failed IOL group (n=3) compared with the ECS group (n=3) (see Table 5.23), and one was significant; and 4 phosphopeptides at least halved in the failed IOL group (see Table 5.24), none of which were significant.

In the SP-CON group: 3 phosphopeptides at least doubled in the failed IOL group (n=3) compared with the ECS group (n=2) (see Table 5.25), none of which were significant; and 7 at least halved in the IOL group (see Table 5.26), 1 of which was significant.

In the OXT-REL group: 18 phosphopeptides at least doubled in the failed IOL group (n=3) compared with the ECS group (n=3) (see Table 5.27), 4 of which were significant; and 22 phosphopeptides at least halved in the failed IOL group (see Table 5.28), 4 of which were significant.

In the OXT-CON group: 9 phosphopeptides at least doubled in the IOL group (n=3) compared with the ECS group (n=2) (see Table 5.29), 2 of which were significant; and 8 at least halved in the IOL group (see Table 5.30), 2 of which were significant.

Of the phosphopeptides which at least significantly halved or doubled in the failed IOL group compared with the ECS group, only one was shared between two conditions: Neuroblast differentiation-associated protein AHNAK (AHNAK) significantly at least halved in both the PRE and OXT-REL conditions. This phosphopeptide also halved in the SP-CON condition, but this was not significant.

As well as investigating the phosphorylation events which were significantly different between the failed IOL group and the ECS group, the phosphorylation events which were replicated across more than one condition were also investigated, even if not significant, as it may be that if the study had increased power these may be significant, and the fact that these changes were seen in multiple conditions may indicate inherent differences in the myometrium of the failed IOL group. Of all the phosphorylation-events, Table 5.31 shows those which at least halved in the failed IOL group under more than one condition, although not all were significant at the $p \leq 0.05$ level. This shows that phosphorylation of Serine 176 on Integrin, alpha 5 (Fibronectin receptor, alpha polypeptide), isoform CRA_b more than halved under both SP-REL (-1.13853

(0.329)) and PRE (-1.698 (0.115)) conditions when compared with the same conditions for the ECS group; phosphorylation of Serine 759 cDNA FLI77630, highly similar to Homo sapiens BPY2 interacting protein 1, mRNA more than halved in both SP-CON and PRE in the failed IOL group; phosphorylation of Serine 151 of cDNA FLI59374, highly similar to Homo sapiens caldesmon 1, transcript variant 2, mRNA at least halved in the failed IOL group in the SP-REL, OXT-REL and PRE conditions; Serine 194 phosphorylation of transforming growth factor beta-1-induced transcript 1 protein was halved in the failed IOL group under SP-CON ($p<0.05$) and OXT-CON; Serine 210 phosphorylation of Neuroblast differentiation-associated protein AHNAK more than halved in the failed IOL group in SP-CON and PRE conditions; Serine 65 phosphorylation of Transcription elongation factor A protein-like 3 was at least halved in the failed IOL group under SP-CON and OXT-CON conditions; and phosphorylation jointly of Serine 1620 and 1621 of 182 kDa tankyrase-1-binding protein was at least halved under OXT-REL and OXT-CON conditions.

Table 5.32 shows those phosphorylation events which at least doubled in the failed IOL group under more than one condition, but again not all significant at the $p\leq0.05$ level. Phosphorylation of Serine 478 of cDNA FLJ53399, highly similar to Monocarboxylate transporter 1 at least doubled in the failed IOL group under SP-REL, OXT-REL, SP-CON and PRE conditions; phosphorylation of Serine 2180 of

Filamin-A at least doubled in the failed IOL group under SP-REL ($p \leq 0.05$), OXT-REL and OXT-CON conditions; phosphorylation of Serine 65 of Transcription elongation factor A protein-like 3 more than doubled in the failed IOL group under OXT-REL ($p \leq 0.05$) and PRE conditions; and Serine 384 of Protein Lunapark more than doubled in the failed IOL group under OXT-CON and PRE conditions.

Table 5.21: Phosphorylation events which at least doubled in the failed IOL group in the PRE condition.

Protein	Gene	Accession	Sequence detected	Site	log 2 fc	p value
Cortactin, isoform CRA_c OS=Homo sapiens	CTTN	Q14247	[K].TQTPPVSPAPQPTEER.[L]	Threonine 401	1.617	0.084
Protein lunapark	LNP	Q9C0E8	[K].ASDSEEPEEK.[Q]	Serine 384	1.631	0.096
Transcription elongation factor A protein-like 3	TCEAL3	Q969E4	[K].REDEGEPGDEGQLEDEGSQEK.[Q]	Serine 65	1.115	0.104
Heat shock protein HSP 90-beta	HSP90AB1	P08238	[K].EISDDEAEEEK.[G]	Serine 226	1.090	0.397
cDNA FLJ53399, highly similar to Monocarboxylate transporter 1		B4E106	[K].DTEGGPKEEESPV.[-]	Serine 478	2.181	0.425
Mutant desmin		A5Z217	[R].TFGGAPGFPLGSPLSSPVFPR.[A]	Serine 28 Serine 32	1.388	0.543
Mutant desmin		A5Z217	[R].TSGGAGGLGSLR.[A]	Ambiguous	1.025	0.545
Mutant desmin		A5Z217	[R].TFGGAPGFPLGSPLSSPVFPR.[A]	Serine 32	1.384	0.604

Table 5.22: Phosphopeptides which at least halved in the failed IOL group in the PRE condition.

Protein Descriptions	Gene Name	Accession	Sequence detected	Phosphosites	log 2 fc	p value
Integrin, alpha 5 (Fibronectin receptor, alpha polypeptide), isoform CRA_b	ITGA5	A0A024RB01	[R].LLESSLSSSEGEEPVEYK.[S]	Serine 176	-1.698	0.115
SAM and SH3 domain-containing protein 1 (Fragment)	SASH1	O94885	[R].EQSDDETEESVK.[F]	Serine 248	-1.456	0.209
Neuroblast differentiation-associated protein AHNAK	**AHNAK**	Q09666	[R].LPSGSGAASPTGSAVDIR.[A]	Serine 210	-1.425	0.034
cDNA FLJ59374, highly similar to Homo sapiens caldesmon 1 (CALD1), transcript variant 2, mRNA	CALD1	B4DPW5	[K].RGSIGENQIKDEK.[I]	Serine 151	-1.205	0.087
Eukaryotic translation initiation factor 5B	EIF5B	A0A087WUT6	[K].NKPGPNIESGNEDDDASFK.[I]	Serine 214	-1.186	0.232
cDNA FLJ77630, highly similar to Homo sapiens BPY2 interacting protein 1, mRNA		A8K940	[K].AVPMAPAPASPGSSNDSSAR.[S]	Serine 759	-1.174	0.174
Nuclear ubiquitous casein and cyclin-dependent kinase substrate 1 OS=Homo sapiens	NUCKS1	Q9H1E3	[R].KVVDYSQFQESDDADEDYGR.[D]	Serine 19	-1.115	0.166

Table 5.23: Phosphopeptides which at least doubled in the failed IOL group in the SP-REL condition.

Protein Descriptions	Gene Name	Accession	Annotated Sequence	Phosphosites	LOG2 FC	p value
Filamin-A	FLNA	P21333	[K].IPEISIQDMTAQVTSPSGK.[T]	Serine 2180	1.550	0.034
Vacuolar protein sorting-associated protein 4A	VPS4A	Q9UN37	[K].GSDSDSEGDNPEK.[K]	Serine 97	1.032	0.071
Filamin-A	FLNA	P21333	[K].FNEEHIPDSPFVVPVASPSGDAR.[R]	Serine 2327	1.600	0.117
cDNA FLJ53399, highly similar to Monocarboxylate transporter 1		B4E106	[K].DTEGGPKEEESPV.[-]	Serine 478	1.931	0.311

Table 5.24: Phosphopeptides which at least halved in the failed IOL group in the SP-REL condition.

Protein Descriptions	Gene Name	Accession	Annotated Sequence	Site	LOG2 FC	p value
Phosphatidate cytidylyltransferase		B3KM95	[R].VAHEPVAPPEDKESESEAKVDGETASDSESR.[A]	Ambiguous	-1.35554	0.053193
Testicular secretory protein Li 7		A0A140VK07	[R].EPAPASPAPAGVEIR.[S]	Serine 102	-1.00651	0.123959
cDNA FLJ59374, highly similar to Homo sapiens caldesmon 1 (CALD1), transcript variant 2, mRNA		B4DPW5	[K].RGSIGENQIKDEK.[I]	Serine 151	-1.01966	0.291106
Integrin, alpha 5 (Fibronectin receptor, alpha polypeptide), isoform CRA_b	ITGA5	A0A024RB01	[R].LLESSLSSSEGEEPVEYK.[S]	Serine 176	-1.13853	0.329186

Table 5.25: Phosphopeptides which at least doubled in the failed IOL group in the SP-CON condition.

Protein Descriptions	Gene Name	Accession	Annotated Sequence	Site	Log2-FC	p-value
28 kDa heat- and acid-stable phosphoprotein	PDAP1	Q13442	[K].KSLDSDESEDEEDDYQQK.[R]	Serine 60 Serine 63	1.199	2.89E-01
Membrane-associated progesterone receptor component 1	PGRMC1	O00264	[K].EGEEPTVYSDEEEPK.[D]	Serine 181	1.491	3.36E-01
cDNA FLJ53399, highly similar to Monocarboxylate transporter 1		B4E106	[K].DTEGGPKEEESPV.[-]	Serine 478	2.607	3.62E-01

Table 5.26: Phosphopeptides which at least halved in the failed IOL group in the SP-CON condition.

Protein Descriptions	Gene Name	Accession	Sequence Detected	Site	Log2-FC	p-value
Phosphatidate cytidylyltransferase		B3KM95	[R].VAHEPVAPPEDKESESEAKVDGETASDSESR.[A]	Ambiguous	-1.481	0.248
Lon protease homolog, mitochondrial	LONP1	E5KMI6	[R].MEMINVSGYVAQEK.[L]	Ambiguous	-1.473	0.150
Plectin	PLEC	Q15149	[R].QQSDHDAER.[L]	Serine 2578	-1.373	0.388
Neuroblast differentiation-associated protein AHNAK	AHNAK	Q09666	[R].LPSGSGAASPTGSAVDIR.[A]	Serine 210	-1.095	0.131
Transforming growth factor beta-1-induced transcript 1 protein	TGFB1I1	O43294	[R].VQNHLPASGPTQPPVVSSTNEGSPSPPEPTGK.[G]	Serine 194	-1.039	0.010
Transcription elongation factor A protein-like 3	**TCEAL3**	Q969E4	[R].EDEGEPGDEGQLEDEGSQEK.[Q]	Serine 65	-1.038	0.078
cDNA FLJ77630, highly similar to Homo sapiens BPY2 interacting protein 1, mRNA		A8K940	[K].AVPMAPAPASPGSSNDSSAR.[S]	Serine 759	-1.035	0.156

Table 5.27: Phosphopeptides which at least doubled in the failed IOL group in the OXT-REL condition.

Protein Descriptions	Gene Name	Accession	Annotated Sequence	Phosphosites	LOG2 FC	p value
Protein phosphatase 1 regulatory subunit 12B	sm-M20	E1CKY7	[R].LESGGSNPTTSDSYGDR.[A]	Serine 43	1.049	0.001
Synaptopodin 2	SYNPO2	B9EG60	[R].GTGAGGDSGPEEDYLSLGAEACNFMQSSSAK.[Q]	Serine 729	1.405	0.029
SH3 and PX domain-containing protein 2A	SH3PXD2A	Q5TCZ1	[K].EAEEGPTGASESQDSPR.[K]	Serine 547	1.016	0.031
Transcription elongation factor A protein-like 3	TCEAL3	Q969E4	[K].REDEGEPGDEGQLEDEGSQEK.[Q]	Serine 65	1.205	0.036
Mitogen-activated protein kinase	MAPK1	Q1HBJ4	[R].VADPDHDHTGFLTEYVATR.[W]	Threonine 185	1.029	0.054
Alpha B crystallin	CRYAB	C3VMY8	[R].APSWFDTGLSEMR.[L]	Serine 59	1.207	0.087
Talin-1	TLN1	A0A1S5UZ07	[R].GSQAQPDSPSAQLALIAASQSFLQPGGK.[M]	Ambiguous	1.018	0.100
Membrane-associated progesterone receptor component 1	PGRMC1	O00264	[R].GDQPAASGDSDDDEPPPLPR.[L]	Serine 57	1.080	0.117
Mutant desmin		A5Z217	[R].TSGGAGGLGSLR.[A]	Serine 68	1.444	0.143

Synaptopodin 2	SYNPO2	B9EG60	[R].APPPVAYNPIHSPSYPLAALK.[S]	Serine 930	1.181	0.146
Eukaryotic translation initiation factor 5B	EIF5B	A0A087WUT6	[K].WDGSEEDEDNSK.[K]	Serine 164	2.041	0.160
Filamin-A	FLNA	P21333	[K].IPEISIQDMTAQVTSPSGK.[T]	Serine 2180	1.013	0.175
Neuroblast differentiation-associated protein AHNAK	AHNAK	Q09666	[R].DDGVFVQEVTQNSPAAR.[T]	Serine 41	1.492	0.214
Microtubule-associated protein 1B, isoform CRA_b	MAP1B	A0A024RAM4	[K].GEAEQSEEEADEEDKAEDAR.[E]	Serine 1016	1.041	0.230
WD repeat-containing protein 44	WDR44	Q5JSH3	[K].EYVSNDAAQSDDEEKLQSQPTDTDGGR.[L]	Serine 403	1.211	0.254
cDNA FLJ53399, highly similar to Monocarboxylate transporter 1		B4E106	[K].DTEGGPKEEESPV.[-]	Serine 478	1.670	0.279
Lon protease homolog, mitochondrial OS=Homo sapiens GN=LONP1 PE=3 SV=1	LONP1	E5KMI6	[R].MEMINVSGYVAQEK.[L]	Serine 659	1.053	0.281
Filamin-A	FLNA	P21333	[K].VHSPSGALEECYVTEIDQDK.[Y]	Serine 2370	1.191	0.309

Table 5.28: Phosphopeptides which at least halved in the failed IOL group in the OXT-REL condition.

Protein Descriptions	Gene Name	Accession	Annotated Sequence	Phosphosites	LOG2 FC	p-value
Smoothelin	SMTN	A0A087X1R1	[R].STSFGVPNANSIK.[Q]	Serine 877	-1.283	0.008
Neuroblast differentiation-associated protein AHNAK	AHNAK	Q09666	[R].LPSGSGAASPTGSAVDIR.[A]	Ambiguous	-1.002	0.04
Alpha-adducin	ADD1	P35611	[R].KQKGSEENLDEAR.[E]	Serine 586	-1.255	0.043
Serine/threonine-protein kinase 10	STK10	O94804	[K].QVAEQGGDLSPAANR.[S]	Serine 438	-1.016	0.046
Plectin	PLEC	Q15149	[K].GYYSPYSVSGSGSTAGSR.[T]	Serine 4626	-1.076	0.059
Palladin	PALLD	Q8WX93	[R].DSGDENEPIQER.[F]	Serine 1121	-1.228	0.06
Epiplakin	EPPK1	P58107	[RC].QVSASELHTSGILGPETLR.[D]	Serine 2720 Serine 3251 Serine 3785	-1.014	0.072
Inactive phospholipase C-like protein 1	PLCL1	Q15111	[K].KLPSDPDVLEGEVTDEDEEAEMSR.[R]	Threonine 556	-1.529	0.073
Epididymis luminal protein 113	HEL113	V9HWE1	[R].QVQSLTCEVDALK.[G]	Serine 325	-1.526	0.113
CDC42 binding protein kinase beta (DMPK-like), isoform CRA_a	CDC42BPB	A0A024R6N2	[K].HSTPSNSSNPSGPPSPNSPHR.[S]	Serine 1690 Serine 1693	-1.681	0.132
cDNA FLJ77630, highly similar to		A8K940	[R].SASPHDVDLCLVSPCEFEHR.[K]	Ambiguous	-1.392	0.146

Homo sapiens BPY2 interacting protein 1, mRNA						
Melanoma antigen family D, 2, isoform CRA_a	MAGED2	A0A024R9Y7	[K].HLDGEEDGSSDQSQASGTTGGR.[R]	Serine 191 Serine 194	-1.099	0.171
Palladin	PALLD	Q8WX93	[R].SRDSGDENEPIQER.[F]	Serine 1118 Serine 1121	-1.527	0.195
182 kDa tankyrase-1-binding protein	TNKS1BP1	Q9C0C2	[R].VPSSDEEVVEEPQSR.[R]	Serine 1620 Serine 1621	-1.022	0.213
Receptor-type tyrosine-protein phosphatase		B7Z2A4	[K].VVQEYIDAFSDYANFK.[-]	Y 809	-1.368	0.221
Dystrobrevin alpha	DTNA	Q9Y4J8	[K].ELNSEVGSETESNVDSEFAR.[T]	Serine 641	-1.189	0.231
Nuclease-sensitive element-binding protein 1 (Fragment)	YBX1	H0Y449	[K].AADPPAENSSAPEAEQGGAE.[-]	Serine 364	-1.22	0.243
Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5	MACF1	H3BQK9	[R].EVEEELATSGGQSPTGEQIPQFQQR.[Q]	Serine 5733	-1.679	0.26
cDNA FLJ59374, highly similar to Homo sapiens caldesmon 1 (CALD1), transcript variant 2, mRNA		B4DPW5	[K].RGSIGENQIK.[D]	Serine 151	-1.664	0.289
cDNA FLJ55039, moderately similar		B7Z525	[K].GNAEGSSDEEGK.[L]	Serine 155 Serine 156	-1.348	0.361

to Hepatoma-derived growth factor						
cDNA FLJ54732, moderately similar to Sorbin and SH3 domain-containing protein 1		B7Z9B7	[R].ASGSFAPISQTPPSFSPPPPLVPPAPEDLR.[R]	**Serine 440**	-1.145	0.451
Nexilin (F actin binding protein), isoform CRA_b	NEXN	D3DQ79	[K].ESLSPGK.[L]	Serine 179	-1.443	0.513

Table 5.29: Phosphopeptides which at least doubled in the failed IOL group in the OXT-CON condition.

Protein description	Gene Name	Accession	Annotated sequence	Phosphosites	Log2-fc	p-value
Myosin light chain kinase, smooth muscle	MYLK	Q15746	[K].DEVEVSDDDEK.[E]	Serine 1438	3.393	0.008
Myosin light chain kinase, smooth muscle	MYLK	Q15746	[K].DEVEVSDDDEKEPEVDYR.[T]	Serine 1438	2.005	0.019
Filamin-A	FLNA	P21333	[K].IPEISIQDMTAQVTSPSGK.[T]	Serine 2180	1.567	0.067
Protein lunapark	**LNP**	Q9C0E8	[K].ASDSEEPEEK.[Q]	Serine 384	1.911	0.114
Heat shock protein HSP 90-alpha	HSP90AA1	P07900	[R].DKEVSDDEAEEK.[E]	Serine 231	1.138	0.117
Calnexin	CANX	P27824	[K].QKSDAEEDGGTVSQEEEDR.[K]	Serine 564	1.017	0.117
Epididymis luminal protein 113	HEL113	V9HWE1	[R].SSVPGVR.[L]	Serine 73	1.461	0.123
Small glutamine-rich tetratricopeptide repeat-containing protein alpha	SGTA	O43765	[R].TPSASNDDQQE.[-]	Serine 305	1.253	0.527
Monocarboxylate transporter 1	SLC16A1	P53985	[K].DTDGGPKEEESPV.[-]	Serine 498	1.679	0.586

Table 5.30: Phosphopeptides which at least halved in the failed IOL group in the OXT-CON condition.

Protein description	Gene Name	Accession	Annotated sequence	Phosphosites	Log2-fc	p value
WWC family member 3	WWC3	T2C6S4	[R].SDSDSSTLPR.[K]	Serine 1052	-1.473	0.030
Epididymis luminal protein 114	HEL114	V9HWK2	[R].DPSASPGDAGEQAIR.[Q]	Serine 288	-1.109	0.049
182 kDa tankyrase-1-binding protein	TNKS1BP1	Q9C0C2	[R].VPSSDEEVVEEPQSR.[R]	Serine 1620 Serine 1621	-1.516	0.054
Transcription elongation factor A protein-like 3	TCEAL3	Q969E4	[R].EDEGEPGDEGQLEDEGSQEK.[Q]	Serine 65	-1.063	0.103
Rho guanine nucleotide exchange factor 12	ARHGEF12	Q9NZN5	[R].TDCSSGDASRPSSDNADSPK.[S]	Serine 309	-1.080	0.116
Transforming growth factor beta-1-induced transcript 1 protein	TGFB1I1	O43294	[R].VQNHLPASGPTQPPVVSSTNEGSPS PPEPTGK.[G]	Serine 194	-1.354	0.154
Myosin light chain kinase, smooth muscle	MYLK	Q15746	[R].KSSTGSPTSPLNAEK.[L]	Serine 1776 Ambiguous Ambiguous	-1.613	0.204
cDNA FLJ56912, highly similar to Fibulin-2		B7Z9B8	[R].RVTEDSEEEEEEEER.[E]	Serine 303	-1.192	0.235

Table 5.31: Phospho-events which at least significantly halve in the failed IOL group under more than one condition, with log2-fc for each condition (where p ≤ 0.05).

Protein name	Gene name	Accession	Site	REL-SP	REL-OT	CON-SP	CON-OT	PRE
Integrin, alpha 5 (Fibronectin receptor, alpha polypeptide), isoform CRA_b	ITGA5	A0A024RB01	Serine 176	**-1.139** (0.329)				**-1.698** (0.115)
cDNA FLJ77630, highly similar to Homo sapiens BPY2 interacting protein 1, mRNA		A8K940	Serine 759			**-1.035** (0.156)		**-1.174** (0.174)
cDNA FLJ77630, highly similar to Homo sapiens BPY2 interacting protein 1, mRNA		A8K940	Ambiguous		**-1.392** (0.146)			
Phosphatidate cytidylyltransferase		B3KM95	Ambiguous	**-1.356** (0.053)		**-1.481** (0.248)		
cDNA FLJ59374, highly similar to Homo sapiens caldesmon 1 (CALD1), transcript variant 2, mRNA	CALD1	B4DPW5	Serine 151	**-1.020** (0.291)	**-1.664** (0.289)			**-1.205** (0.087)
Transforming growth factor beta-1-induced transcript 1 protein	TGFB1I1	O43294	Serine 194			**-1.039** (0.010)	**-1.354** (0.154)	
Neuroblast differentiation-associated protein AHNAK	AHNAK	Q09666	Serine 210			**-1.095** (0.131)		**-1.425** (0.034)
Neuroblast differentiation-associated protein AHNAK	AHNAK	Q09666	Ambiguous		**-1.002** (0.04)			
Plectin	PLEC	Q15149	Serine 2578			**-1.373** (0.388)		
Plectin	PLEC	Q15149	Serine 4626		**-1.076** (0.059)			
Transcription elongation factor A protein-like 3	TCEAL3	Q969E4	Serine 65			**-1.038** (0.078)	**-1.063** (0.103)	
182 kDa tankyrase-1-binding protein	TNKS1BP1	Q9C0C2	Serine 1620 and Serine 1621		**-1.022** (0.213)		**-1.516** (0.054)	

Table 5.32: Phospho-events which at least double in the failed IOL group under more than one condition, with log2-fc for each condition (p-value).

Protein name	Gene name	Accession	Site	REL-SP	REL-OT	CON-SP	CON-OT	PRE
cDNA FLJ53399, highly similar to Monocarboxylate transporter 1		B4E106	Serine 478	1.931 (0.311)	1.67 (0.279)	2.607 (0.362)		2.181 (0.425)
Filamin-A	FLNA	P21333	Serine 2180	1.55 (0.034)	1.013 (0.175)		1.567 (0.067)	
Filamin-A	FLNA	P21333	Serine 2327	1.6 (0.117)				
Filamin-A	FLNA	P21333	Serine 2370		1.191 (0.309)			
Transcription elongation factor A protein-like 3	TCEAL3	Q969E4	Serine 65		1.205 (0.036)			1.115 (0.104)
Protein lunapark	LNP	Q9C0E8	Serine 384				1.911 (0.114)	1.631 (0.096)

Figure 5.14A and 5.14B shows STRING diagrams (https://string-db.org/) summarising the phosphoproteomic data for proteins for which phosphorylation events at least A. double or B. halve, in the failed IOL group under the two contracted conditions (SP-CON and OXT-CON). Both groups have significantly more protein-protein interaction (PPI) than expected (p<1.0-16). Figure 5.15 shows a STRING diagram summarising the phosphoproteomic data for proteins for which phosphorylation events at least doubled or halved in the failed IOL group under the two contracted conditions (SP-CON and OXT-CON). This group has significantly more protein-protein interaction (PPI) than expected (p<1.0-16) and this suggests that these proteins may interact *in vivo*, and in this case may be associated with a failed IOL profile. Figure 5.16 shows a String diagram summarising the phosphoproteomic data for proteins for which phosphorylation events at least A. double or B. halve, in the failed induction of labour group under the peak relaxation conditions (SP-REL and OXT-REL). Both groups have significantly more protein-protein interaction (PPI) than expected (p<1.0-16). Figure 5.17 shows String diagrams summarising the phosphoproteomic data for proteins for which phosphorylation events at least doubled or halved in the failed IOL group under the peak relaxation conditions (SP-REL and OXT-REL). This group has significantly more protein-protein interaction (PPI) than expected (p<1.0-16).

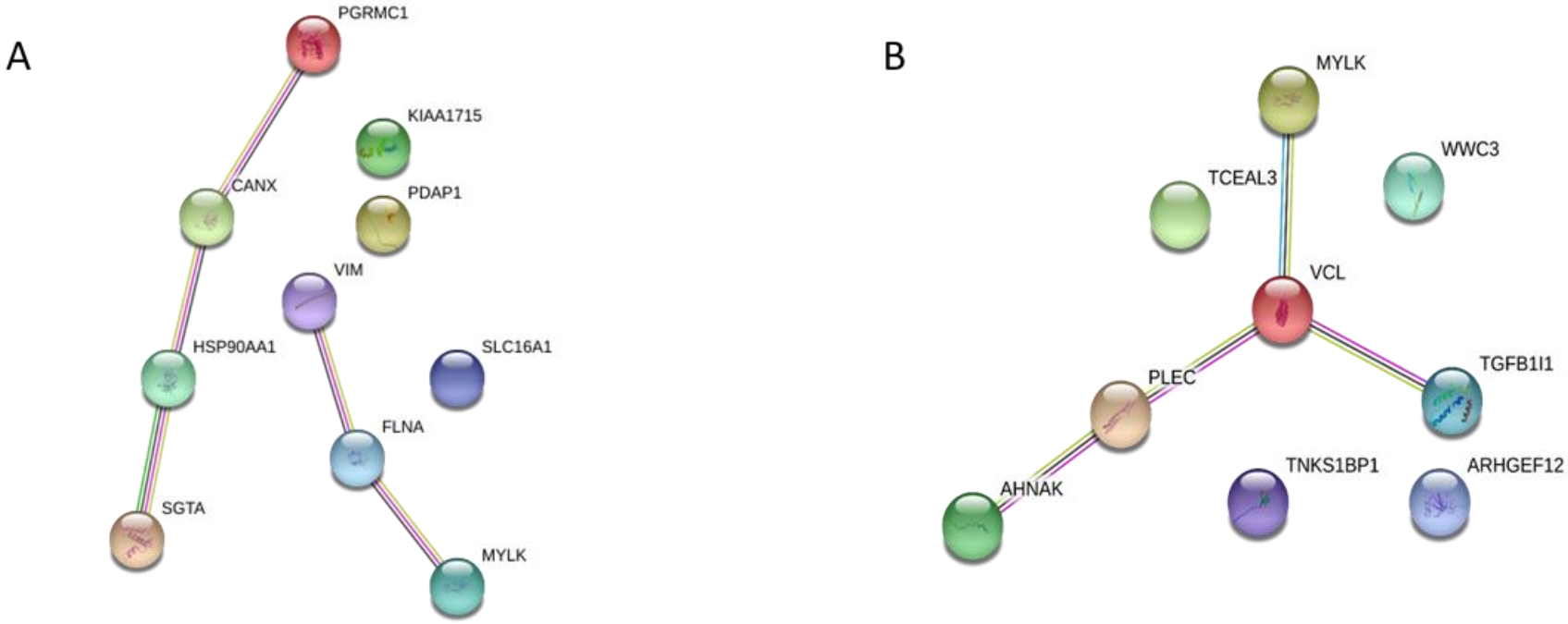

Figure 5.14: STRING diagram summarising the phosphoproteomic data for proteins for which phosphorylation events at least A. double or B. halve, in the failed induction of labour group under contracted condition (SP-CON and OXT-CON) **(**https://string-db.org/). Both groups have significantly more protein-protein interaction (PPI) than expected (p<1.0-16).

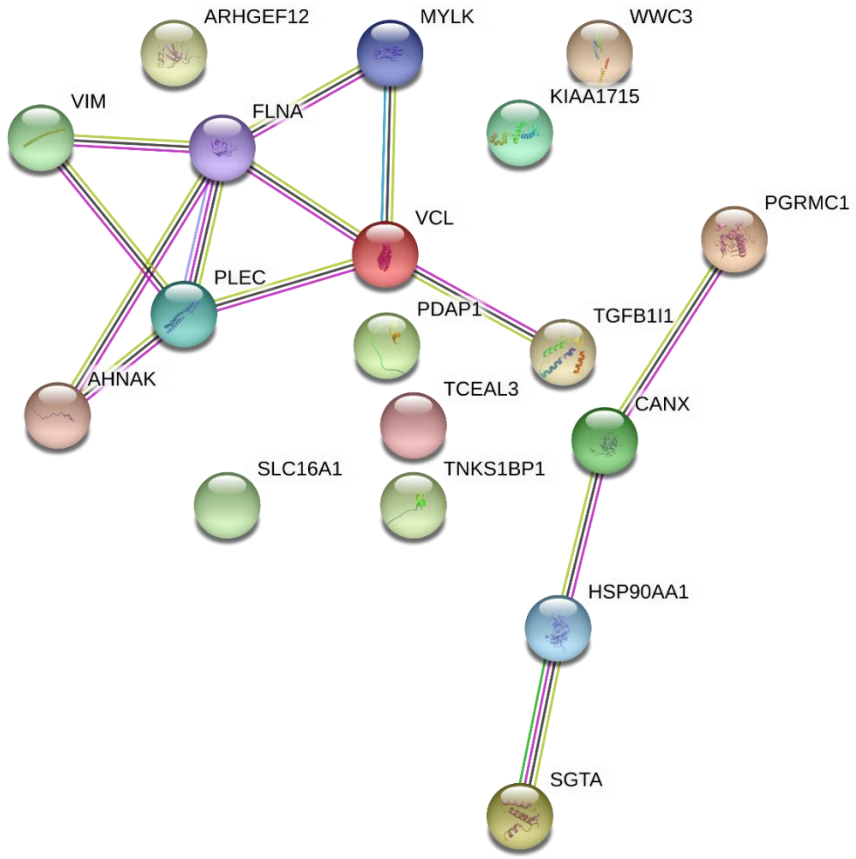

Figure 5.15: STRING diagram summarising the phosphoproteomic data for proteins for which phosphorylation events at least double or halve in the failed induction of labour group under contracted condition (SP-CON and OXT-CON) (https://string-db.org/). This group has significantly more protein-protein interaction (PPI) than expected (p<1.0-16).

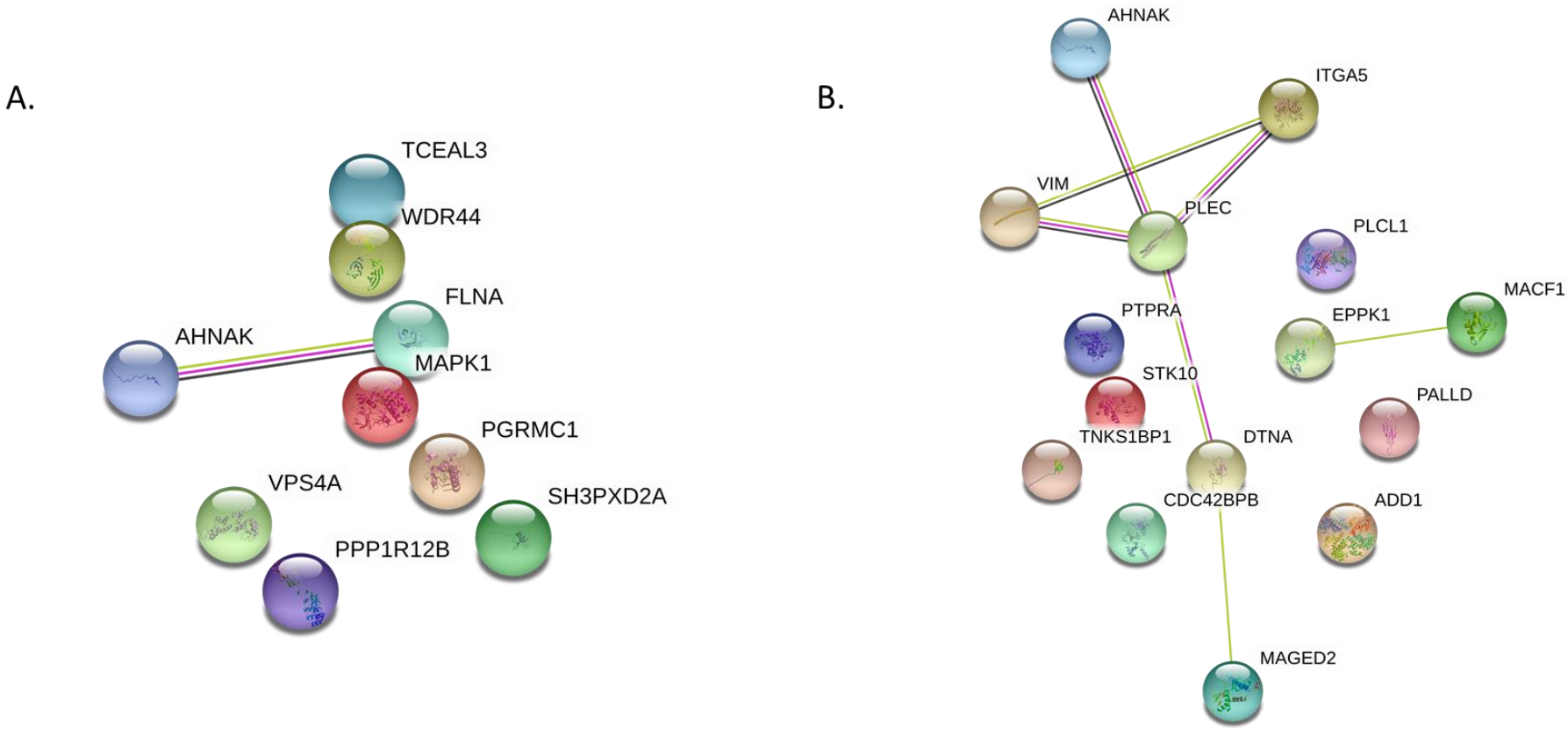

Figure 5.16: STRING diagram summarising the phosphoproteomic data for proteins for which phosphorylation events at least A. double or B. halve, in the failed induction of labour group at peak relaxation (SP-REL and OXT-REL) (https://string-db.org/). Both groups have significantly more protein-protein interaction (PPI) than expected (p<1.0-16).

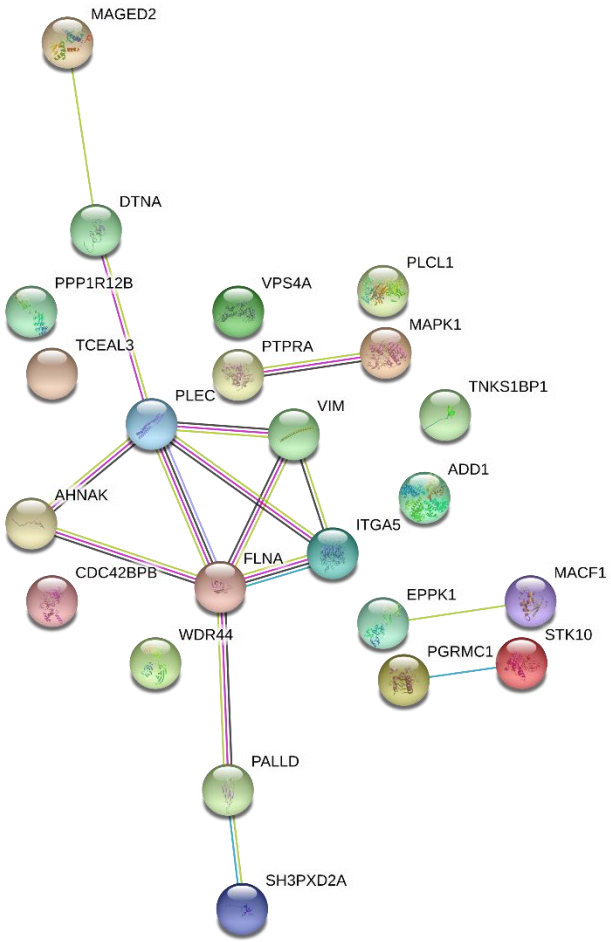

Figure 5.17: STRING diagram summarising the phosphoproteomic data for proteins for which phosphorylation events at least double or halve in the failed induction of labour group at peak relaxation (SP-REL and OXT-REL) (https://string-db.org/). This group has significantly more protein-protein interaction (PPI) than expected (p<1.0-16).

5.8 Discussion

This chapters presents novel data on protein distribution and phosphorylation in fresh human myometrium. The findings from Part 1 indicates that there are differences in the phosphoproteome between the pre-contracting (PRE) and contracting state (SP-REL) of human myometrial tissue; and findings from Part 2 suggests that myometrial proteins and phosphorylation events are differentially expressed in women with failed IOL compared with women having an elective caesarean section. This could reflect differential myometrial phenotypes. These findings have the potential to improve our understanding of the pathways involved in spontaneous labour in humans, and have produced a wealth of data from which future studies can be launched.

As discussed in the introduction and throughout the previous chapters, suggested hypotheses for the trigger of human parturition include a change in balance from a dominantly progesterone environment to one dominated by oestrogen, as well as activation of inflammatory pathways. Progesterone and oestrogen appear to have antagonistic effects on the myometrium, and previous studies have shown that oestrogen promotes transcription of genes such as *COX-2* and the OXT receptor (*OTr*), which stimulate myometrial activity, while progesterone stimulates those which result in decreased myometrial activity[35]. One suggested mechanism for functional progesterone withdrawal is a change

in expression of progesterone and oestrogen receptors on uterine myocyte cell surfaces at the time of labour[97, 100,32].

5.8.1 Part 1 discussion total proteins

5.8.1.1 Complement and coagulation pathways

Overall, in Part 1, 178 proteins at least halved and five at least doubled for all three samples under the SP-REL condition compared with the PRE condition (REL/PRE). By uploading the list of these proteins to the String software programme online, the complement and coagulation pathways were identified as having multiple proteins involved. The resultant KEGG diagram from this search (Figure 5.6) illustrates these pathways and indicates that 11 of the 74 identified proteins in the pathway at least halved between REL and PRE.

This includes CRP, a marker of inflammation and known to be synthesised by smooth muscle cells[298], which was lower in the SP-REL condition compared with the PRE. This could be interpreted as evidence that with contractions comes a paradoxical decrease in inflammatory markers, and on initial review this is surprising as it may be expected that complement and inflammatory markers would increase when the myometrium is contracting. Another explanation for this change, and the finding that complement proteins decreased, is that these proteins may be catabolised. Sharp *et al.* (2016)[3] took myometrial samples at the time of caesarean section from women who had been labouring and women

who were not labouring. Samples were snap frozen and stored at -80 degrees Celsius. Transcription analysis of these samples showed an increase in expression of genes coding for inflammatory markers with active labour along with a decrease in genes involved with muscle-specific pathways[3]. One explanation for the difference in the results of the Sharp *et al.* (2016) study and the present study is that the previous researchers measured RNA rather than protein. Another potential explanation is that the previous researchers processed and snap-froze the myometrium immediately following sample collection. Given the labouring myometrium would likely have been under a high action and potentially inflammatory response when compared with the myometrium from the not-in-labour women, this could have artificially heightened the inflammatory response observed in the myometrium from the emergency caesarean section group. In contrast, for the present study, the myometrium was taken from women at elective caesarean section, and strips were allowed to equilibrate in the Myobath-II prior to further experimentation, and that was the same for all of the samples. The latter experimental model may provide a better representation of the difference in proteins between the pre-contracting and contracting state. However, a further potential explanation is that the PRE condition is not a good representation of pre-contraction, and actually causes the myometrium stress through not being under tension,

thereby artificially increasing the inflammatory response. Nevertheless, in both of these studies the complement and inflammatory pathways appear to be important in contraction, and certainly warrant further investigation.

In further support of an involvement of these pathways with labour, another research group integrated three myometrial transcriptome datasets and found that there were 126 genes which were associated with labour in all three datasets. These included interleukins and cytokines, as well as those related to apoptosis and cell proliferation[32]. These findings support the present phosphoproteomics findings, which themselves are arguably closer to the functionality of the myometrium than the genomic profile.

5.8.1.2 Thrombin

Within the coagulation pathway (Figure 5.7), it is interesting that antithrombin III and heparin cofactor II were found to at least halve in the contracting state (SP-REL) compared with the pre-contracting state (PRE) in all three samples. In the coagulation pathway these proteins act to inhibit thrombin production. If antithrombin III and heparin cofactor II are metabolised or have reduced production in the contracting state of myometrium, it would follow that this may result in a subsequent increase in thrombin levels that are associated with myometrial contraction. Previous studies have shown that bleeding activates the clotting cascade which activates thrombin[299], and bleeding during pregnancy

can be associated with preterm labour and an "irritated uterus"[300, 301]. Thrombin is a protease that interacts with the G protein-coupled membrane protease-activated receptor family[302]. Activation of this receptor by thrombin in rats stimulates myometrial contractions via activation of the phosphatidyl-inositol signalling pathway and subsequent influx of calcium ions into the cytosol both *in vitro*[303] and *in vivo*[302]. These contractions are of a similar strength to those induced by oxytocin[303,302]. Further, in cultured endometrial stromal cells, thrombin stimulates matrix metalloproteinase-1 production, and the source of thrombin is thought to be decidual cells which is suggested to lead to matrix degradation. Studies of human preterm birth show that levels of thrombin-antithrombin complexes are raised in maternal plasma and in amniotic fluid, and increased thrombin-antithrombin plasma complexes have been associated with subsequent premature rupture of membranes. Thrombin was shown to be increased in the amniotic membranes of women who delivered preterm compared with term delivery[304]. In primary amnion mesenchymal cells, thrombin has been shown to upregulate MMP mRNA as well as PGE2 mRNA[304]. A previously suggested mechanism for rupture of membranes involving thrombin is that the mechanical structure of the amnion is dependent upon interstitial collagens, and degradation of these collagens by MMPs has been shown to precede membrane rupture[304].The findings from part 1 in the present

study that lower levels of proteins which inhibit thrombin were associated with the contracting state would fit with this theory, as the potential subsequent increase in thrombin levels could promote contractions in the contracting state. Previous evidence suggests that thrombin is a potential trigger for spontaneous labour and may particularly be involved with preterm birth. This could potentially be stimulated by changes in the myometrial or amniotic membrane vasculature which causes release of thrombin and warrants further investigation.

5.8.1.3 Acid-sensing ion channel 5

The greatest overall mean decreases in protein in the contracting state (SP-REL) were in acid-sensing ion channel (ASIC) 5 and Putative Heat Shock protein β4. ASICs (ASIC 1-5) are a family of degenerin/epithelial sodium ion (Na^+) channel receptors which are expressed in the nervous system. ASICs have primarily been researched in their role in fear behaviour and pain sensation, and their contribution to neurodegeneration following a stroke[305]. However, ASIC 5 appears to have a different role to the other ASIC channels[306], with only 30% similarity with the other ASIC proteins[307]. ASICs 1-4 are activated by protons when extracellular pH becomes more acidic, and participate in pathological processes involving hypoxia such as pain, malignancy and apoptosis[308]. When a proton binds to the extracellular region of the ASIC 1-4 ion channels residing on

a neurone, conformational change occurs to allow passage of Na^+ into the cell which results in membrane depolarisation and activation of voltage-gated Ca^{2+}-channels. The resulting influx of Ca^{2+} depolarises the neuron causing an excitatory response[309]. In contrast, ASIC 5 does not respond to low pH[307] and its physiological function in humans remains poorly understood[310]. ASIC 5 has been named the brain, liver, intestinal Na^+ channel (BLINaC), bile acid-sensitive ion channel (BASIC), and human intestinal Na^+ channel (hINaC). ASIC 5 mRNA is highly expressed in the liver and brain, and in the liver is activated by bile acids[307, 310], and has also been found in the intestinal tract, lung, kidney and testes[310]. Recent electrophysiological studies of the function of human ASIC 5 in HEK293 cells using the patch clamp technique revealed that extracellular Ca^{2+} blocks the receptor and renders the channels inactive at rest; and the channels are activated by bile acids[310].

Although there appear to be no previous published studies which describe ASIC 5 in the myometrium, this is potentially an important protein for normal functioning of the human myometrium given the finding in the present study of lower levels in the relaxed contracting state compared to pre-contracting. Of note, obstetric cholestasis is a condition of pregnancy for which the pathology is not well understood and which is associated with increased maternal plasma bile acids, maternal itching and increased risk for still birth[311]. If ASIC 5 were to

be directly involved in activating labour, it would be expected that women with obstetric cholestasis were highly likely to deliver preterm as the bile acids may activate the receptor. However, although there is evidence of a slight increase in preterm birth rate for pregnancies affected by obstetric cholestasis, a sizeable proportion of these are iatrogenic, and there does not appear to be a large increase in risk of preterm birth[272, 311]. Given these findings, ASIC channel receptors require further investigation in relation to myometrial contraction, human labour, including potential association with some of the poor obstetric outcomes associated with obstetric cholestasis.

5.8.1.4 Heat Shock Proteins

The protein with the second biggest difference between the SP-REL group and the PRE group in Part 1 was Putative Heat Shock Protein (HSP) 90-β4. In addition, other HSPs were found differentially phosphorylated in Part 2, including the phosphorylation of HSP 90-β at Serine 226 which non-significantly more than doubled in all three samples in the failed IOL group under the PRE condition, and phosphorylation of HSP-90α at Serine 231 which more than doubled in the failed IOL group under OXT-CON conditions.

HSPs have been previously been studied at different stages of pregnancy. One group found a 69% reduction in sHSP αB-crystallin in labouring human myometrium when compared with non-labouring (myometrial samples snap-

frozen at the time of sampling) and changes in the phosphorylation of HSP27 with labour. Further investigation have shown these proteins to be associated with remodelling of actin at the time of labour, and that HSP27 moved from the perinuclear region to the actin cytoskeleton with labour[312]. In the ewe, HSP 90 and HSP 70 were increased in both the myometrium and endometrium during labour when compared with pre-labour. Further, treating ewe myometrium with oestrogen stimulated a similar increase in HSP-90 and -70 mRNA, while treatment with progesterone suppressed this response[313]. Moreover, it has been suggested that oestrogen and progesterone may regulate HSPs, and HSP 90 and 70 have been shown to bind to progesterone receptors (PR), rendering the PR inactive, and dissociation of HSP from the PR results in reactivation of the PR. In addition, oestrogen has been shown to stimulate expression of HSP 90α in cell culture of endometrial cells, and progesterone depresses expression[314]. Therefore it has been suggested that in humans increased HSP 70 and HSP 90 may stimulate oestrogen receptor (ER) function and inhibit PR function, thereby causing an effective progesterone withdrawal and triggering myometrial contraction and labour[313].

Moreover, small glutamine-rich tetratricopeptide repeat-containing protein alpha (SGTA) binds to HSP 70 and 90[315], and in Part 2 of the present study phosphorylation of SGTA at Serine 305 was non-significantly more than twice as

high in the failed IOL group when contracting under stimulation with oxytocin (OXT-CON) than in the ECS group under the same conditions (Table 5.28). In addition, the receptors of steroid hormones require assembly of the co-chaperones HSP 70 and HSP 90 in order to form a functional conformation. Other co-chaperones are also required to support this role and a feature of many of these proteins is the tetratricopeptide repeat (TPR) that is highly conserved and allows interaction with the c-domain of HSP 90 and HSP 70. SGTA down-regulates expression of androgen receptors through localisation of the receptors in the cytoplasm and reducing the amount reaching the nucleus. *In vitro*, PR requires HSPs 40, 70 and 90, p23 and Hop in order to fold properly and bind to progesterone. SGTA also down-regulates expression of PR, and SGTA knockdown in cell culture results in increased expression of PR, whereas overexpression of SGTA decreases the activity of PR. This effect does not occur with the ER[316]. One group found that phosphorylation of SGTA at Serine 305 was essential for stabilisation of Platelet-Derived Growth Factor Receptor alpha (PDGFR-α) in cancer cells dependent on PDGFR-α for survival[317].

In Part 1 of the present study, HSPβ total protein levels were twice as high in the PRE samples as the REL samples, which would indicate a decrease of HSPβ in the contracting phase compared with the pre-contracting phase. This doesn't directly fit with the idea of a relationship between HSPs and functional

progesterone withdrawal and contraction, as suggested by the studies discussed above, however these previous studies compared labouring myometrium immediately snap-frozen at the time of elective caesarean section with non-labouring myometrium, and therefore it is possible that the findings may be confounded by the immediate preceding physiological stress that the myometrium had been subjected to. The differential phosphorylations seen in the failed IOL group under OXT-CON and PRE conditions in Part 2 of the present study may alter the usual binding activity of HSP-90 to the PRs, thereby affecting contractility. The observed doubling in phosphorylation of small glutamine-rich tetratricopeptide repeat-containing protein alpha at Serine 305 in the failed IOL group is interesting; phosphorylation at this site may make it more or less likely to bind with PR, thereby affecting the myometrial response to oxytocin during induction of labour. However, there may be another explanation, for example the increase in HSP and phosphorylation of SGTA may instead be a response to increased PR activity and an attempt to decrease this activity, or it may be that there is stabilisation of another cell surface receptor that we do not yet know of that may contribute to failed IOL. It is also possible that because the myometrium has already laboured that there is a prolonged effect from this, despite the time given for equilibration in the organ bath.

5.8.2 Phosphorylation events in Part 1

In study 1, phosphorylation of AHNAK at Serine 5552, and of 182 kDa tankyrase-1-binding protein at Serine 1297 were at least halved in all three samples in the SP-REL condition compared with PRE; and phosphorylation of Filamin-A at Serine 968, of Leiomodin-1 at Serine 555, and of DYSL3 protein at Serine 518, Serine 522 and Threonine 514, all doubled in the REL condition compared with PRE. None of these phosphorylation sites were found to be significantly different between the failed IOL group and ECS group in Part 2, although phosphorylation events on other sites of the same proteins were significantly different.

5.8.2.1 Leiomodin-1

Leiomodin-1 has previously been found within smooth muscle, including the uterus. Leiomodin-1 is a member of the tropomodulins (Tmod) family, which are proteins involved in actin-capping, and which work with tropomyosin to prevent depolymerisation at the pointed end of actin filaments. It is thought that Leiomodin-1 may also cap actin, but this is yet to be proven. Leiomodin-1 has also been shown to bind to tropomyosin in its N-terminal[318]. However, the N-terminal does not seem to be the site phosphorylated in the present study (including those phosphorylations which only occurred within one sample).

Leiomodin-1 is under transcriptional control of myocardin (MYOCD) and is thought to play a role in contractility. In a study investigating the role of LMOD1

in pulmonary arterial hypertension (PAH), a condition where there is excessive proliferation of smooth muscle cells resulting in remodelling and occlusion of vessels, there were reduced levels of LMOD1 and MEF2C mRNA in smooth muscle cells derived from people with PAH when compared to a control group. This was also associated with an increase in microRNA (miR)-214[319].

The phenotype of smooth muscle cells is dictated by the set of genes expressed, and it is thought that differential gene expression allows smooth muscle cells to adapt to the organ in which they are involved. Dysfunction in the expression of these genes have been observed in pathologies such as cancer, atherosclerosis, obstructive bladder and reproductive disease. SMC (smooth muscle cell)-restricted genes is a term used for those genes specific for SMC differentiation[320], and it has previously been reported that a large proportion of SMC-restricted genes are under the control of the transcription factor Serum Response Factor (SRF)[320]. MYOCD is a known co-factor of SRF, and Myocyte Enhancer Factor 2C (MEF2C) contributes an additional regulatory influence. Interaction of MEF2C-MYOCD is believed to upregulate genes to coordinate a contractile SMC phenotype[319], and LMOD-1 is believed to be an important gene for this phenotype[319, 320].

In Part 1 of the present study, phosphorylation of LMOD-1 at serine 555 was at least doubled in the REL condition compared to the PRE condition and this may

be important in promoting the effect on myometrial contractile activity of LMOD-1. If this phosphorylation is impaired, contractility may not be so effective. A search of Phosphosite and Uniprot indicates that research into this phosphorylation site has not been performed in myocytes, making it a novel target for future research.

5.8.2.2 Dihydropyrimidinase-related protein 3

Further consistent phosphorylation changes observed in all three samples between PRE and REL were three phosphorylations on the Dihydropyrimidinase-related protein 3 (DPYSL3 protein). DPYSL3, also known as Collapsin response mediator protein (CRMP)-4, is a hydrolase belonging to the DHOase family (www.phosphosite.org). Uniprot (www.uniprot.org) describes this protein as being essential for class 3 semaphorins signalling and the resultant cytoskeleton remodelling. This protein is involved with actin crosslink formation, and also part of a cellular response to cytokine stimulus (www.phosphosite.org). Further, in a previous study, mRNA from DPYSL3 was found to more than double in pregnant myometrium when compared with non-pregnant myometrium[321]. The DPYSL3 gene has also been found to be upregulated in “ripe” (Bishops score of 5 or more) when compared with “unripe” cervical samples in women at term but not in labour[322].

Glycogen Synthase Kinase 3 (GSK3) is an unusual protein kinase as it requires a prior priming phosphorylation of the protein at another site before it will act. GSK3 is known to cause the phosphorylation of CRMP-4, but only once it has already been primed through phosphorylation of S-522 by another kinase. CRMP-2 is in the same family as CRMP-4 and is better studied; and the same phosphorylation sites in CRMP-2 are present in CRMP-4, including S-518, T-514, and T-509, as well as the priming site of S-522. In neuroblastoma cells in culture, cyclin-dependent kinase 5 and dual tyroxine-regulated kinase (DYRK)-2 were able to phosphorylate CRMP-4 at S522, and thereby prime CRMP4 for phosphorylation by GSK3 at the subsequent sites. This group also determined that stimulation of neuroblastoma cells with IGF1 (Insulin-like growth factor 1) decreased CRM4 phosphorylation, likely through inhibition of GSK3 activity[323]. In Part 1 of the present study, the phosphorylation events which were at least doubled in all three samples were T-514, S-518 and S-522; therefore, this could reflect an increase in CDK5 or DYRK2 and/or GSK3 activity.

5.8.2.3 Filamin-A

Serine 968 of Filamin-A had an average 2.435-fold increase in phosphorylation in the contracting state (SP-REL) compared with pre-contracting (PRE). Filamin-A is a ubiquitous protein of the cytoskeleton involved in linkage of actin filaments (Phosphosite.org). It also has an essential role in cell migration within

the embryo. Phosphorylation of Serine 968 has previously been identified with Mass Spectrometry phosphoproteomics but has not previously been further investigated.

In summary, the phosphorylation events which at least doubled in the contracting state (REL) compared with pre-contracting (PRE) in Part 1 of this study involve proteins that interact with actin, and the effect of phosphorylation at these sites on the function of these proteins warrant further investigation. It is possible that these changes are simply the result of increased contractile activity, however they may also represent a part of the pathway for the trigger for labour in humans.

5.8.2.4 Neuroblast differentiation-associated protein AHNAK

Phosphorylation of neuroblast differentiation-associated protein AHNAK (AHNAK) at Serine 5552 halved under the SP-REL condition compared with the PRE condition in Part 1 of the present study; and phosphorylation of AHNAK at Serine 210 was significantly reduced in the failed IOL group under the PRE condition. This is a recognised phosphorylation site of AHNAK although its action has not been investigated (phosphosite.org). In a previous study, gene expression of *AHNAK* was found to be lower in labouring samples than non-labouring myometrial samples that were processed and snap-frozen at the time of delivery, and therefore this change may reflect the physiological stress of

labour rather than being related to the trigger for labour[3]. AHNAK has also been investigated in cardiac smooth muscle, and binds to the L-type Ca^{2+}-channel, conferring a link between Ca^{2+}-channels and the actin cytoskeleton[145, 324]. AHNAK is therefore another protein which warrants investigation in its role in myometrial contraction and the trigger for labour, including the effect of its phosphorylation at Serine 5552 and Serine 210.

5.8.2.5 182 tankyrase-1-binding protein

Phosphorylation of 182 kDa tankyrase-1-binding protein halved under the REL condition compared with under the PRE condition in Part 1, and was seen to be doubled in the OXT-REL and OXT-CON conditions of the failed IOL group in study 2. If the SP-REL/PRE condition can be considered to show the difference between resting state and contracting state, and as these phosphorylation events halve in the contracting state but in the contracting state of the oxytocin stimulated conditions of the failed IOL group these phosphorylation events more than double, this suggests that this protein is of potential functional interest.

5.8.3 Part 2 discussion total proteins

The number of significant differences in the proteome and phosphoproteome between the myometrium taken from women with failed IOL and myometrium taken from women at elective caesarean section support the hypothesis that

failed IOL and failure to progress may be associated with a specific phenotype that confers myometrial dysfunction, thereby providing clues as to proteins required for spontaneous labour in humans. All of the myometrial samples in this study were allowed to equilibrate in buffer solution in the Myobath-II prior to being treated under the same conditions in the organ bath. Theoretically this could mean that the comparisons between the two maternal sample groups are more reliably attributed to inherent differences in the myometrium rather than potentially confounded by the immediately preceding stress of labour experienced by the failed IOL group, as could have been the case had the myometrium been processed immediately following collection.

5.8.3.1 Matrix Gla Protein

The mean concentration of Matrix Gla Protein (MGP) was at least doubled in the failed IOL group, under all of the experimental conditions (PRE, SP-REL, SP-CON, OXT-REL, OXT-CON). MGP is an inhibitor of vascular smooth muscle calcification that is dependent upon vitamin-K, and is present in vascular smooth muscle and bone[325]. There appears to be limited prior knowledge of the action of MGP in the myometrium in the published literature, however a published abstract by Stetson *et al.* (2018) [326] described a study whereby MGP myometrial expression was measured using immunohistochemistry and RNAseq with qRT-PCR validation in the following groups: pregnant women at term and in labour;

women at term and not in labour; women in preterm labour; and women who were preterm and not in labour. Maternal MGP samples were also analysed using ELISA. Further, MGP was assessed within pregnant and non-pregnant myometrial cell lines. The group found that pregnant myometrial cells in culture had a higher expression of MGP than the non-pregnant cell lines; that maternal serum MGP levels were unaffected by labour at term; and that there were significantly higher levels of MGP gene expression in the not-in-labour myometrial samples than both those labouring at term and those labouring preterm. Further, that immunohistochemistry showed a co-localisation of MGP with smooth-muscle actin monofilaments[326]. In a further study[327] investigating MGP and sperm maturation, MGP and γ-glutamyl carboxylase (GGCX) were found to be present in vesicles that allowed intercellular communication, and that this was dependent upon a low-Ca^{2+} environment: in a high Ca^{2+} concentration environment MGP is unable to bind to proteins[327]. In Part 2 of the present study, MGP was more than twice as high in the failed IOL group, matching the higher levels associated with no labour when compared with labour in the Stetson *et al.* (2018) study. Therefore it is possible that elevated MGP is associated with a non-labouring myometrium phenotype, and perhaps via an effect on Ca^{2+} transportation and/or an effect on actin, or potentially

through impaired intercellular communication associated with increased extracellular Ca^{2+}.

5.8.3.2 Lysyl oxidase homolog 2 and transforming growth factor-beta-induced protein ig-h3

Lysyl oxidase homolog 2 (LOXL2) was found to at least double in all five conditions in the failed IOL group when compared with the ECS group. LOXL2 is a member of the lysine tyrosylquinone (LTQ)-dependent lysyl oxidase (LOX) family, which are copper-dependent amine oxidases[328]. As with other members of the LOX family, LOXL2 is thought to promote stiffening of the extracellular matrix (ECM) through its catalysing of cross-linking of the ECM proteins, including elastin and collagen. This in turn leads to changes in the cellular mechano-transduction. The LOX family have been particularly investigated as they are thought to play a role in breast cancer, and have been implicated in cancer cell migration, via activation of FAK/Src signalling pathways as a result of the hydrogen peroxide produced following ECM substrate oxidation[328]. LOXL2 is found in many human tissues, with greatest expression in the reproductive organs, including uterus, placenta and prostate. LOXL2 has been implicated in many cancers, and has been found to be upregulated in cancer cells and tissues[328]. Hypoxia induces the expression of LOXL2, and it is a transcriptional target of Hypoxia Inducible Factor 1 Subunit Alpha (HIF1A). The expression of

LOXL2 has also been associated with the upregulation of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 (TIMP-1), thereby promoting extracellular matrix (ECM) degradation which has been implicated in promotion of dissemination of metastases in breast cancer. Inhibitors of LOXL2 have been developed and these result in a reduction in tumour and fibrotic microenvironment formation in mice[328].

LOXL2 concentrations are also increased by Transforming Growth Factor (TGF)-β, which is known to be a promoter of accumulation of collagen and fibrotic disease of different tissues, including the lung and heart[329]. Interestingly, Transforming Growth Factor-Beta-induced Protein ig-h3 (TGFBI or βig-H3) also significantly at least doubled in the failed IOL group when compared with the ECS group in all conditions excepting PRE. βig-H3 is an extracellular matrix protein that is induced by TGF-β[330], and secreted from smooth muscle cells, fibroblasts, and chondrocytes[330, 331]. It is known to be involved in both physiological and pathological processes such as adhesion and migration, inflammation and morphogenesis. It enables linkage of different ECM proteins, and can bind to collagen types I, II and IV and proteoglycans. βig-H3 is present in the kidneys and urine of people with diabetes, and has been suggested as a marker of nephropathy severity[330]. Further, βig-H3 was found in the urine of women with pre-eclampsia but was not detectable in the urine of women with

both normal pregnancies and pregnancy induced hypertension, indicating that TGF-β may be involved in the renal pathology caused by pre-eclampsia[332]. βig-H3 is thought to be a tumour suppressor, as unlike LOXL2, it is reduced in breast, prostate and lung cancer cells. However, very high levels of βig-H3 are also associated with worsened cancer prognosis and metastases in some cases, including in the deposition of ECM involved in the invasive growth of melanoma cells. Interestingly, peritoneal cells in culture were found to abundantly secrete βig-H3 which promoted motility and adhesion to the peritoneal cells of ovarian cancer cells; and therefore are implicated in ovarian cancer metastasis to the peritoneum, although βig-H3 was downregulated in ovarian cancer cells[330, 333]. In mice, βig-H3 was found to peak at day 4, when trophoblastic invasion occurs [334]. In Part 2, both βig-H3 and LOXL2 significantly at least doubled in the failed IOL group compared with the ECS group, and on the background of the previous findings this could be a result of increased TGF-β. This in turn could potentially have two implications in its association with failed IOL, in that it could indicate that there is increased stiffness of the myometrium rendering the myometrium less able to coordinate contractions in such a way as to allow labour; or it may be that as a similar profile is observed with pre-eclampsia, that the observed failure to progress in labour may be secondary to impaired vascularity of the myometrium leading to impaired coordination of contraction. In addition,

phosphorylation of Serine 194 of TGFBI halved in the SP-CON condition in the failed IOL group.

5.8.3.3 Atrial natriuretic peptide-converting enzyme

Atrial natriuretic peptide (ANP)-converting enzyme total protein levels significantly at least halved between the failed IOL group compared with the elective caesarean section group in all conditions other than OXT-REL. Yan *et al.* (1999)[335] identified Corin, a predicted type II transmembrane protein, in the human heart[335]. Yan *et al.* (2000)[336] later determined that Corin was the sought-after ANP-converting enzyme[336]. In the murine heart, ANP-converting enzyme mRNA is present from embryo day 9.5 (E9.5). Using Fluorescent in situ Hybridisation (FISH), ANP-converting enzyme mRNA was also found in the uterus of pregnant mice, and this was in highest concentrations in decidual cells near to the embryo implantation site[335]. A further study illustrated that ANP receptors are functionally active in myometrium of pregnant women, as treatment of human myometrial membrane with ANP increased production of cyclic guanosine monophosphate (cGMP)[337].

Atrial Natriureteric Peptide (ANP) is well studied in cardiac tissues and is a hormone which reduces blood pressure through excretion of sodium ions and thereby reduces blood volume. ANP is converted into its active form from the precursor pro-ANP by ANP-converting enzyme[336, 338]. In mice, reduced ANP

results in increased blood pressure[338]. As ANP-converting enzyme is a transmembrane protein, it is expected to have its action at the site where it is found. Cui *et al.* (2012)[338] created an ANP-converting enzyme Knock-out (KO) mouse which had a cardiac promoter able to convert pro-ANP to ANP in the heart but not in the uterus, and normal blood pressure was maintained in the non-pregnant mouse. However, in the pregnant ANP-converting enzyme-KO mouse, blood pressure increased during pregnancy and only returned to normal post-partum. In the ANP-converting enzyme-KO mice, spiral artery remodelling and trophoblast invasion were impaired. Further, uterine ANP-converting enzyme mRNA levels were lower in women with pre-eclampsia than those from normal pregnancies. This suggests that ANP-converting enzyme is important for physiological changes at the feto-maternal interface, and specifically in this case with pre-eclampsia [338].

Further, Cootauco *et al.* (2008)[339] performed a myometrial organ bath study and observed that in myometrium primed with oxytocin, adding increasing concentrations of ANP reduced the frequency of myometrial contractions. Myometrial cell culture taken from myometrium of women at elective caesarean section showed an increasing ANP concentration within these cells with increased gestational age. The concentration of ANP decreased when the cultured cells were treated with oxytocin but increased when the cultured cells

were treated with Nifedipine (a tocolytic agent). The authors concluded that ANP causes myometrial relaxation in a dose-dependent manner[339]. In another study, Soloff *et al.* (2011)[340] immortalised myometrial cells prepared from women in late gestation and treated the cells with increasing doses of progesterone, followed by Affymetrix GeneChip analysis. The authors found that the expression of ANP-converting enzyme was upregulated nearly 8-fold following treatment with progesterone[340].

In summary, it seems that ANP causes myometrial relaxation, and increased ANP levels reduce the frequency of myometrial contractions in oxytocin-treated human myometrium [339], and that ANP-converting enzyme is required to activate ANP. In Part 2 of the present study, levels of ANP-converting enzyme in the failed IOL labour group was found to be at least half that measured in the ECS group in all conditions other than OXT-CON, which would theoretically result in a reduction of ANP production and, theoretically, increased frequency of myometrial contractions. Or, as reduced ANP and ANP-converting enzyme is associated with hypertension and impaired trophoblast invasion and spiral artery remodelling, this reduced ANP-converting enzyme in the failed IOL group could indicate that impaired response to IOL is associated with impaired spiral artery formation, potentially through impaired vascularity.

5.8.3.4 Membrane metallo-endopeptidase

Membrane metallo-endopeptidase (MME) was also found to significantly at least halve in the failed IOL group compared with the ECS group, in all groups other than OXT-REL (as with ANP). In the heart, MME is known to break down ANP[341]. This warrants further investigation given the previously discussed findings in the literature.

5.8.3.5 Pleckstrin homology-like domain, family A, member 5

Pleckstrin homology-like domain, family A, member 5 (PHLDA5) total protein significantly doubled in the failed IOL group compared with the ECS group under SP-REL conditions. And phosphorylation of this protein non-significantly halved under both contracting conditions. Another member of this family, PHLDA2, has been associated with birthweight and growth restriction, and in cell culture of primary trophoblast cells overexpression of PHLDA2 inhibited proliferation and induced apoptosis[342], and therefore also warrants further investigation in future studies.

5.8.4 Phosphorylation events Part 2

The most significant phosphorylation events in the failed IOL group when compared with the ECS group are summarised in the String diagram presented in Figure 5.14A, which shows those proteins for which phosphorylation events doubled in the contracted state. This shows two main clusters: one linking

Membrane-associated progesterone receptor component 1 (PGRMC1), calnexin, Heat shock protein HSP 90-β, and Small glutamine-rich tetratricopeptide repeat-containing protein alpha; the second linking Vimentim, Filamin A and Myosin Light Chain Kinase. These have significantly more interactions than that which would be expected if proteins were randomly selected, with a PPI of <1.0e-16. These differences suggest that phosphorylation changes in these proteins may interfere with normal myometrial function. Figure 5.14B shows proteins for which the phosphorylation events halved in the contracted states, and Figure 5.15 shows proteins for which phosphorylation events both halved and doubled in the contracted state.

5.8.4.1 Myosin light chain kinase

Two phosphorylation events occurred for myosin light chain kinase (MYLK), both in the OXT-CON group: phosphorylation of MYLK at serine 1438 more than doubled in the failed IOL group under OXT-CON. Under the same condition, phosphorylation of MYLK at Serine 1776 halved in the failed IOL group .Using data from the same experiments as Part 1 of the present study, Hudson *et al.* (2017)[145] found that phosphorylation of serine 1760 increased during spontaneous contraction (SP-CON) and oxytocin driven contractions (OXT-CON), which was verified with Western blotting[145]. This site was not differentially phosphorylated between the failed IOL group and ELSCS group. While 1776 is

recognised as a phosphorylation site by software such as Uniprot and Phosphosite, little else is known about it other than it is close to the calmodulin-binding region at 1711-1774 residues (www.uniprot.com and www.phosphosite.com). Serine 1438 is recognised as a phosphorylation site, but again little is known about its functional significance. This is the phosphorylation site that significantly increased the most in the failed IOL group when compared with the ECS group, and seems to increase only in response to oxytocin stimulated contraction in the present study. Given that IOL usually involves infusion of oxytocin to stimulate contractions, if there is a phosphorylation of MYLK at this site which somehow impedes normal function of the kinase, it could be an area to target in future studies. Future work is therefore required to investigate the functional effects, if any, of these phosphorylation events.

5.8.4.2 Membrane-associated progesterone receptor component 1

Membrane-associated progesterone receptor component 1 (PGRMC1) is a member of the membrane-associated progesterone receptor family. PGRMC1 levels have been found to be reduced in term myometrium, either in labour or not in labour, when compared to preterm myometrium; and treatment of myometrial strips with an antibody to PGRMC1 suppressed the ability of progesterone to induce relaxation in myometrial strips[343]. Wang *et al.* (2016)[344] investigated differences in mRNA expression of PGRMC1 in myometrium taken

from women at term not in labour and those having an emergency caesarean section and found that there was significantly decreased PGRMC1 mRNA and protein in the myometrium taken from the women who were at term not in labour. The authors suggested that this could be a pathway for functional withdrawal of progesterone in women at term [344]. In Part 2 of the present study, phosphorylation of PGRMC1 at Serine 181 doubled in the failed IOL group when compared with the ECS group under the SP-CON condition and at Serine 57 under the OXT-REL condition. Although not significant, this doubling could indicate an increase of the activity of this receptor in the failed IOL group which may render the myometrium less likely to contract and more likely to remain under the influence of progesterone and in the quiescent state; this could contribute to the failure to progress. However this is speculative and would require further study.

5.8.4.3 Calnexin

Calnexin is a nexin on the endoplasmic reticulum which interacts with Ca^{2+} pumps on the endoplasmic reticulum that are mainly found at the mitochondria-associated membrane (MAM). In addition, calnexin is also found in varying concentrations on the cell membrane. Under normal conditions, approximately 80% of calnexin is found in the endoplasmic reticulum, again with the majority of these on the MAM. Phosphofurin Acidic Cluster Sorting Protein 2 (PACS-2) is

a cytosolic sorting protein which is involved in normal distribution of calnexin throughout the cell. In cell culture, if PACS-2 is knocked down, the proportion of calnexin at the cell surface increases. Interestingly, phosphorylation of calnexin at both Serine 554 and 564 decrease the binding ability of PACS-2 to calnexin, and serine 554/564 Asp phosphomimic mutation results in a similar redistribution of Calnexin as with PACS-2 knock down, so that more calnexin is found at the cell surface[345]. This site had increased phosphorylation in the failed IOL group under OXT-CON conditions, and therefore may have resulted in increased calnexin at the myocyte cell surface. As calnexin can also act as a chaperone protein, it is possible that this could have had some effect on the cell interactions and contractility.

5.8.5 Comparisons of part 1 and part 2

As both part 1 and part 2 investigated PRE and SP-REL conditions, the results can be compared to determine the consistency of the conclusions. Reassuringly, proteins for which the REL/PRE ratio at least halved in all three samples for part 1 were also found to halve for the elective caesarean section group of part 2, including complement component C8 alpha chain, cryocrystalglobulin CC1 heavy chain variable region, and cDNA FLJ55673, highly similar to Complement factor B. Other proteins followed the same direction of change but did not always reach a doubling or halving between the two conditions.

5.9 Conclusions

This study provides a wealth of novel proteomic and phosphoproteomic data identifying proteins and phosphorylation events which can be targeted in future research into the molecular mechanism of labour. Some results confirm findings in the literature, but most are novel findings which warrant further investigation. Importantly, these results support the hypothesis that there is a myometrial phenotype associated with failed IOL and failure to progress in labour that is potentially associated with dysfunctional myometrium. Particularly interesting pathways include those involving actin, thrombin, vascular formation and cell adhesion.

5.10 Limitations

Many of the potential conclusions discussed are speculative and would require further investigation. The conclusions may also be limited by there only being three women in each of the groups. Moreover, the apparent change in myometrial phenotype in Part 2 may be the consequence of the difficult induction of labour rather than the underlying physiopathological cause of the failure to progress. More research is needed at a functional level, combining myometrial contractility measurements and molecular studies. The strength of the work presented in this chapter is that it proves that the phosphoproteomic

approaches are a potentially powerful resource to understand the complex process of human labour.

CHAPTER 6. DISCUSSION

6.1 Summary

The overarching aim of my thesis was to further knowledge of the mechanism of human parturition. The four studies described in this thesis have utilised 'omics technologies in both clinical case-control and cohort settings to improve our understanding of the trigger for labour in humans. This includes identification of metabolites, proteins and myometrial protein phosphorylation sites that are linked to different modes of labour onset and delivery, as well as gestational age at delivery. The techniques used allow measurement of metabolite changes between labour and non-labour within the uterine environment immediately following delivery.

Chapters 2 and 3 showed the efficacy, reliability and consistency of the Metabolon, Inc., Mass Spectrometry metabolomics platform in this setting, despite there only being ten women included in each group. For example, in Chapter 2, all the women who had an elective caesarean section were administered the antibiotic Metronidazole, whereas it was not administered to any in the vaginal delivery group and this was directly reflected in the results (see section 2.9.5).

The results indicate that spontaneous labour involves signals generated by the fetus associated with matched changes in the placenta, resulting in specific

ratios of steroid metabolites between the cord artery and cord vein. On the other hand, there were fewer differences in the intervillous and maternal blood with labour, which fits with previous research. This is important because it suggests that the difficulties experienced with the current management of preterm labour and induction of labour is because medications given systemically to the mother do not address underlying issues in the fetal circulation. These results provide important novel leads to investigate for future research.

The fetus is enclosed within the myometrium and membranes, with its circulation separated from the maternal circulation by the placenta. It is well established that there are no obvious changes in the maternal circulating levels of progesterone and oestrogens with labour; and it remains difficult to elucidate the triggering pathways involved with labour by studying only the maternal circulation. The results presented in this thesis suggest that the trigger for labour relates to changes in metabolite production by the fetus; with increased progesterone and oestriol produced by the fetus compared to that produced by the placenta. And that this is associated with reduced progesterone produced by the placenta, potentially in a negative feedback mechanism.

The reason for the increased expression of progesterone by the fetus may well go back to fetal adrenal maturation, and specifically related to expression, or

activation, of the enzyme 3beta hydroxysteroid dehydrogenase. This enzyme catalyses conversion of pregnenolone to progesterone; 17alpha hydroxy pregnenolone to 17 alpha-hydroxy progesterone; and DHEA to androstenedione. This provides androgen substrates for aromatisation to oestrone and oestradiol (from the second trimester high placental aromatase activity converts increasing amounts of androgen to oestrogen[346]). The results of this thesis show that 17alpha hydroxyprogesterone follows a similar pattern to progesterone in terms of CA/CV ratios and supports the concept of fetal adrenal maturation. A previous study investigated activation of the fetal adrenal gland with regards to fetal virilisation. Differentiation to male or female sex occurs during the first trimester, as a result of male hormones secreted by the fetal testis. Overexposure of a female (46 XX) fetus to androgens results in virilised genitalia. This happens if there is a deficiency of cytochrome p450 21-hydroxylase, as with congenital adrenal hyperplasia, resulting in reduced fetal cortisol production, resulting in a reduction in negative feedback on ACTH production normally instigated by cortisol. The excess ACTH leads to excessive androgen production are produced[346].

The results in chapter 2 showed an increase in cortisol in the cord of the labouring group (VC/EC), however it could not be excluded that this was secondary to the effect of labour itself on the fetus compared with non-labour.

Subsequently, in chapter 3, while the concentration of cortisol in the cord vein was higher in the labouring groups than the non-labouring groups, the amount in the CV of the IOL group was significantly lower than that in the CV of the SL group (0.41-FC, p=0.028), despite both groups going through the contractions of labour and vaginal delivery. This suggests that spontaneous labour is associated with increased activation of adrenal enzymes, for example 3βHSD.

6.2 Linking the findings of the different studies

Although the studies in this thesis approached the overarching question of the thesis from different angles, there are general agreements between them. Chapter 2 resulted in the generation of a hypothesis for the potential pathway for labour, which was largely supported by the results from Chapter 3, suggesting that the pathway for labour involves elevated endocannabinoids in the cord would result in increased production of sphingolipids and ceramides, which could in turn stimulate prostaglandin production, resulting in labour. Moreover, this pathway may involve uterine lymphocytes. Progesterone has been shown to decrease FAAH activity in human lymphocytes *in vitro*, and FAAH breaks down endocannabinoids including AEA, PEA and OEA[184, 188, 190, 197, 347-349]. A reduction in progesterone concentration or sensitivity immediately prior to labour could result in an increase in endocannabinoid, sphingolipid, ceramide and prostaglandin production, triggering myometrial contractions and labour.

One of the most important findings of this thesis is a difference in certain metabolites between the cord artery and cord vein with different modes of labour onset and delivery. These results clearly show that the metabolic profiles of these two compartments are distinct, providing valuable insight into metabolites differentially produced by the fetus and placenta with spontaneous labour. The differences in the metabolome of these compartments are particularly consequential between spontaneous and induced labour given both groups had undergone the prolonged stress of labour. For example, with regards to the mean concentrations of oestriol and progesterone in cord artery and cord vein, the induction group was more alike the elective caesarean section group than the spontaneously labouring group. Further, eleven per cent of the measured metabolites were significantly different between the cord vein of the spontaneously labouring and induction of labour groups, and 10.1% different in the cord artery. This was compared with just 3.1% difference between the maternal latent phase samples, and 2.9% difference in the intervillous maternal samples.

These differences in progesterone and oestriol may represent a type of local progesterone withdrawal: less progesterone produced by the placenta with spontaneous labour could be perceived by myometrial cells as a form of progesterone withdrawal, even if progesterone levels remain high in the

maternal blood. In addition, this could in part explain why progesterone administered to the mother does not prevent preterm labour and preterm birth[125]. In many previous studies where cord blood was sampled as a whole and not defined as arterial or venous, as was done in chapter 2, such differences would have been missed. It would be worthwhile to replicate these findings in larger studies.

In addition, the results from Chapter 3 also indicate that there may be a link between spontaneous labour and a pathway involving the balance between ceramide, sphingosine and sphingosine 1-phosphate, and the resultant effect on cAMP, calcium homeostasis and actin disassembly. Alterations in this balance may influence myometrial contractility, and may be involved in spontaneous labour, potentially linking with the changes observed in endocannabinoid concentrations and the onset of spontaneous labour.

6.2.1 Association of maternal metabolites during pregnancy with gestational age at delivery

The maternal metabolome during pregnancy has been systematically investigated in the studies included in this thesis, particularly in Chapters 3 and 4, and numerous metabolites are shown to be associated with gestational age at delivery, and with preterm birth. The ORA analysis indicates that pathways that are most over-represented and associated with gestational age at time of

spontaneous labour involve fatty acid metabolism. This includes a potential association with dyslipidaemia and earlier gestational age at delivery. However, as with the other findings, further investigation using other approaches, such as Mendelian Randomisation, are essential to determine whether any of the differences in metabolites observed in these studies may be part of a causal pathway.

6.2.2 Vascular dysfunction

There were also indicators of changes in vascular function potentially being involved with the trigger for spontaneous labour, this included findings from Chapters 4 and 5. Specifically, that the trigger for labour, particularly preterm spontaneous labour, could be associated with dyslipidaemia, inflammation, and potentially vascular dysfunction. Further, these results suggest potentially modifiable risk factors that could be prioritised for further investigation as fruitful targets to prevent preterm labour. Subsequently, the next steps will involve replicating results and using other approaches to strengthen causal inference on the relation of these maternal risk factors with gestational age at delivery. Vascular dysfunction has also been associated with other pathologies of pregnancy such as pre-eclampsia and gestational diabetes; it is likely that all these pathologies are linked, and it may be that spontaneous labour occurs at a point when vascular function in the placenta decreases, and once a certain

threshold is crossed labour occurs. In women where there is already some vascular dysfunction, such as in women with pre-existing dyslipidaemia, diabetes or hypertension, it may be that it takes less for the threshold to be reached and these women are more likely to have preterm birth. It is important that in Chapter 4 the association between dyslipidaemia and shorter mean gestational age at delivery persisted even after adjustment for the identified potential confounders.

6.2.3 Myometrial phenotype associated with failed induction of labour

The number of significant differences in the proteome and phosphoproteome between the myometrium of women with failed IOL and myometrium of women at elective caesarean section support an hypothesis that failed IOL and failure to progress may be associated with a specific myometrial phenotype that confers myometrial dysfunction, thereby providing clues as to proteins required for spontaneous labour in humans.

The proteins and phosphorylation events identified include those that are involved in calcium homeostasis, actin and cytoskeleton function, and vascular function. Some results confirm findings in the literature, but most are novel findings which warrant further investigation. Evaluating the functional effects of these findings are vital next steps. This study provides a wealth of novel proteomic and phosphoproteomic data identifying proteins and

phosphorylation events which can be targeted in future research into the molecular mechanism of labour.

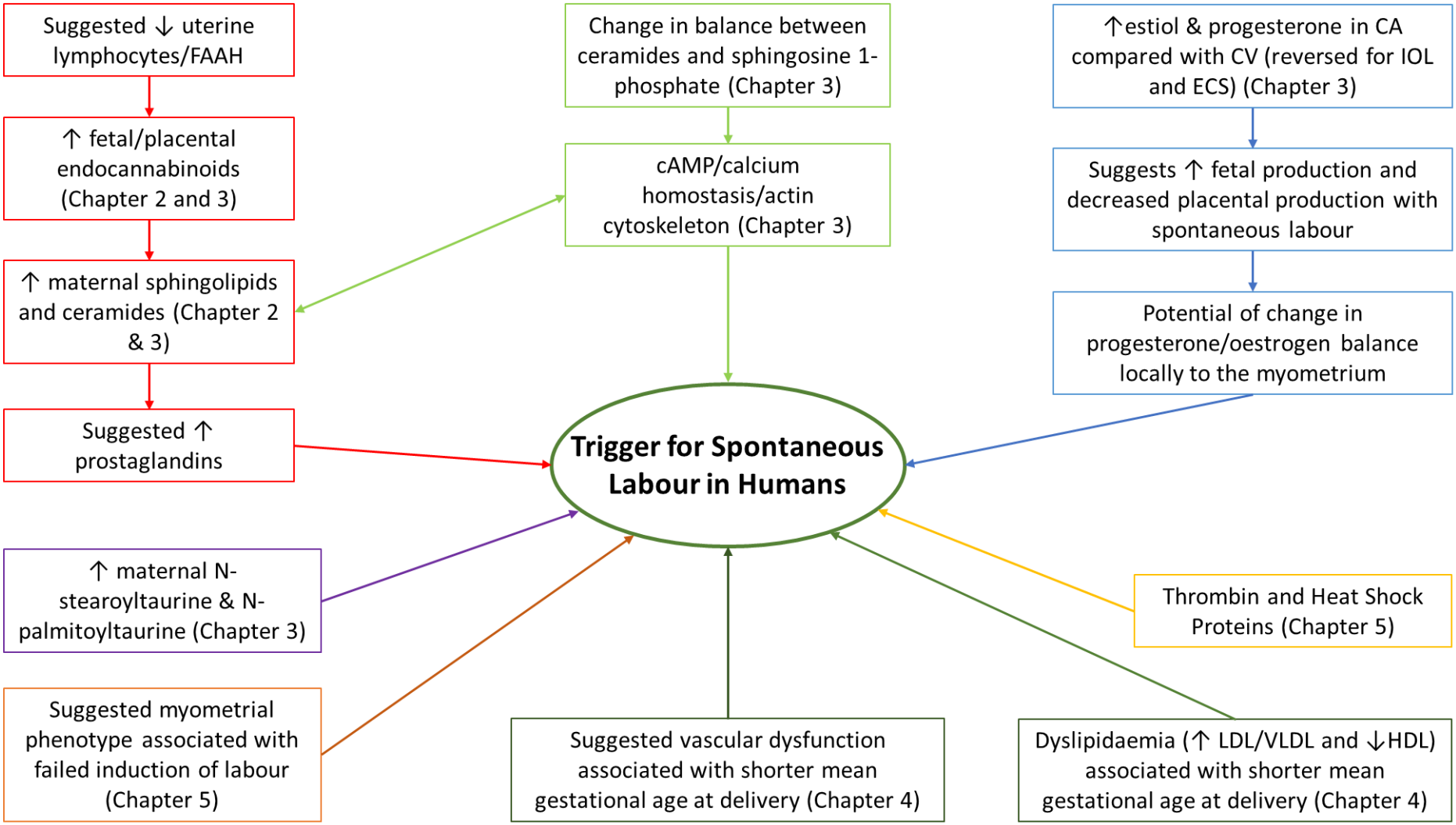

Figure 6.1: Diagram illustrating highlights of findings of the thesis relating to improving understanding of the trigger for labour in humans

6.3 Study limitations

A potential limitation of my thesis is the small sample sizes for the clinical studies. However, while future work will involve larger studies to confirm the results of this thesis, it was an important finding that the metabolomic profiles observed in Chapters 2 and 3 were consistent between the groups, with relative tight 95% confidence intervals. This suggests that the differences observed in this thesis may be due to the differences in mode of labour onset, delivery, and gestational age at delivery that these studies were designed to investigate.

In the BiB study, residual confounding and missing data may have introduced bias in the results. However, to the extent that these have been explored in the analysis models used, there is no indication that the missing data represents a major source of bias. For example, the comparison of outcomes between the total recruited pregnancies (n=13 776), the eligible sample (n=12 308), and the analysis sample (n=7 440) (the latter of which had no missing data), showed no significant differences in outcomes and results between the three groups (see section 4.6.1).

A further limitation regarding the myometrial studies is that the Myobath II is not a perfect representation of what occurs *in vivo*. It is obviously difficult to experiment on myometrium within pregnant women, however using fresh

tissue would seem to be more reliable than cell culture techniques; nevertheless, there remains the potential for misrepresentation in organ bath experiments.

6.4 Clinical implications

The observed differences in cord artery and cord vein metabolites between spontaneous and induced labour may help explain why exogenous progesterone does not prevent pregnancy loss or PTB, and provide novel targets for drug development. However, given the limited sample size of the current studies, replication of these findings in follow-on studies is needed.

The ineffectiveness and inefficiencies of the current management of both PTB and IOL may in part be due to spontaneous labour involving specific signals from the fetus that act locally on the fetal membranes, cervix and myometrium. The current management options for PTB prevention and IOL include maternal administration of progesterone and oxytocin, respectively, and the reason these options are relatively ineffective and inefficient could be due to such methods not altering or matching the fetal signals of spontaneous labour.

Novel drugs targeting mechanisms directly implicated in human labour may allow us to induce labour in a way that better reproduces spontaneous labour and increase the likelihood of normal vaginal delivery. Further, if there is a specific myometrial phenotype associated with failed induction of labour there may be better techniques to recognise this early or to modify the way in which

the myometrial contractions are stimulated. Both approaches could reduce the increased risk for adverse pregnancy outcomes that are currently associated with induction of labour[30].

The findings from Chapter 4 suggest that a general pattern of dyslipidaemia may be associated with shorter gestational age at delivery. As such dyslipidaemia has been investigated and treated for reduction of cardiovascular disease, it may be that specific drugs can be developed to target these pathways, or lifestyle changes recommended to women who are at higher risk, in order to reduce the risk for preterm birth.

6.5 Future studies

The studies included in this thesis have generated several hypotheses on the potential key molecular mechanisms involved in the trigger for human labour. These hypotheses can be followed up in subsequent studies which may inform the development of drugs for better treatment and prevention of preterm birth, and to better induce labour when indicated. To this end, future studies to continue the research presented in this thesis could include:

1. A large study to investigate metabolomic differences between the cord artery and cord vein with different types of labour onset. Numbers needed for appropriate power for this study would be guided by Chapters 2 and 3.

2. Large cohort study to further investigate metabolome changes at different gestations of pregnancy. Particularly investigating potential differences and similarities between women according to age, past medical history, BMI, dyslipidaemia, and other demographic features, and the effect this may have on gestational age at delivery.
3. The findings from Chapter 4 show that certain metabolites are associated with gestational age at delivery, and the next step will be to determine if these are likely to reflect a causal relationship. Mendelian randomisation (MR) is a method that uses genetic variants to investigate the effect of modifiable causes on diseases[350-352]. MR can be used to further investigate possible causal links between metabolites and gestational age at delivery. This would involve two steps. First, one needs to identify common genetic variants that are associated with elevations in certain blood metabolites, which are available from metabolomics genome-wide association studies (GWAS). Second, an MR study would test whether people with such genetic variants have a higher incidence of PTB than those who do not have these genetic variants. Importantly, MR studies are more robust to the common limitations of epidemiological studies, such as reverse causation (i.e. genetic variants are determined from conception) and confounding (i.e. genetic variants are usually not related to exposure-

outcome confounders). However, it must be noted that MR in this model would assume that the genetic variant influences PTB only by affecting metabolite levels, and that MR assumes long-term exposure to altered metabolite levels. Therefore, if labour is influenced by transient metabolite changes, MR in this setting may not be informative. However, it may also be that certain genetic variants confer increased susceptibility to changes in metabolites that results in having a lower threshold at which labour occurs.

4. Study to test the hypothesis that immune cells within the uterus are involved in the trigger for spontaneous human labour, including the involvement of FAAH within lymphocytes and the control of endocannabinoids. This will involve characterising the lymphocyte populations in cord arterial, cord vein and maternal blood, and determining whether there are differences in these populations between women who labour and women who do not labour at the time of delivery.
5. Further Myobath II studies to confirm the phosphoproteomic findings of Chapter 5, including use of specific phosphoantibodies to validate the phosphorylation changes. Experiments would be designed with cellular biochemistry experts to investigate of the functional effects of the identified phosphorylation events.

APPENDICES

APPENDIX 2A1: Publication associated with this thesis and adapted for Chapter 2 (written by Katherine Birchenall with support from Professor Lopez Bernal and Dr Welsh).

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Metabolite Changes in Maternal and Fetal Plasma Following Spontaneous Labour at Term in Humans Using Untargeted Metabolomics Analysis: A Pilot Study

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Abstract: The mechanism of human labour remains poorly understood, limiting our ability to manage complications of parturition such as preterm labour and induction of labour. In this study we have investigated the effect of labour on plasma metabolites immediately following delivery, comparing cord and maternal plasma taken from women who laboured spontaneously and delivered vaginally with women who were delivered via elective caesarean section and did not labour. Samples were analysed using ultra high-performance liquid chromatography-tandem mass spectrometry. Welch's two-sample t-test was used to identify any significant differences. Of 826 metabolites measured, 26.9% (222/826) were significantly altered in maternal plasma and 21.1% (174/826) in cord plasma. Labour involves changes in many maternal organs and poses acute metabolic demands in the uterus and in the fetus and these are reflected in our results. While a proportion of these differences are likely to be secondary to the physiological demands of labour itself, these results present a comprehensive picture of the metabolome in the maternal and fetal circulations at the time of delivery and can be used to guide future studies. We discuss potential causal pathways for labour including endocannabinoids, ceramides, sphingolipids and steroids. Further work is necessary to confirm the specific pathways involved in the spontaneous onset of labour.

Keywords: human parturition; endocannabinoid; steroids; energy metabolism

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1. Introduction

Our understanding of the mechanisms of human parturition remains incomplete and consequently management options for complications of pregnancy such as preterm labour (PTL), and common interventions such as induction of labour (IOL) [1,2], remain clinically unsatisfactory [3].

A better understanding of human labour at term (defined as 37–42 weeks' gestation) would allow development of methods for improved prediction and prevention of PTL, as well as more effective strategies for IOL [4–6]. The aetiology of PTL is heterogeneous and frequently pathological, however it may result in stimulation of the same triggers for parturition as spontaneous labour at term [1,7]. The proportion of pregnancies with IOL in England has increased from 20.3% in 2006–2017 to 29.4% in 2016–2017 [4,6,8–10]. IOL can be inefficient, with potentially severe complications for the mother and neonate, including lengthy hospital stays at high cost to the National Health Service and a negative impact on the birth experience [3,11,12]. The use of clinical and molecular markers to identify women at risk of complications of pregnancy remains challenging due to their poor predictive value [13,14].

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There is a need to identify the metabolic changes underlying the physiological onset of spontaneous human labour and to develop new clinically useful predictive markers and better drug targets.

Metabolomics allows the investigation of a wide range of metabolites across the main biochemical pathways [15]. The advantage of studying the metabolome or metabolic profile of a system is that it reveals the current activity rather than a prediction of what may happen, as is the case with genomic studies [16–18]. The most commonly used metabolomic techniques are mass spectrometry (MS) and nuclear magnetic resonance (NMR), paired with data processing using advanced computational programmes [18,19]. NMR can measure a broad range of metabolites however it is less sensitive than MS. Gas chromatography (GC) or liquid chromatography (LC) coupled to MS provides excellent separation of molecules within a sample according to their mass-to-charge ratio and analysis at a wide range of concentrations. As MS is more sensitive, it can be utilised to measure more metabolites, but it has a longer analysis time than NMR [19]. Metabolomics has been used to investigate pregnancy related changes in maternal and cord blood, fetal membranes, cervico-vaginal secretions, urine and amniotic fluid; with the potential of identifying metabolic profiles or biomarkers associated with different outcomes, such as PTL [20,21], missed miscarriage [22], diagnosis of exposure to chorioamnionitis in the neonate [23], and the effect of maternal diet on amniotic fluid composition [20,21,24]. However, to our knowledge there are no previous studies focusing on parturition.

Here we present novel MS data from a pilot study designed to investigate the effect of parturition on plasma metabolites in the fetal and maternal circulation at the time of delivery and demonstrate that even with a relatively small sample size significant and robust changes can be identified providing new insights into the metabolic responses of the maternal-feto-placental unit in spontaneous labour.

2. Materials and Methods

2.1. Participants

Nine women with a vaginal delivery (VD) following active labour of spontaneous onset and ten women who had an elective caesarean section (CS) for reasons not related to maternal or fetal disease gave informed, written consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and ethical approval for the study was gained from the National Research Ethics Service Committee-South West, Bristol (reference number: E5431). The inclusion criteria were: women with uncomplicated singleton term pregnancies; age 18–40 years; no significant past medical history. The exclusion criteria were: multiple pregnancies; age

under 18 years or over 41 years of age; taking medications likely to affect metabolomics; diabetes; pre-eclampsia; other metabolic conditions; raised temperature or signs of fetal distress during labour. The following demographic information was collected: ethnicity, age, BMI, smoking status, time last eaten, time of delivery, duration of delivery, drugs given during hospital stay, Apgar scores of the baby, and cord gases if taken.

2.2. Collection of Samples

Cord blood and maternal (intervillous) blood samples were obtained within 30 minutes of delivery of the baby and placenta, as described in detail previously [25], following delayed cord clamping which is routinely carried out at St Michael's Hospital for both VD and elective CS provided there are no contraindications. Samples were collected using a sterile 21 Gauge needle and syringe, transferred into Vacutainer tubes containing EDTA, then centrifuged at 1000× g for ten minutes. 200 µL of the clear upper plasma layer was transferred into chilled propylene tubes and stored at −80 ◦C.

2.3. Metabolomic Analysis

The samples were transported on dry ice to Metabolon, Inc. (Morrisville, NC, USA) for ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC/MS) [26–28]. All samples were analysed together in order to reduce risk of batch bias. This included the measurement of 826 known metabolites for each sample, grouped into 108 sub-pathways and the following eight super-pathways: Lipid, Amino Acid, Peptide, Energy, Nucleotide, Cofactors and Vitamins, and Xenobiotics. Briefly, plasma samples were subjected to methanol extraction then split into aliquots for analysis by UHPLC/MS in the positive (involving two methods, one optimized for hydrophilic, and the other hydrophobic compounds), negative or polar ion mode. Metabolites were identified by automated comparison of ion features to a reference library of chemical standard, followed by visual inspection for quality control [29]. Multiple water blanks were included on each plate of experimental samples to identify any compounds resulting from storage or handling. Compounds which were detected at a level at least triple that found in the water blanks and which were confirmed to be present relative to a chemical reference standard were included in the final analysis. For quality assurance and quality control (QC), pooled QC plasma replicates, as well as several internal standards, were assessed to determine instrument variability, with representative relative standard deviation (RSD) = 3% for internal standards and 7% for endogenous biochemicals.

2.4. Statistical Analysis

The two experimental groups were labelled VC/EC and VM/EM. VC/EC represents the difference in the means between VC, the metabolite quantity identified in cord plasma from women who laboured and delivered vaginally, and EC, the metabolite quantity identified in cord plasma from women who did not labour and delivered via elective CS. VM/EM represents the difference in the means between VM, the metabolite quantity identified in intervillous plasma from women who laboured and delivered vaginally, and EM, the metabolite quantity contained in intervillous plasma from women who did not labour and delivered via elective CS. Welch's two-sample t-test was used to identify metabolites for which the means were significantly different between VC and EC or VM and EM. Analysis was performed on log-transformed data, considered significant if $p \leq 0.05$. A q-value was also calculated to control for false discovery rate and account for the multiple comparisons which occur in metabolomic-based studies, where a q-value of <0.10 gives high confidence that a significant difference is not due to chance.

3. Results

3.1. Demographics

Table 1 presents the characteristics of the labouring and non-labouring groups. All nine women in the vaginal delivery group had confirmed labours of spontaneous onset. All women were of white ethnicity and there were no significant differences in age, parity, employment status, Apgar scores at birth, birthweight or gender of fetus. None of the women had experienced medical complications during the pregnancy, nor had there been concern for any of the fetuses. The mean BMI at booking was 21.6 in the VD group and 25.6 in the elective CS group (p = 0.02), due to an outlier in the elective CS group with BMI 38.3. Further analysis was performed and confirmed her inclusion did not affect the spread of results. The median gestation at delivery was 40 weeks in the VD group and 39 weeks and 2 days in the elective CS group (p = 0.04), an expected difference as planned CSs are routinely booked between 39 and 40 weeks' gestation whereas spontaneous deliveries may occur up to three weeks later.

Table 1. Characteristics of included human participants.

Demographic	VD n = 9	Elective CS n = 10	p Value (at ≤ 0.05)
Age (years)	32.4 (24–39) a	34.6 (30–40) a	NS
BMI at pregnancy booking	21.6 (19.9–23.1) a	25.56 (21.7–38.3) a	0.02
Ethnicity	White British: 9	White British: 9 White Romanian: 1	NS
Smoking history:			NS
Never	8	4	
Ex	0	5	
Current	1 (4/day)	1 (10/day)	
Maternal past medical history	Eczema: 1 ICSI pregnancy (own sperm/egg): 1	Previous postnatal depression:1 Well controlled asthma: 1 (MTHFR gene 1): 1	NA
Maternal employment status at pregnancy booking:			NS
Employed	8	8	
Home maker	1	2	
Gravida	1.2 (0–2) a	2.4 (0–5) a	NS
Parity	0.6 (0–2) a	1.7 (0–7) a	NS
Significant pregnancy complications	Nil	Nil	NA

Indication for elective CS	NA	Breech: 3 Previous CS: 3 Maternal request: 1 Previous 3rd degree vaginal tear: 1 Previous traumatic delivery: 1 Tocophobia: 1	NA
Duration of labour in minutes	312 min (70–650) a	NA	NA
Gestation at delivery (weeks+days)	40 (38+1–41+6) a	39+2 (38+4–40+3) a	0.04
Apgar scores at 1, 5 and 10 min:			NS
8,9,10	1	0	
9,10,10	8	10	
Management of third stage:			NS
Syntometrine or Carbetocin	8	10	
Physiological	1	0	
Interval between delivery and freezing of sample (minutes)	38 (36–60) b	28.5 (24–45) b	0.02
Gender of fetus:			NS
Female	5	4	
Male	4	6	
Apgar scores at 1, 5 and 10 min:			NS
8,9,10	1	0	
9,10,10	8	10	
Birth weight (Kilograms):	3.4 (3.2–3.9) a	3.6 (3.1–4.1) a	NS

a Mean (range); b Median (range); VD = Vaginal Delivery; CS = Caesarean Section; BMI = Body Mass Index; NS = not significant; NA = non-applicable; ICSI = intracytoplasmic sperm injection.

3.2. Metabolomics

All the results refer to ten women in the elective CS group and nine women in the VD group. It was not possible to obtain intervillous blood from one placenta, reducing the VM samples to eight. The numbers of metabolites with significant ($p \leq 0.05$) differences between VM and EM, and VC and EC, in the eight super-pathways are presented in Table 2. Of the 108 metabolite sub-pathways measured, 83 contained metabolites with significant VC/EC and/or VM/EM differences. For the following analysis, xenobiotic metabolites will be considered separately.

Table 2. Number of metabolites which significantly increased or decreased at the time of delivery between women who spontaneously laboured and delivered vaginally and women who did not

labour and delivered via elective caesarean section in the maternal (intervillous) plasma (VM/EM) and cord plasma (VC/EC) for each of the super pathways.

Super Pathway	Number Metabolites Measured	Number Significant Changes VM/EM			Number Significant Changes VC/EC		
		↑	↓	Total (%)	↑	↓	Total (%)
Lipid	405	139	14	153 (37.8)	73	12	85 (21.0)
Amino Acid	177	9	15	24 (13.6)	15	14	29 (16.4)
Xenobiotics	108	18	3	21 (19.4)	16	5	21 (19.4)
Carbohydrate	29	7	0	7 (24.1)	9	0	9 (31.0)
Cofactors and Vitamins	31	3	4	7 (22.6)	3	5	8 (25.8)
Energy	11	3	0	3 (27.3)	6	0	6 (54.6)
Nucleotide	38	2	2	4 (10.5)	9	3	12 (31.6)
Peptide	24	0	1	1 (4.2)	3	5	8 (33.3)
Partially characterised	3	2	0		2	0	

VM = Maternal (intervillous) plasma from women who laboured and delivered vaginally (n = 8); EM = maternal plasma from women who did not labour and delivered via caesarean section (n = 10); VC = cord plasma from women who laboured and delivered vaginally (n = 9); EC= cord plasma from women who did not labour and delivered via elective caesarean section (n = 10); ↑ = Number of metabolites significantly elevated; ↓ = Number of metabolites significantly decreased.

3.3. Changes in Cord Plasma Metabolome

Table S1 shows the changes in cord plasma (VC/EC) of specific metabolites within the sub-pathways ordered according to the magnitude of fold-change. 120 metabolites significantly increased in the cord plasma of women who laboured, 102 of which also had a significant q value (<0.10), indicating a high likelihood that the significant change was not due to chance. These comprised 58 lipid metabolites, 14 amino acid metabolites, nine carbohydrate metabolites, eight nucleotide metabolites, six energy metabolites, three cofactors and vitamins metabolites, two peptide metabolites and two partially characterised molecules. The metabolite with the largest VC/EC fold-change was heme, which had an average 14.30-fold increase in the cord plasma ($p = 0.024$) taken from women who laboured.

Thirty-nine metabolites significantly decreased in the cord plasma of women who laboured

(VC/EC), and 27 of these had a significant q value: eleven amino acids, six lipids, four peptides, three cofactors and vitamins and three nucleotides.

3.4. Changes in Maternal Plasma Metabolome

Table S2 shows the corresponding changes in maternal plasma metabolites (VM/EM). Overall, 165 metabolites significantly increased in labouring women, all of which had a significant q value. These

included: nine amino acids, seven carbohydrates, three cofactors and vitamins, three energy metabolites, 139 lipids, two nucleotides, and two partially characterised molecules. The metabolite with the greatest significant-fold increase in maternal plasma of women who laboured was the partially characterised molecule glucuronide of C10H18O2 (8), with an average 10.85-fold increase in VM plasma. The dicarboxylate fatty acids maleate and adipate (C6-DC) increased 10.42-fold and

8.06-fold, respectively.

Thirty-six metabolites were significantly decreased in the maternal plasma of women who laboured when compared with women who did not labour (VM/EM), with a corresponding significant q value, comprising fifteen amino acids, four cofactors and vitamins, fourteen lipids, two nucleotides and one peptide. Among these were steroid sulphates, including 3-dehydrocholate ($p = 0.002$), 16α-hydroxy DHEA 3-sulfate ($p = 0.007$) and androsteroid monosulfate C19H28O4S ($p = 0.017$), with 0.13, 0.22 and

0.26 fold-changes, respectively.

3.5. Xenobiotics

The findings within the xenobiotic metabolites were as expected, reflecting the drugs known to be administered to the women. For example, metronidazole was given to all ten women who were delivered via elective CS but not to any of the women who delivered vaginally, and this was reflected in the results (Tables S3 and S4).

3.6. Lipid Super-Pathway

The lipid super-pathway contained the most differences between labouring and non-labouring women (Figure 1g and Table 2). Most of these changes were significant increases in maternal plasma (VM/EM). 405 metabolites were identified in this super-pathway: 34.3% (139/405) of the metabolites significantly increased and 3.5% (14/405) significantly decreased in the maternal plasma of women who laboured when compared to non-labour; correspondingly, 18.0% (73/405) of the metabolites significantly increased in the cord plasma of women who laboured, and 3.0% (12/405) significantly decreased. Of note, 22 sphingolipids (Table S5) significantly increased in maternal plasma of labouring women with only one corresponding increase found in cord plasma; whereas the sub-pathway with the most significant changes seen in the cord plasma within the lipid super-pathway were fatty acid metabolism (acyl carnitine), long chain fatty acids and polyunsaturated fatty acids (Figure 1g). There were significant increases in the medium and long chain fatty acids in both cord and maternal plasma and there were selective changes in the maternal and fetal circulation for acyl carnitines, acyl cholines, and eicosanoids (12-HETE and 23-HHTrE) (Tables S1 and S2).

Of the steroid metabolites, cholesterol was significantly increased in the maternal plasma of women who laboured (VM/EM) (Table S2) but did not change in the cord plasma (VC/EC). 17α-hydroxypregnenolone 3-sulfate and 21-hydroxypregnenolone monosulfate were significantly decreased in the maternal plasma of women who laboured (VM/EM), with no corresponding changes seen in the cord plasma (Table S6). Conversely, progesterone significantly increased in the cord plasma (VC/EC), with no change seen in maternal plasma (VM/EM). There were significant increases in 5α-pregnan-3β-ol,20-one sulfate, 5α-pregnan-3β,20β-diol monosulfate, 5α-pregnan-3β,20α-diol monosulfate, 5α-pregnan-3β,20α-diol monosulfate and pregnanediol-3-glucuronide in maternal plasma, which were not seen in the cord (Table S7).

Of the endocannabinoid metabolites, three were significantly increased in the cord plasma of women who laboured when compared with those who did not (VC/EC); and one significantly

increased, and one significantly decreased in the maternal plasma of women who laboured (VM/EM) (Table S8). Twelve of seventeen ceramide metabolites significantly increased in the maternal plasma (VM/EM), however there were no such significant differences seen in the cord plasma samples (VC/EC) (Table S9). Corticosterone significantly increased in maternal plasma (VM/EM) and in cord plasma (VC/EC) (5.41 and 3.09-fold, respectively) of women who laboured, and the corresponding increases for cortisol were 5.02-fold and 3.57-fold. There were also significant increases in cortisone and cortisone 21-sulfate in cord plasma but not in maternal plasma (Tables S1 and S2).

Androstenediol (3β,17β) disulfate and androsteroid monosulfate C19H28O4S significantly decreased in maternal plasma of women who laboured (VM/EM). 16a-hydroxy DHEA 3-sulfate significantly decreased in both the cord and maternal plasma. Androsterone glucuronide, epiandrosterone sulfate, 5α-androstan-3β and 17β-diol disulfate significantly increased in the maternal plasma, and androsterone sulfate and androstenediol (3α, 17α) monosulfate significantly increased in both maternal and cord plasma (Tables S1 and S2).

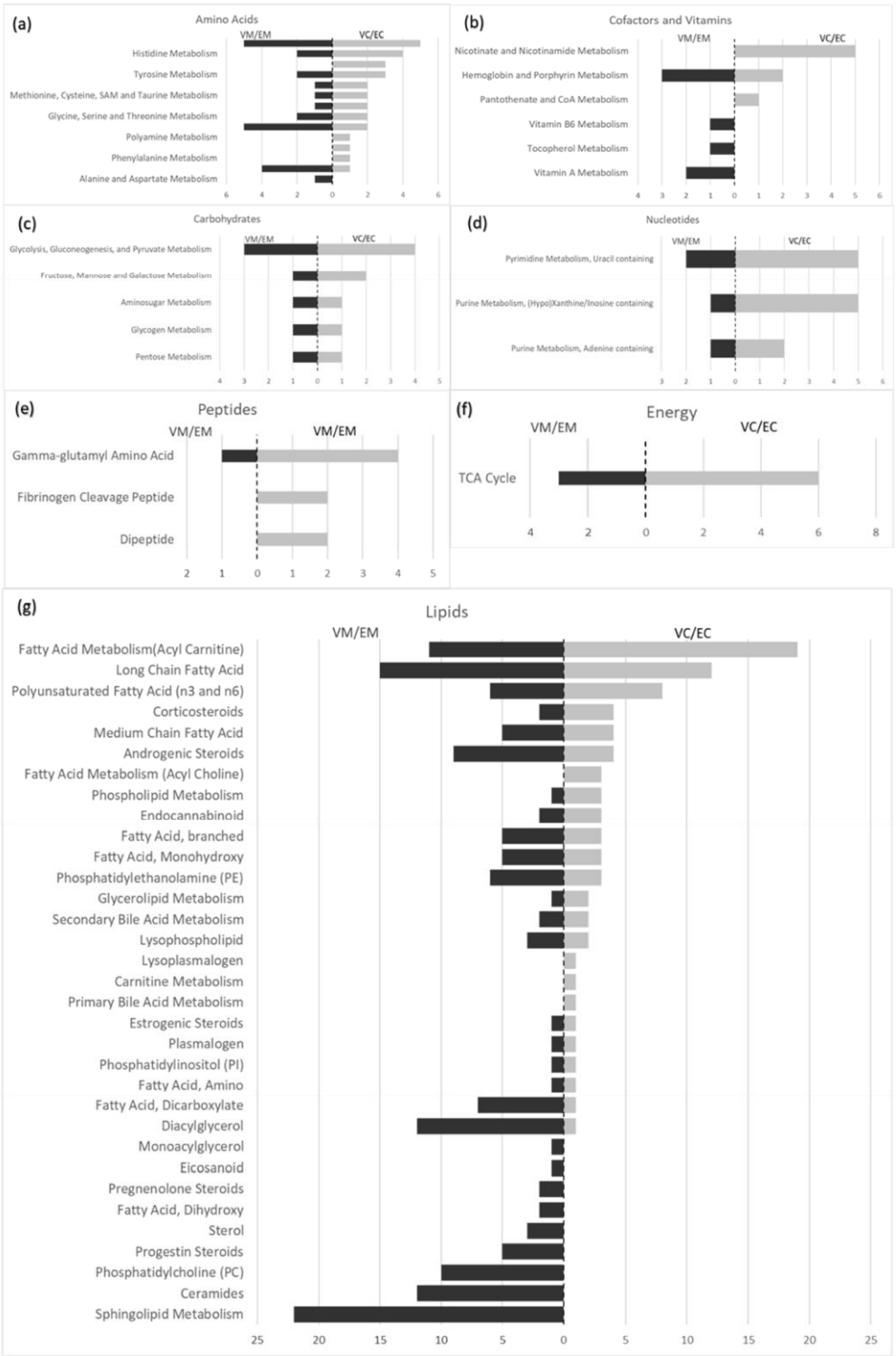

Figure 1. Comparative histograms showing the number of metabolites with significant changes between maternal (intervillous) plasma taken from women who laboured and maternal plasma (intervillous) taken from women who did not labour (VM/EM) compared with cord plasma taken from women who laboured and cord plasma taken from women who did not labour (VC/EC) for each if the sub-pathways within the following super-pathways: (a) Amino Acids; (b) Cofactors and Vitamins; (c) Carbohydrates; (d) Nucleotides; (e) Peptides; (f) Energy; (g) Lipids.

Of the oestrogenic steroids, estrone 3-sulfate significantly increased in the cord plasma with no change in the maternal plasma, while estriol 3-sulfate significantly decreased in the maternal plasma

with no change in the cord (Tables S1 and S2).

3.7. Nucleotides

The majority of the significant changes seen within the nucleotide super-pathway were within

VC/EC (Figure 1d), including significant increases in inosine, hypoxanthine, and xanthine, and a significant decrease in N1-methylinosine. 5-methyluridine (ribothymidine) significantly decreased in both cord (VC/EC) and maternal plasma (VM/EM), while 5,6-dihydrouracil and xanthosine significantly increased (Tables S1 and S2).

3.8. Amino Acids

Unlike the other super-pathways, most of the significant changes within the amino-acid super-pathway were decreases in VC/EC and VM/EM (Figure 1a). Serine, histidine, tryptophan, N-acetylkynurenine, picolinate, indole-3-carboxylic acid, citrulline and arginine significantly decreased in both VC/EC and VM/EM.

Carnosine, N-acetyltyrosine and xanthurenate significantly decreased in VC/EC only, while S-1-pyrroline-5-carboxylate, indolebutyrate, ornithine, homoarginine, threonine, lysine and trans-4-hydroxyproline decreased in VM/EM only. By contrast, N-acetylaspartate increased 7.14-fold in VM/EM only, and cysteine-glutathione disulfide increased 5.21-fold in VC/EC (Tables S1 and S2).

3.9. Peptides

Twenty metabolites were measured in this super-pathway (Figure 1e and Table 2). Two of the four fibrinogen cleavage peptide metabolites measured were significantly increased in cord plasma of women who laboured compared with non-labour: fibrinopeptide A des-ala increased 12.12-fold and fibrinopeptide A phosphono-ser increased 11.34-fold. There were no corresponding changes in maternal plasma.

3.10. Carbohydrate Metabolism

Twenty nine metabolites were measured in this super-pathway (Figure 1c and Table 2), and of these glucose, maltose, fructose, ribulonate/xylulonate, pyruvate and lactate were significantly increased in both the cord and maternal plasma taken from women who laboured compared with non-labour. 3-phosphoglycerate was significantly increased in cord plasma.

3.11. Energy Super-Pathway

Eleven metabolites were measured in this super-pathway (Table 2). Six metabolites of the TCA cycle were significantly increased in cord plasma of labouring women, but only three increased in maternal plasma (Tables S1 and S2). There were no significant changes in oxidative phosphorylation.

3.12. Co-Factors and Vitamins

Thirty-one metabolites were measured in this super-pathway (Figure 1b and

Table 2). Of these, 1-methylnicotinamide, N1-methyl-2-pyridone-5-carboxamide and N1-methyl-4-pyridone-3-carbox-amide from nicotinate and nicotinamide metabolism were significantly decreased in the cord plasma taken from women who laboured (VC/EC), and adenosine 5’-diphosphoribose (ADP-ribose) significantly increased (Table S1). There were no such changes in the maternal plasma.

Heme significantly increased 14.3-fold in cord plasma (VC/EC), with no change seen in the maternal plasma, and two metabolites of vitamin A metabolism and one metabolite of vitamin B6 metabolism were significantly increased with labour in maternal plasma (VM/EM), with no corresponding changes

in the cord (Tables S1 and S2).

4. Discussion

To our knowledge, this study is unique in its comparison of the metabolomic profile of cord and maternal plasma at the time of delivery between women who have laboured and delivered vaginally and women who have not laboured and delivered via elective caesarean section. The clear-cut differences in metabolite levels between the two groups confirm the strong impact that active labour has on both mother and neonate. A proportion of these changes may be due to the stress of labour itself, but we believe that within these metabolites there are clues as to which pathways trigger spontaneous parturition in women. In addition, the observed changes in xenobiotics such as metronidazole were as expected, supporting the robustness of metabolomic methodology in this setting. While it cannot be ruled out that the intervillous blood samples had some degree of fetal blood contamination, the very distinct metabolic profiles that we obtained in cord and intervillous plasma confirms the fetal and maternal circulations were sampled separately [25], thereby confirming the reliability of this non-invasive method for experimental sampling of maternal blood after delivery.

It could be argued that the stress of labour resembles strenuous exercise, and it would be useful to look to studies which investigate metabolic changes due to exercise to see if they match any of the changes observed here. Medium- and long-chain acetylcarnitines increase up to 9-fold following intense exercise in men [30]. A major source of acetylcarnitine is acetyl Co-A, formed from pyruvate oxidation, and this increase following exercise is likely due to a switch to increased fatty acid oxidation in skeletal muscle instead of glycolysis during exercise [31,32]. The significant increases in acylcarnitine fatty acid metabolism observed in both the cord and maternal plasma of labouring women (VC/EC and VM/EM) (Tables S1 and S2) could in part be explained by fatty acid oxidation for energy used by the woman and neonate during labour and delivery. However, while the source of this may be from working skeletal muscle in the labouring mother, this explanation is less likely in the fetus/neonate who is not thought to be using their skeletal muscles during labour and delivery. The increased levels of stress metabolites observed in labour, including cortisol, are likely to be generating fatty acid mobilisation [33]. The increase in sphingomyelins observed in labouring maternal plasma and the increase in acylcarnitine fatty acid metabolism in both the cord and maternal plasma of labouring women could indicate a considerable shift in intermediary metabolism at the time of labour following the relative decrease of these metabolites in pregnancy [34].

Labour and vaginal delivery would be expected to affect metabolites of fetal and maternal adrenal origin, particularly glucocorticoids, as part of the physiological stress response [35,36]. In support of this theory, we found cortisol and its major metabolites cortisone, cortisone 21-sulfate and corticosterone were significantly increased in the cord plasma of women who spontaneously laboured and delivered vaginally (VC) when compared with those who did not labour (EC). Similarly, cortisol and corticosterone were significantly increased in the maternal plasma taken from women who laboured and delivered vaginally (VM) when compared with those who did not labour (EM) (Tables S1 and S2). Our data confirm that there is activation of the fetal and maternal adrenal cortex during labour and vaginal delivery, and while this may be part of the mechanism initiating spontaneous labour, further studies are needed to investigate this hypothesis.

Within cord plasma (VC/EC), heme was the metabolite with the greatest fold-change (Table S1). Heme is a complex of iron with protoporphyrin IX, present in haemoglobin and myoglobin. Its levels in the plasma can increase rapidly following haemolysis or tissue injury [37]. It is possible that the increased heme present in the cord taken from women who laboured is a marker of the increased trauma and tissue damage which a neonate experiences during vaginal delivery when compared with delivery via caesarean section.

4.1. Endocannabinoids, Ceramides and Sphingolipids

Endocannabinoids have been investigated as biomarkers for reproductive events, with both predictive and diagnostic uses [38]. Formed from membrane phospholipids, endocannabinoids are described as non-classical neurotransmitters [38]. Of the two characterised cannabinoid receptors, CB1 is present in areas of the brain, peripheral nerve terminals and extraneural sites including the uterus, whereas CB2 is largely limited to cells and organs of the immune system [39]. N-arachidonoylethanolamine (anandamide or AEA) binds to CB1 and CB2 and is broken down by the enzyme Fatty Acid Amide Hydrolase (FAAH) [39]. Cannabis exogenously acts on the endocannabinoid system, and its use has been associated with pregnancy complications such as preterm birth, placental abruption, fetal growth restriction and spontaneous miscarriage [40]. Elevated plasma AEA in early pregnancy (<8 weeks) and lower peripheral lymphocyte expression of FAAH has been associated with miscarriage [40,41], and the CB1-knock out mouse has elevated corticotrophin-releasing hormone (CRH) and spontaneous preterm labour [42]. The uterus can produce its own AEA [39], and myometrial studies show a concentration-dependent relaxation effect of AEA on human myometrial contraction in vitro [43]. Of note, FAAH converts AEA to ethanolamine and releases arachidonic acid for prostaglandin synthesis, providing a potential link between elevated AEA and labour [42]. Ceramide is a ubiquitous sphingolipid second messenger released rapidly in response to apoptosis and stress [44]. CB1 activation induces sphingomyelin hydrolysis and acute production of ceramide, while CB2 activation leads to de novo sustained ceramide production [39]. The pro-inflammatory cytokine TNFα is involved in CB1 and CB2 activation and ceramide production [39,44,45], whereby inflammatory events may stimulate the onset of labour.

In this study, AEA, oleoyl ethanolamide (OEA), and palmitoyl ethanolamide (PEA) were significantly increased in the cord plasma taken from the women who laboured (VC/EC) (Table S8). PEA was significantly increased in the corresponding maternal plasma (VM/EM), while N-oleoylserine significantly decreased in maternal plasma. Interestingly, while in previous studies maternal plasma AEA has been shown to decline throughout pregnancy until a sharp increase at the time of spontaneous or induced labour [40,42], we found no difference in the maternal plasma (VM/EM) immediately following delivery between women who laboured and women who did not; however there was a significant increase in endocannabinoids, including AEA, in the cord plasma of women who laboured (VC/EC). It is possible that any increase in AEA is localised to the placenta during labour, and it has previously been shown that AEA concentrations are significantly higher in the umbilical vein than the umbilical arteries, which suggests either production by or transportation across the placenta [40]. In addition, we found 71% (12/17) of the measured ceramides increased significantly in the maternal plasma (VM/EM) (Table S9), while none increased in the cord plasma (VC/EC). Correspondingly, 40% (22/55) of measured sphingolipids were increased in the maternal plasma (VM/EM) while only 2% (1/55) significantly changed in the cord plasma (VC/EC). This finding supports previous reports of increased ceramide levels and expression of serine palmitoyl transferase, the rate-limiting enzyme for ceramide synthesis de novo, in the placentas of women who laboured compared with those who delivered by elective CS. This group also reported an increase in interleukin-6, suggesting the involvement of ceramide signalling and inflammatory

mechanisms in labour [46]. Our data support the view that the endocannabinoid/ceramide/sphingolipid pathway has the potential to stimulate labour [40,42,46].

4.2. Prostaglandins

Prostaglandins are involved in the early preparation for parturition including cervical ripening (prostaglandin E2) and stimulation of contractility (prostaglandin F2α), as well as rupture of membranes. Increases in prostaglandins, prostacyclin and their metabolites during advancing labour have been demonstrated in maternal plasma, amnion and amniotic fluid [47–49]. Prostacyclin and thromboxane metabolites increase in maternal urine in threatened preterm labour [50], and the prostacyclin metabolite 6-ketoprostaglandin F1α increases in maternal urine both during vaginal delivery and elective CS [51].

In our study, the eicosanoid 12-HETE was significantly increased in the maternal plasma of women who laboured and delivered vaginally (VM/EM) (Table S2), however no prostaglandins were detected. This may be due to the concentrations of prostaglandins in plasma being too low to detect using this metabolomic platform, or because the samples were collected following placental separation with a potentially rapid turnover resulting in these eicosanoid metabolites being excreted into the urine prior to sample collection.

4.3. Progesterone and Pregnenolone

Functional progesterone withdrawal has been linked to the onset of labour [42]. Although there were no significant differences in progesterone levels in the cord (VC/EC) or maternal (VM/EM) plasma in our study, there was a significant increase in metabolites of the progestin sub-pathway in the maternal plasma of labouring women (VM/EM). There is a strong positive correlation of 3β- and 16α-hydroxysteroids with gestational age [52]. This reflects maturation of the fetal adrenal zone and potential involvement of these metabolites in the preparation for labour.

Parturition has long been associated with changes in progesterone and pregnenolone metabolism in many species [53,54]. Our findings have uncovered a rich and complex pattern of steroid metabolites in spontaneous labour and vaginal delivery reflected in both maternal and fetal circulations (Tables S6 and S7). Of interest is the relative increase in VC/EC of pregnenolone and hydroxypregnenolone sulfates which are mirrored by changes in the opposite direction in the maternal circulation (Table S6). Moreover, progesterone and pregnandiol metabolites increased in both the maternal and fetal circulations (Table S7). These changes indicate increased turnover of C21 compounds, which are the first steroids derived from cholesterol through the P450scc and 3β-hydroxysteroid dehydrogenase pathway during labour. We have previously demonstrated changes in cholesterol-transporting lipoproteins in the maternal circulation in women in spontaneous labour [55], confirming the need for high cholesterol turnover during parturition. Our results present, for the first time, a comprehensive picture of C21 metabolites in the maternal and fetal circulations in relation to labour and vaginal delivery that can be used to guide future studies investigating steroid changes at parturition.

4.4. Estrogen

Estrogen synthesis in pregnancy results from the integration of maternal, placental and fetal tissues [56]. Placental Corticotrophin Releasing Hormone (CRH) stimulates the fetal adrenals to produce DHEA-sulfate (DHEA-S), which is 16-hydroxylated in the fetal liver and converted to estriol and estrone in the placenta [57,58]. In our study, 16a-hydroxy DHEA 3-sulfate significantly decreased in both the cord (VC/EC) and maternal plasma (VM/EM), while estriol 3-sulfate significantly decreased

in the maternal plasma (VM/EM) and estrone 3-sulfate significantly increased in the cord plasma (VC/EC) of women who laboured (Tables S1 and S2). This provides an interesting insight into the effect of labour on the metabolism of estrogens. It is likely that these changes are related to substrate availability, including DHEA and its sulfated derivatives, as well as local changes in enzyme activity involving sulfatases, sulfotransferases, hydroxylases and aromatases, and their redox cofactors including NADH and NADPH in the uterus, placenta, adrenals, liver and other organs. The regulatory effect of hormones and growth factors such as CRH [58,59] and epidermal growth factor [60] on each pathway will be modulated by acute changes in cofactor availability during the process of labour which places strong energy and redox demands on the uterus. Previously reported increases in the estriol/estradiol ratio in maternal plasma occur gradually over several weeks before labour onset [59]. Our findings of a decrease in 16a-hydroxy DHEA 3-sulfate in the cord and maternal plasma and a decrease in estriol 3-sulfate in the maternal plasma of women who laboured may indicate a sudden decrease in production of DHEA-S by the fetal adrenals at the time of or during spontaneous labour. This adds further depth to our knowledge of the complex steroid pathways involved in the spontaneous onset of human labour. In addition, changes in estrogenic metabolites in the maternal and fetal circulations may not necessarily reflect local tissue changes, such as within the myometrium, which may also have a functional effect on uterine contractility [61]. While there are many practical advantages in sampling the peripheral circulation, and our study provides clear cut overall differences in steroid metabolites between labouring and non-labouring women, the limitation of metabolomic studies using peripheral blood is that the dilution effect and the contribution of many organs to steroid metabolism may obscure local changes in individual tissues. Further work is necessary to understand these mechanisms, combining peripheral plasma metabolomics with measurements of enzyme activity in specific tissues. A potential limitation to this study is the relatively low number of participants in each group. However, clear and significant changes in metabolite levels were identified within this pilot group with relatively little noise, demonstrating the reliability of this approach. The platform used is very robust

with 26.9% (222/826) of metabolites significantly changing in maternal plasma and 21.1% (174/826) in cord plasma with labour. The method is unlikely to show false positive results: for example metronidazole was given at the time of knife-to-skin for all women in the elective CS group, but none of the women in the VD group received it; reassuringly, all the maternal and cord plasma of the non-labour group contained metronidazole and none of the labouring group contained it (Tables S3 and S4). Another limitation is the fact that the study is cross sectional, rather than serial, so it is difficult to know whether the changes observed with labour are part of the mechanism of the initiation of labour or a consequence of the stress and metabolic demands of several hours of active labour. Nevertheless, our data show that metabolomic techniques are a powerful approach to identify biochemical changes associated with labour.

5. Conclusions

In this pilot study we have demonstrated a wide range of metabolic changes at the time of delivery between women who laboured spontaneously and women who did not labour. The data will be useful to investigate the biochemical pathways involved in the physiological mechanism of labour in normal term pregnancy and provide a pattern against which pathological changes in preterm labour can be compared. Our results demonstrate an interplay between metabolites in the maternal and fetal circulations which point to the involvement of the endocannabinoid, sphingolipid, ceramide and steroid systems in the mechanism of active labour. Further studies are now warranted, where serial maternal samples are taken throughout pregnancy to determine when these changes take place, especially focusing on the weeks and days immediately prior to spontaneous labour.

Supplementary Materials: The following are available online at http://www.mdpi.com/1660-4601/16/9/1527/s1,

Table S1: Fold-change in cord plasma (VC/EC) for metabolites (xenobiotics excluded) with significant p (≤0.05) and q (≤0.10), ordered according to fold-change (greatest to smallest), with corresponding sub-pathway and super-pathway, Table S2: Fold-change in maternal (intervillous) plasma (VM/EM) for metabolites (xenobiotics excluded) with significant p (≤0.05) and q (≤0.10), ordered according to fold-change (greatest to smallest), with corresponding sub-pathway and super-pathway, Table S3: Fold-change in cord plasma (VC/EC) for xenobiotics with significant p (≤0.05) and q (≤0.10), ordered according to fold-change (greatest to smallest), with corresponding sub-pathway and super-pathway (of note, the fold-change for Metronidazole is recorded as 0 as there was 0 quantity recorded in the VC group, however the p-value is the smallest), Table S4: Fold-change in maternal (intervillous) plasma (VM/EM) for xenobiotics with significant p (≤0.05) and q (≤0.10), ordered according to fold-change (greatest to smallest) (of note, the fold-change for Metronidazole is recorded as 0 as there was 0 quantity recorded in the VC group, however the p-value is the smallest), Table S5: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for sphingolipids, with corresponding p and q values (significant when p ≤ 0.05 and q ≤ 0.10), Table S6: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for pregnenolone steroids, with corresponding p and q values (significant when p ≤ 0.05 and q ≤ 0.10), Table S7: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for progestin steroids, with corresponding p and q values (significant when p ≤ 0.05 and q ≤ 0.10), Table S8: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for endocannabinoid steroids, with corresponding p and q values (significant when p ≤ 0.05 and q ≤ 0.10), Table S9: Fold-change in cord plasma (VC/EC) and maternal (intervillous) plasma (VM/EM) for ceramides, with corresponding p and q values (significant when p ≤ 0.05 and q ≤ 0.10).

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K.A.B., G.I.W. and A.L.B.; Project administration, K.A.B.; Resources, K.A.B., G.I.W. and A.L.B.; Supervision,

G.I.W. and A.L.B.; Validation, K.A.B., G.I.W. and A.L.B.; Visualization, K.A.B.; Writing—original draft, K.A.B.; Writing—review & editing, K.A.B., G.I.W. and A.L.B.

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References

Lopez Bernal, A. Overview. Preterm labour: Mechanisms and management. BMC Pregnancy Childbirth 2007, 7, S2. [CrossRef] [PubMed]

Chang, H.H.; Larson, J.; Blencowe, H.; Spong, C.Y.; Howson, C.P.; Cairns-Smith, S.; Lackritz, E.M.; Lee, S.K.; Mason, E.; Serazin, A.C.; et al. Preventing preterm births: Analysis of trends and potential

reductions with interventions in 39 countries with very high human development index. Lancet 2013, 381, 223–234. [CrossRef]

Lozano, R.; Naghavi, M.; Foreman, K.; Lim, S.; Shibuya, K.; Aboyans, V.; Abraham, J.; Adair, T.; Aggarwal, R.; Ahn, S.Y.; et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010:

A systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012, 380, 2095–2128. [CrossRef]

Middleton, P.; Shepherd, E.; Crowther, C.A. Induction of labour for improving birth outcomes for women at or beyond term. Cochrane Database Syst. Rev. 2018, 5, Cd004945. [CrossRef] [PubMed]

National Collaborating Centre for Women's and Children's Health. National Collaborating Centre for

Women's and Children's Health. National Institute for Health and Care Excellence: Clinical Guidelines. In Preterm Labour and Birth; National Institute for Health and Care Excellence (UK) Copyright (c) 2015; National Collaborating Centre for Women's and Children's Health: London, UK, 2015.

National Collaborating Centre for Women's and Children's Health. National Collaborating Centre for

Women's and Children's Health. National Institute for Health and Care Excellence: Clinical Guidelines. In Intrapartum Care: Care of Healthy Women and Their Babies During Childbirth; National Institute for Health and Care Excellence (UK) Copyright (c) 2014; National Collaborating Centre for Women's and Children's Health: London, UK, 2014.

Menon, R.; Bonney, E.A.; Condon, J.; Mesiano, S.; Taylor, R.N. Novel concepts on pregnancy clocks and alarms: Redundancy and synergy in human parturition. Hum Reprod Update 2016, 22, 535–560. [CrossRef] [PubMed]

National Health Service Digital. NHS Maternity Statistics 2016–2017; Health and Social Care Information Centre: Leeds, UK, 2017.

Grobman, W.A.; Rice, M.M.; Reddy, U.M.; Tita, A.T.N.; Silver, R.M.; Mallett, G.; Hill, K.; Thom, E.A.;

El-Sayed, Y.Y.; Perez-Delboy, A.; et al. Labor Induction versus Expectant Management in Low-Risk Nulliparous Women. N. Engl. J. Med. 2018, 379, 513–523. [CrossRef]

Keirse, M.J. Elective induction, selective deduction, and cesarean section. Birth 2010, 37, 252–256. [CrossRef] [PubMed]

Mealing, N.M.; Roberts, C.L.; Ford, J.B.; Simpson, J.M.; Morris, J.M. Trends in induction of labour, 1998-2007: A population-based study. Aust. N. Z. J. Obstet. Gynaecol. 2009, 49, 599–605. [CrossRef] [PubMed]

National Institute for Clinical and Health Excellence. Inducing Labour Clinical Guideline CG70; National Institute for Clinical and Health Excellence: Manchester, UK, 2008.

Georgiou, H.M.; Di Quinzio, M.K.; Permezel, M.; Brennecke, S.P. Predicting Preterm Labour: Current Status and Future Prospects. Dis. Markers 2015, 2015, 435014. [CrossRef]

Kruit, H.; Heikinheimo, O.; Sorsa, T.; Juhila, J.; Paavonen, J.; Rahkonen, L. Cervical biomarkers as predictors of successful induction of labour by Foley catheter. J. Obstet. Gynaecol. 2018, 1–6. [CrossRef] [PubMed]

Roberts, L.D.; Souza, A.L.; Gerszten, R.E.; Clish, C.B. Targeted metabolomics. Curr. Protoc. Mol. Biol. 2012. [CrossRef] [PubMed]

Lindon, J.C.; Holmes, E.; Nicholson, J.K. Metabonomics in pharmaceutical R&D. FEBS J. 2007, 274, 1140–1151. [CrossRef] [PubMed]

Nicholson, J.K.; Lindon, J.C.; Holmes, E. 'Metabonomics': Understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 1999, 29, 1181–1189. [CrossRef] [PubMed]

Wishart, D.S. Emerging applications of metabolomics in drug discovery and precision medicine. Nat. Rev. Drug Discov. 2016, 15, 473–484. [CrossRef] [PubMed]

Atzori, L.; Barberini, L.; Santoru, M.L.; Antonucci, R.; Fanos, V. Metabolomics explained to perinatologists and pediatricians. J. Matern. Fetal Neonatal Med. 2012, 25, 10–12. [CrossRef]

Caboni, P.; Meloni, A.; Lussu, M.; Carta, E.; Barberini, L.; Noto, A.; Deiana, S.F.; Mereu, R.; Ragusa, A.; Paoletti, A.M.; et al. Urinary metabolomics of pregnant women at term: A combined GC/MS and NMR approach. J. Matern. Fetal Neonatal Med. 2014, 27, 4–12. [CrossRef]

Virgiliou, C.; Gika, H.G.; Witting, M.; Bletsou, A.A.; Athanasiadis, A.; Zafrakas, M.; Thomaidis, N.S.; Raikos, N.; Makrydimas, G.; Theodoridis, G.A. Amniotic Fluid and Maternal Serum Metabolic Signatures in the Second Trimester Associated with Preterm Delivery. J. Proteome Res. 2017, 16, 898–910. [CrossRef]

Wu, Z.; Jin, L.; Zheng, W.; Zhang, C.; Zhang, L.; Chen, Y.; Guan, J.; Fei, H. NMR-based serum metabolomics study reveals a innovative diagnostic model for missed abortion. Biochem. Biophys. Res. Commun. 2018, 496, 679–685. [CrossRef]

Fattuoni, C.; Pietrasanta, C.; Pugni, L.; Ronchi, A.; Palmas, F.; Barberini, L.; Dessi, A.; Pintus, R.; Fanos, V.; Noto, A.; et al. Urinary metabolomic analysis to identify preterm neonates exposed to histological chorioamnionitis: A pilot study. PLoS ONE 2017, 12, e0189120. [CrossRef]

Fotiou, M.; Fotakis, C.; Tsakoumaki, F.; Athanasiadou, E.; Kyrkou, C.; Dimitropoulou, A.; Tsiaka, T.; Chatziioannou, A.C.; Sarafidis, K.; Menexes, G.; et al. (1)H NMR-based metabolomics reveals the effect of maternal habitual dietary patterns on human amniotic fluid profile. Sci. Rep. 2018, 8, 4076. [CrossRef]

Yuan, W.; Heesom, K.; Phillips, R.; Chen, L.; Trinder, J.; Lopez Bernal, A. Low abundance plasma proteins in labour. Reproduction 2012, 144, 505–518. [CrossRef]

Montrose, D.C.; Zhou, X.K.; Kopelovich, L.; Yantiss, R.K.; Karoly, E.D.; Subbaramaiah, K.; Dannenberg, A.J. Metabolic profiling, a noninvasive approach for the detection of experimental colorectal neoplasia. Cancer Prev. Res. 2012, 5, 1358–1367. [CrossRef]

Hiltunen, T.P.; Rimpela, J.M.; Mohney, R.P.; Stirdivant, S.M.; Kontula, K.K. Effects of four different antihypertensive drugs on plasma metabolomic profiles in patients with essential hypertension. PLoS ONE 2017, 12, e0187729. [CrossRef]

Shin, S.Y.; Fauman, E.B.; Petersen, A.K.; Krumsiek, J.; Santos, R.; Huang, J.; Arnold, M.; Erte, I.; Forgetta, V.;

Yang, T.P.; et al. An atlas of genetic influences on human blood metabolites. Nat. Genet. 2014, 46, 543–550. [CrossRef]

Dehaven, C.D.; Evans, A.M.; Dai, H.; Lawton, K.A. Organization of GC/MS and LC/MS metabolomics data into chemical libraries. J. Cheminform. 2010, 2, 9. [CrossRef]

Lehmann, R.; Zhao, X.; Weigert, C.; Simon, P.; Fehrenbach, E.; Fritsche, J.; Machann, J.; Schick, F.; Wang, J.; Hoene, M.; et al. Medium chain acylcarnitines dominate the metabolite pattern in humans under moderate intensity exercise and support lipid oxidation. PLoS ONE 2010, 5, e11519. [CrossRef]

Peake, J.M.; Tan, S.J.; Markworth, J.F.; Broadbent, J.A.; Skinner, T.L.; Cameron-Smith, D. Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise. Am. J. Physiol. Endocrinol. Metab. 2014, 307, E539–E552. [CrossRef]

Yin, P.; Lehmann, R.; Xu, G. Effects of pre-analytical processes on blood samples used in metabolomics studies. Anal. Bioanal. Chem. 2015, 407, 4879–4892. [CrossRef]

Manolopoulos, K.N.; O'Reilly, M.W.; Bujalska, I.J.; Tomlinson, J.W.; Arlt, W. Acute Hypercortisolemia Exerts Depot-Specific Effects on Abdominal and Femoral Adipose Tissue Function. J. Clin. Endocrinol. Metab. 2017, 102, 1091–1101. [CrossRef]

Lindsay, K.L.; Hellmuth, C.; Uhl, O.; Buss, C.; Wadhwa, P.D.; Koletzko, B.; Entringer, S. Longitudinal

Metabolomic Profiling of Amino Acids and Lipids across Healthy Pregnancy. PLoS ONE 2015, 10, e0145794. [CrossRef]

Hill, M.; Paskova, A.; Kanceva, R.; Velikova, M.; Kubatova, J.; Kancheva, L.; Adamcova, K.; Mikesova, M.; Zizka, Z.; Koucky, M.; et al. Steroid profiling in pregnancy: A focus on the human fetus. J. Steroid. Biochem. Mol. Biol. 2014, 139, 201–222. [CrossRef] [PubMed]

Petraglia, F.; Imperatore, A.; Challis, J.R. Neuroendocrine mechanisms in pregnancy and parturition. Endocr. Rev. 2010, 31, 783–816. [CrossRef] [PubMed]

Immenschuh, S.; Vijayan, V.; Janciauskiene, S.; Gueler, F. Heme as a Target for Therapeutic Interventions.

Front. Pharmacol. 2017, 8, 146. [CrossRef] [PubMed]

Rapino, C.; Battista, N.; Bari, M.; Maccarrone, M. Endocannabinoids as biomarkers of human reproduction. Hum. Reprod. Update 2014, 20, 501–516. [CrossRef]

Cianchi, F.; Papucci, L.; Schiavone, N.; Lulli, M.; Magnelli, L.; Vinci, M.C.; Messerini, L.; Manera, C.; Ronconi, E.; Romagnani, P.; et al. Cannabinoid receptor activation induces apoptosis through tumor necrosis factor alpha-mediated ceramide de novo synthesis in colon cancer cells. Clin. Cancer Res. 2008, 14, 7691–7700. [CrossRef] [PubMed]

Marczylo, T.H.; Lam, P.M.; Amoako, A.A.; Konje, J.C. Anandamide levels in human female reproductive tissues: Solid-phase extraction and measurement by ultraperformance liquid chromatography tandem mass spectrometry. Anal. Biochem. 2010, 400, 155–162. [CrossRef]

Maccarrone, M.; Valensise, H.; Bari, M.; Lazzarin, N.; Romanini, C.; Finazzi-Agro, A. Relation between decreased anandamide hydrolase concentrations in human lymphocytes and miscarriage. Lancet 2000, 355, 1326–1329. [CrossRef]

Nallendran, V.; Lam, P.M.; Marczylo, T.H.; Bankart, M.J.; Taylor, A.H.; Taylor, D.J.; Konje, J.C. The plasma levels of the endocannabinoid, anandamide, increase with the induction of labour. Bjog 2010, 117, 863–869. [CrossRef]

Dennedy, M.C.; Friel, A.M.; Houlihan, D.D.; Broderick, V.M.; Smith, T.; Morrison, J.J. Cannabinoids and the human uterus during pregnancy. Am. J. Obstet. Gynecol. 2004, 190, 2–9. [CrossRef]

Melland-Smith, M.; Ermini, L.; Chauvin, S.; Craig-Barnes, H.; Tagliaferro, A.; Todros, T.; Post, M.; Caniggia, I.

Disruption of sphingolipid metabolism augments ceramide-induced autophagy in preeclampsia. Autophagy 2015, 11, 653–669. [CrossRef]

Habayeb, O.M.; Taylor, A.H.; Evans, M.D.; Cooke, M.S.; Taylor, D.J.; Bell, S.C.; Konje, J.C. Plasma levels of the endocannabinoid anandamide in women—A potential role in pregnancy maintenance and labor? J. Clin. Endocrinol. Metab. 2004, 89, 5482–5487. [CrossRef]

Signorelli, P.; Avagliano, L.; Reforgiato, M.R.; Toppi, N.; Casas, J.; Fabrias, G.; Marconi, A.M.; Ghidoni, R.; Caretti, A. De novo ceramide synthesis is involved in acute inflammation during labor. Biol. Chem. 2016, 397, 147–155. [CrossRef]

Keirse, M.J.; Mitchell, M.D.; Turnbull, A.C. Changes in prostaglandin F and

13,14-dihydro-15-keto-prostaglandin F concentrations in amniotic fluid at the onset of and during labour. Br. J. Obstet. Gynaecol. 1977, 84, 743–746. [CrossRef]

Sellers, S.M.; Hodgson, H.T.; Mitchell, M.D.; Anderson, A.B.; Turnbull, A.C. Raised prostaglandin levels in the third stage of labor. Am. J. Obstet. Gynecol. 1982, 144, 209–212. [CrossRef]

Lee, D.C.; Romero, R.; Kim, J.S.; Yoo, W.; Lee, J.; Mittal, P.; Kusanovic, J.P.; Hassan, S.S.; Yoon, B.H.; Kim, C.J. Evidence for a spatial and temporal regulation of prostaglandin-endoperoxide synthase 2 expression in human amnion in term and preterm parturition. J. Clin. Endocrinol. Metab. 2010, 95, E86–E91. [CrossRef]

Kurki, T.; Viinikka, L.; Ylikorkala, O. Urinary excretion of prostacyclin and thromboxane metabolites in threatened preterm labor: Effect of indomethacin and nylidrin. Am. J. Obstet. Gynecol. 1992, 166, 150–154. [CrossRef]

Ylikorkala, O.; Paatero, H.; Suhonen, L.; Viinikka, L. Vaginal and abdominal delivery increases maternal urinary 6-keto-prostaglandin F1 alpha excretion. Br. J. Obstet. Gynaecol. 1986, 93, 950–954. [CrossRef] [PubMed]

Hill, M.; Parizek, A.; Kancheva, R.; Duskova, M.; Velikova, M.; Kriz, L.; Klimkova, M.; Paskova, A.; Zizka, Z.; Matucha, P.; et al. Steroid metabolome in plasma from the umbilical artery, umbilical vein, maternal cubital vein and in amniotic fluid in normal and preterm labor. J. Steroid. Biochem. Mol. Biol. 2010, 121, 594–610. [CrossRef]

Hill, M.; Parizek, A.; Cibula, D.; Kancheva, R.; Jirasek, J.E.; Jirkovska, M.; Velikova, M.; Kubatova, J.; Klimkova, M.; Paskova, A.; et al. Steroid metabolome in fetal and maternal body fluids in human late pregnancy. J. Steroid. Biochem. Mol. Biol. 2010, 122, 114–132. [CrossRef]

Pavlicev, M.; Norwitz, E.R. Human Parturition: Nothing More Than a Delayed Menstruation. Reprod. Sci. 2018, 25, 166–173. [CrossRef]

Phillips, R.J.; Heesom, K.J.; Trinder, J.; Bernal, A.L. Human maternal plasma proteomic changes with parturition. EuPA Open Proteom. 2014, 5, 10–20. [CrossRef]

Diczfalusy, E. Endocrine functions of the human fetoplacental unit. 1964. Am. J. Obstet. Gynecol. 2005, 193,

2024–2025. [CrossRef]

Rock, R.C. Endocrinology of the Fetoplacental Unit: Role of Estrogen Assays. Lab. Med. 1984, 15, 95–97. [CrossRef]

Smith, R.; Butler, T.; Chan, E.-C. Do estrogen receptor variants explain the enigma of human birth? EBioMedicine 2018, 39, 25–26. [CrossRef]

Smith, R.; Smith, J.I.; Shen, X.; Engel, P.J.; Bowman, M.E.; McGrath, S.A.; Bisits, A.M.; McElduff, P.; Giles, W.B.; Smith, D.W. Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor. J. Clin. Endocrinol. Metab. 2009, 94, 2066–2074. [CrossRef]

Hudson, C.A.; McArdle, C.A.; Lopez Bernal, A. Steroid receptor co-activator interacting protein (SIP) mediates EGF-stimulated expression of the prostaglandin synthase COX2 and prostaglandin release in human myometrium. Mol. Hum. Reprod. 2016, 22, 512–525. [CrossRef]

Anamthathmakula, P.; Kyathanahalli, C.; Ingles, J.; Hassan, S.S.; Condon, J.C.; Jeyasuria, P. Estrogen receptor alpha isoform ERdelta7 in myometrium modulates uterine quiescence during pregnancy. EBioMedicine 2019, 39, 520–530. [CrossRef]

APPENDIX 3A1: Copy of patient information leaflet used for the study outlined in Chapters 2, 3 and 5.

http://www.bris.ac.uk/Depts/ObsGyn

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23 September 2016

Patient Information Sheet: Investigating Human Labour

You are being invited to contribute samples for a research study. Before you decide whether you wish to contribute, it is important for you to understand why the research is being done, and what it will involve.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Please read the following information carefully and ask us if there is anything that is unclear, or on which you would like more information. Take time to decide whether you wish to take part.

Part 1:

What is the purpose of the study?
The aim of the project is to identify certain substances in the womb that may trigger labour, in order that we can identify and eventually prevent women from going into premature labour or facilitate induction of labour when indicated.

Why have I been chosen?
The samples you give us will help us understand how labour begins, with the long-term aim of being able to predict and prevent premature labour or to facilitate induction of labour in late pregnancy.

Do I have to take part?
No, you are under no obligation to contribute samples to this study. It is up to you whether you decide to take part. If you do decide to take part, you are free to withdraw your consent at any time. Your delivery will be unaffected whether or not you decide to take part. If you withdraw your consent after samples have been taken, they will be disposed of under strict Trust guidelines which govern the disposal of such samples with the provision that it will not affect your treatment.

What will happen to me if I take part?

We would like to take a small sample from your placenta, once your baby has been delivered. The samples will be about the size of a peanut. We would also like to take blood samples from the cord and a matching sample from you if possible. As the placenta will already be delivered, this process will involve no pain or potential harm to either yourself or your baby. This tissue would normally be disposed of after delivery.
If you happen to require a Caesarean section, we would also like to take a small sample of tissue, again about the size of a peanut, from your womb during the operation. Taking the sample would have no effect on your operation, or subsequent recovery.

What are the possible disadvantages and risks of taking part?
Very rarely taking tissue from the womb will cause a little extra bleeding but there are no long term effects from this. Taking placental and cord blood samples has no known risks.

What are the possible benefits of taking part?
The procedure will be of no direct benefit to you or your baby. However, the samples you give us will help us understand how labour begins, with the long-term aim of being able to predict and prevent preterm labour.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed.

Contact Details:
If you would like to contact a member of our team, if you have any concerns or would like any further information, you can do so by contacting Dr Kate Birchenall

This completes Part 1 of the Information Sheet.
If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2:
Complaints:
If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

Will my taking part in this study be kept confidential?
If you do decide to contribute to the study, the researchers and clinicians will record some details of your delivery (for example the gestational age at delivery and mode of delivery). These details will be collected from your maternity records and are essential to the project, enabling us to distinguish between term and preterm samples, in labour and not in labour samples, for example. Only the researchers involved with the study will have access to this information. Our procedures for handling, processing, storage and destruction of the data are compliant with the Data Protection Act 1998. The data and samples will be given a laboratory number and will be anonymised. The samples, including DNA, may be retained after the study is completed to contribute to further studies on preterm labour, or disposed of under strict Trust and Human Tissue Authority guidelines. The laboratory information gained from the samples will have no effect on any subsequent treatment you may have.

What will happen to any samples I give?
The samples will be analysed in the University of Bristol, but some extracts may be analysed in other centres through existing collaborators with specific methods to investigate the mechanism of human labour.

What will happen to the results of the research study?
You will not be notified of the results of the study. The results will be published in medical journals and presented at national and international scientific meetings and medical conferences. You will not be identified in any report/publication.

Who is organising and funding the research?
Professor López Bernal is leading the research and it is funded by Bristol Research Charities (David Telling Trust; Mother and Baby Trust)

Who has reviewed the study?
This study was given a favourable ethical opinion by the NRES Committee South West – Central Bristol.

Thank you for taking the time to read this information sheet. You may keep this information sheet and if you are happy to be involved in the study you will also be given a copy of the signed consent form.

Patient information sheet Version 10; 23/9/2016

APPENDIX 3A2: Copy of patient consent form used for studies presented in Chapter 2, 3 and 5.

http://www.bris.ac.uk/Depts/ObsGyn

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Patient Consent Form: Investigating Human Labour

Head of Research Project: Professor A. López Bernal
Consultant, Lead for Day Assessment Unit:
Researcher, Dr Kate Birchenall
Research Midwife:

Patient Identifier _____

1. I confirm that I have read the Patient information sheet Version 10 dated 23 September 2016 for the above study. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. If I withdraw my consent after the samples have been taken, they will be disposed of under UHB Trust / HTA guidelines. ☐
3. I give permission for my medical records to be looked at and the information taken from them to be analysed in strict confidence by responsible people from the research team. ☐
4. I agree to take part in the above study. ☐
5. I agree to allow any remaining samples after completion of this study to be retained in an anonymised form for similar studies with ethical approval. ☐

_____	_____	_____
Name of Patient (BLOCK CAPITALS)	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Consent form. Version 6 – 23 September 2016

APPENDIX 3A3: Table presenting metabolites for which the fold-change is significant (p≤0.05) between: 28 and 34 weeks' gestation samples (34/28); 28 weeks' gestation and latent stage samples (Latent/28 weeks); 34 weeks' gestation and latent stage (Latent/34). Corresponding p-values and q-values are shown.

Biochemical Name	Sub Pathway	Super Pathway	34 weeks 28 weeks (fold-change)	p-value	q-value	Latent 28 weeks (fold-change)	p-value	q-value	Latent 34 weeks (fold-change)	p-value	q-value
4-acetamidophenylglucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**22.26**	0.0065	0.0437	**325.10**	0.0003	0.0012	**14.60**	0.0024	0.0066
4-acetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**14.51**	0.0116	0.0654	**480.17**	0.0003	0.0014	**33.08**	0.0029	0.0074
2-hydroxyacetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**9.27**	0.0473	0.1447	**188.77**	0.0002	0.0010	**20.36**	0.0020	0.0060
2-isopropylmalate	Food Component/Plant	Xenobiotics	**5.16**	0.0267	0.1035	**1.56**	0.0200	0.0255			
21-hydroxypregnanolone disulfate	Pregnenolone Steroids	Lipid	**3.03**	0.0003	0.0049	**3.52**	0.0001	0.0008			
2,3-dihydroxyisovalerate	Food Component/Plant	Xenobiotics	**2.91**	0.0259	0.1024	**3.52**	0.0108	0.0160			
octadecadienedioate (C18:2-DC)	Fatty Acid, Dicarboxylate	Lipid	**2.87**	0.0255	0.1018						
5-hydroxyindoleacetate	Tryptophan Metabolism	Amino Acid	**2.74**	0.0216	0.0930						
taurochenodeoxycholic acid 3-sulfate	Secondary Bile Acid Metabolism	Lipid	**2.27**	0.0242	0.1006	**2.36**	0.0009	0.0028	**1.04**	0.0083	0.0147
4-hydroxyglutamate	Glutamate Metabolism	Amino Acid	**2.22**	0.0001	0.0033	**3.09**	0.0000	0.0002	**1.39**	0.0109	0.0177
taurohyocholate	Secondary Bile Acid Metabolism	Lipid	**2.06**	0.0269	0.1035	**3.74**	0.0210	0.0263			
testosterone sulfate	Androgenic Steroids	Lipid	**2.00**	0.0002	0.0034	**7.11**	0.0000	0.0004	**3.56**	0.0001	0.0011
glycohyocholate	Secondary Bile Acid Metabolism	Lipid	**1.91**	0.0007	0.0086	**2.25**	0.0264	0.0314			

isocitric lactone	TCA Cycle	Energy	**1.88**	0.0072	0.0474	**4.20**	0.0031	0.0064	**2.24**	0.0174	0.0244
5alpha-androstan-3alpha,17alpha-diol disulfate	Androgenic Steroids	Lipid	**1.73**	0.0105	0.0604						
oleoyl-oleoyl-glycerol (18:1/18:1) [2]	Diacylglycerol	Lipid	**1.72**	0.0013	0.0121	**2.28**	0.0003	0.0012			
glycochenodeoxycholate 3-sulfate	Primary Bile Acid Metabolism	Lipid	**1.72**	0.0096	0.0580	**2.03**	0.0043	0.0078			
5alpha-pregnan-diol disulfate	Progestin Steroids	Lipid	**1.71**	0.0002	0.0040	**1.98**	0.0020	0.0047			
5alpha-androstan-3alpha,17beta-diol disulfate	Androgenic Steroids	Lipid	**1.71**	0.0127	0.0673	**5.18**	0.0022	0.0049	**3.04**	0.0017	0.0054
phenylalanylhydroxyproline	Dipeptide	Peptide	**1.70**	0.0007	0.0086	**1.68**	0.0208	0.0261			
glycocholate	Primary Bile Acid Metabolism	Lipid	**1.69**	0.0469	0.1441	**3.52**	0.0413	0.0448			
1,3-dimethylurate	Xanthine Metabolism	Xenobiotics	**1.68**	0.0132	0.0680	**1.94**	0.0014	0.0036			
5alpha-pregnan-3beta,20alpha-diol disulfate	Progestin Steroids	Lipid	**1.64**	0.0000	0.0012	**1.66**	0.0002	0.0008			
estriol 3-sulfate	Estrogenic Steroids	Lipid	**1.63**	0.0012	0.0120	**2.57**	0.0019	0.0045	**1.58**	0.0243	0.0294
17alpha-hydroxypregnanolone glucuronide	Pregnenolone Steroids	Lipid	**1.59**	0.0000	0.0004	**3.33**	0.0000	0.0002	**2.10**	0.0001	0.0010
21-hydroxypregnenolone monosulfate (2)	Pregnenolone Steroids	Lipid	**1.56**	0.0050	0.0358	**3.11**	0.0000	0.0001	**1.99**	0.0003	0.0025
tauroursodeoxycholic acid sulfate (1)	Secondary Bile Acid Metabolism	Lipid	**1.56**	0.0376	0.1249	**2.19**	0.0495	0.0515			
dimethylguanidino valeric acid (DMGV)	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**1.55**	0.0036	0.0285	**1.81**	0.0107	0.0160			
pregnanolone/allopregnanolone sulfate	Progestin Steroids	Lipid	**1.54**	0.0002	0.0035	**1.87**	0.0001	0.0006			
estriol 16-glucuronide	Estrogenic Steroids	Lipid	**1.54**	0.0002	0.0040	**3.04**	0.0002	0.0010	**1.97**	0.0005	0.0033

5alpha-pregnan-3beta-ol,20-one sulfate	Progestin Steroids	Lipid	**1.53**	0.0006	0.0073	**1.97**	0.0000	0.0004			
indole-3-carboxylate	Tryptophan Metabolism	Amino Acid	**1.53**	0.0117	0.0654						
progesterone	Progestin Steroids	Lipid	**1.51**	0.0002	0.0034	**1.82**	0.0000	0.0001	**1.21**	0.0014	0.0051
4-cholesten-3-one	Sterol	Lipid	**1.44**	0.0000	0.0003	**1.37**	0.0001	0.0005			
5alpha-pregnan-3beta,20alpha-diol monosulfate (1)	Progestin Steroids	Lipid	**1.43**	0.0000	0.0020	**1.41**	0.0023	0.0050			
andro steroid monosulfate C19H28O6S (1)	Androgenic Steroids	Lipid	**1.43**	0.0006	0.0076	**2.58**	0.0000	0.0001	**1.81**	0.0000	0.0002
3-methylglutaconate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.42**	0.0034	0.0275	**1.60**	0.0028	0.0059			
pro-hydroxy-pro	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**1.41**	0.0000	0.0004	**1.53**	0.0004	0.0016			
sphinganine	Sphingolipid Synthesis	Lipid	**1.41**	0.0230	0.0980	**1.38**	0.0264	0.0314			
glutamine conjugate of C6H10O2 (1)	Partially Characterized Molecules	Partially Characterized Molecules	**1.40**	0.0006	0.0072	**3.08**	0.0000	0.0005	**2.19**	0.0008	0.0038
glycoursodeoxycholic acid sulfate (1)	Secondary Bile Acid Metabolism	Lipid	**1.40**	0.0071	0.0472						
N-stearoyl-sphinganine (d18:0/18:0)	Dihydroceramides	Lipid	**1.39**	0.0284	0.1072	**1.53**	0.0375	0.0415			
17beta-estradiol	Estrogenic Steroids	Lipid	**1.38**	0.0000	0.0003	**1.83**	0.0002	0.0009	**1.33**	0.0105	0.0173
glutamate	Glutamate Metabolism	Amino Acid	**1.38**	0.0083	0.0524						
5alpha-pregnan-3beta,20beta-diol monosulfate (1)	Progestin Steroids	Lipid	**1.37**	0.0000	0.0003	**1.65**	0.0000	0.0000	**1.21**	0.0000	0.0001

2-hydroxyoctanoate	Fatty Acid, Monohydroxy	Lipid	**1.37**	0.0345	0.1205						
pelargonate (9:0)	Medium Chain Fatty Acid	Lipid	**1.36**	0.0002	0.0040	**1.28**	0.0034	0.0068			
N1,N12-diacetylspermine	Polyamine Metabolism	Amino Acid	**1.35**	0.0002	0.0040				**0.66**	0.0000	0.0001
estriol-3-glucuronide	Estrogenic Steroids	Lipid	**1.35**	0.0291	0.1086	**1.78**	0.0123	0.0178			
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	Phosphatidylethanolamine (PE)	Lipid	**1.34**	0.0000	0.0012	**1.58**	0.0000	0.0001	**1.18**	0.0052	0.0114
adenosine	Purine Metabolism, Adenine containing	Nucleotide	**1.33**	0.0157	0.0763	**1.34**	0.0134	0.0190			
estrone 3-sulfate	Estrogenic Steroids	Lipid	**1.33**	0.0393	0.1291	**1.84**	0.0053	0.0092			
isocaproate (i6:0)	Fatty Acid, Branched	Lipid	**1.32**	0.0026	0.0223	**1.86**	0.0001	0.0007	**1.41**	0.0054	0.0118
5,6-dihydrouracil	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.32**	0.0180	0.0834	**1.25**	0.0261	0.0313			
indolin-2-one	Food Component/Plant	Xenobiotics	**1.32**	0.0387	0.1279						
N-acetyl-isoputreanine	Polyamine Metabolism	Amino Acid	**1.31**	0.0000	0.0004	**1.39**	0.0000	0.0003			
adipoylcarnitine (C6-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	**1.31**	0.0005	0.0066	**2.11**	0.0000	0.0005	**1.61**	0.0295	0.0336
GlcNAc sulfate conjugate of C21H34O2 steroid	Partially Characterized Molecules	Partially Characterized Molecules	**1.31**	0.0144	0.0722	**1.55**	0.0275	0.0324			
2-oxoarginine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**1.31**	0.0267	0.1035						

4-hydroxyphenylacetylglutamine	Acetylated Peptides	Peptide	**1.31**	0.0416	0.1330	**1.73**	0.0001	0.0007	**1.32**	0.0291	0.0334
cortisone	Corticosteroids	Lipid	**1.30**	0.0000	0.0003	**1.54**	0.0000	0.0001	**1.19**	0.0411	0.0422
pregnanediol-3-glucuronide	Progestin Steroids	Lipid	**1.30**	0.0000	0.0003	**1.46**	0.0000	0.0003	**1.12**	0.0089	0.0151
3-methylglutarate/2-methylglutarate	Fatty Acid, Dicarboxylate	Lipid	**1.30**	0.0029	0.0242	**1.58**	0.0005	0.0017			
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	Phosphatidylethanolamine (PE)	Lipid	**1.29**	0.0000	0.0003	**1.68**	0.0000	0.0000	**1.30**	0.0000	0.0001
oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	Diacylglycerol	Lipid	**1.29**	0.0041	0.0322	**1.43**	0.0056	0.0095			
20a-dihydroprogesterone	Progestin Steroids	Lipid	**1.28**	0.0001	0.0031	**1.94**	0.0000	0.0002	**1.52**	0.0014	0.0051
ribitol	Pentose Metabolism	Carbohydrate	**1.28**	0.0012	0.0120	**1.37**	0.0001	0.0007			
suberoylcarnitine (C8-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	**1.28**	0.0116	0.0654	**4.63**	0.0000	0.0001	**3.60**	0.0001	0.0014
hexadecasphingosine (d16:1)	Sphingosines	Lipid	**1.28**	0.0328	0.1166				**0.74**	0.0075	0.0140
17alpha-hydroxyprogesterone	Progestin Steroids	Lipid	**1.27**	0.0126	0.0673	**2.12**	0.0010	0.0028	**1.67**	0.0030	0.0077
1-stearoyl-2-oleoyl-GPI (18:0/18:1)	Phosphatidylinositol (PI)	Lipid	**1.25**	0.0001	0.0031	**1.55**	0.0000	0.0005	**1.24**	0.0008	0.0039
oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]	Diacylglycerol	Lipid	**1.25**	0.0327	0.1166	**1.71**	0.0021	0.0049	**1.37**	0.0243	0.0294
4-acetamidobutanoate	Polyamine Metabolism	Amino Acid	**1.25**	0.0415	0.1330	**1.41**	0.0016	0.0041			
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	Phosphatidylethanolamine (PE)	Lipid	**1.24**	0.0000	0.0013	**1.47**	0.0000	0.0001	**1.19**	0.0002	0.0018
5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	Progestin Steroids	Lipid	**1.24**	0.0002	0.0034	**1.63**	0.0000	0.0001	**1.32**	0.0016	0.0053
3-hydroxybutyroylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**1.24**	0.0094	0.0571	**1.97**	0.0009	0.0027	**1.59**	0.0200	0.0265

sphingomyelin (d18:0/18:0, d19:0/17:0)	Dihydrosphingomyelins	Lipid	**1.24**	0.0253	0.1018	**1.14**	0.0023	0.0052			
N-delta-acetylornithine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**1.24**	0.0374	0.1248	**1.53**	0.0102	0.0155			
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	Phosphatidylethanolamine (PE)	Lipid	**1.23**	0.0000	0.0003	**1.55**	0.0000	0.0002	**1.26**	0.0007	0.0036
erythritol	Food Component/Plant	Xenobiotics	**1.23**	0.0245	0.1011	**1.49**	0.0005	0.0019	**1.21**	0.0127	0.0193
quinolinate	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**1.22**	0.0001	0.0031						
aconitate [cis or trans]	TCA Cycle	Energy	**1.22**	0.0130	0.0680	**1.59**	0.0000	0.0001	**1.30**	0.0007	0.0036
N6-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide	**1.21**	0.0054	0.0376						
dodecadienoate (12:2)	Fatty Acid, Dicarboxylate	Lipid	**1.21**	0.0125	0.0673	**1.50**	0.0044	0.0079			
N4-acetylcytidine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**1.21**	0.0298	0.1091	**1.63**	0.0045	0.0082	**1.35**	0.0050	0.0112
sphingosine 1-phosphate	Sphingosines	Lipid	**1.21**	0.0351	0.1206	**1.36**	0.0160	0.0215			
androstenediol (3beta,17beta) disulfate (2)	Androgenic Steroids	Lipid	**1.20**	0.0012	0.0120	**1.84**	0.0013	0.0034	**1.54**	0.0012	0.0049
carotene diol (3)	Vitamin A Metabolism	Cofactors and Vitamins	**1.20**	0.0298	0.1091	**1.36**	0.0005	0.0018	**1.14**	0.0247	0.0297
beta-citrylglutamate	Glutamate Metabolism	Amino Acid	**1.20**	0.0355	0.1206						
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)	Phosphatidylethanolamine (PE)	Lipid	**1.19**	0.0001	0.0031	**1.27**	0.0004	0.0015			

5,6-dihydrothymine	Pyrimidine Metabolism, Thymine containing	Nucleotide	**1.19**	0.0004	0.0053	**1.29**	0.0095	0.0145			
N-acetylneuraminate	Aminosugar Metabolism	Carbohydrate	**1.19**	0.0077	0.0498						
trans-2-hexenoylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**1.19**	0.0259	0.1024	**2.58**	0.0018	0.0044	**2.17**	0.0054	0.0118
1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6)	Phosphatidylethanolamine (PE)	Lipid	**1.18**	0.0001	0.0031	**1.24**	0.0005	0.0018			
hydroxyasparagine	Alanine and Aspartate Metabolism	Amino Acid	**1.18**	0.0004	0.0062	**1.31**	0.0009	0.0027	**1.12**	0.0407	0.0418
(N(1) + N(8))-acetylspermidine	Polyamine Metabolism	Amino Acid	**1.18**	0.0009	0.0103				**0.85**	0.0032	0.0081
1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)	Phosphatidylethanolamine (PE)	Lipid	**1.18**	0.0010	0.0109	**1.18**	0.0304	0.0351			
N-acetylputrescine	Polyamine Metabolism	Amino Acid	**1.18**	0.0012	0.0120	**1.35**	0.0254	0.0307			
cortolone glucuronide (1)	Corticosteroids	Lipid	**1.18**	0.0104	0.0604	**1.70**	0.0003	0.0012	**1.45**	0.0008	0.0039
5-(galactosylhydroxy)-L-lysine	Lysine Metabolism	Amino Acid	**1.17**	0.0012	0.0120	**1.44**	0.0000	0.0003	**1.24**	0.0002	0.0018
16a-hydroxy DHEA 3-sulfate	Androgenic Steroids	Lipid	**1.17**	0.0236	0.0994	**1.66**	0.0001	0.0006	**1.42**	0.0001	0.0010
methionine sulfone	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.17**	0.0443	0.1390	**1.56**	0.0000	0.0005	**1.33**	0.0016	0.0053
N-acetylhistidine	Histidine Metabolism	Amino Acid	**1.16**	0.0023	0.0205	**1.60**	0.0000	0.0001	**1.39**	0.0000	0.0000
N-acetyltaurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.16**	0.0047	0.0344	**1.25**	0.0049	0.0086			

O-sulfo-L-tyrosine	Chemical	Xenobiotics	**1.16**	0.0086	0.0528	**1.34**	0.0001	0.0005	**1.16**	0.0003	0.0025
dimethylglycine	Glycine, Serine and Threonine Metabolism	Amino Acid	**1.15**	0.0002	0.0040	**1.24**	0.0197	0.0252			
C-glycosyltryptophan	Tryptophan Metabolism	Amino Acid	**1.15**	0.0008	0.0093	**1.13**	0.0384	0.0420			
erythronate	Aminosugar Metabolism	Carbohydrate	**1.15**	0.0008	0.0093	**1.39**	0.0000	0.0001	**1.21**	0.0000	0.0002
3-methoxytyrosine	Tyrosine Metabolism	Amino Acid	**1.15**	0.0013	0.0122	**1.31**	0.0002	0.0010	**1.13**	0.0078	0.0141
1-stearoyl-2-oleoyl-GPC (18:0/18:1)	Phosphatidylcholine (PC)	Lipid	**1.15**	0.0213	0.0925	**1.25**	0.0012	0.0034	**1.09**	0.0331	0.0363
alpha-ketoglutaramate	Glutamate Metabolism	Amino Acid	**1.15**	0.0333	0.1178	**1.29**	0.0043	0.0079			
3-(3-amino-3-carboxypropyl)uridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.15**	0.0365	0.1232						
tetradecadienoate (14:2)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.15**	0.0447	0.1390	**2.02**	0.0018	0.0044	**1.76**	0.0486	0.0474
urate	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**1.14**	0.0001	0.0030	**1.35**	0.0000	0.0001	**1.18**	0.0002	0.0018
N2,N2-dimethylguanosine	Purine Metabolism, Guanine containing	Nucleotide	**1.14**	0.0003	0.0040	**1.34**	0.0002	0.0010	**1.17**	0.0181	0.0250
7-methylguanine	Purine Metabolism, Guanine containing	Nucleotide	**1.14**	0.0045	0.0339	**1.46**	0.0000	0.0002	**1.28**	0.0000	0.0002
2S,3R-dihydroxybutyrate	Fatty Acid, Dihydroxy	Lipid	**1.14**	0.0062	0.0423	**1.31**	0.0068	0.0111			

1-palmitoyl-2-oleoyl-GPI (16:0/18:1)	Phosphatidylinositol (PI)	Lipid	**1.14**	0.0103	0.0604	**1.50**	0.0001	0.0005	**1.31**	0.0000	0.0001
sphingomyelin (d18:0/20:0, d16:0/22:0)	Dihydrosphingomyelins	Lipid	**1.14**	0.0203	0.0892						
orotidine	Pyrimidine Metabolism, Orotate containing	Nucleotide	**1.14**	0.0350	0.1206	**1.43**	0.0001	0.0008	**1.25**	0.0002	0.0018
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	Phosphatidylethanolamine (PE)	Lipid	**1.13**	0.0004	0.0062	**1.15**	0.0042	0.0078			
N6-carbamoylthreonyladenosine	Purine Metabolism, Adenine containing	Nucleotide	**1.13**	0.0005	0.0067	**1.40**	0.0000	0.0000	**1.24**	0.0000	0.0006
N2-acetyl,N6,N6-dimethyllysine	Lysine Metabolism	Amino Acid	**1.13**	0.0016	0.0153	**1.29**	0.0018	0.0044			
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)	Phosphatidylethanolamine (PE)	Lipid	**1.13**	0.0027	0.0232	**1.37**	0.0001	0.0005	**1.21**	0.0000	0.0002
3-methylcytidine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**1.13**	0.0178	0.0833						
N-acetylglucosamine/N-acetylgalactosamine	Aminosugar Metabolism	Carbohydrate	**1.12**	0.0005	0.0067	**1.24**	0.0000	0.0000	**1.11**	0.0024	0.0066
N-acetylglutamine	Glutamate Metabolism	Amino Acid	**1.12**	0.0155	0.0761	**1.43**	0.0005	0.0018	**1.27**	0.0038	0.0090
N-palmitoyl-sphinganine (d18:0/16:0)	Dihydroceramides	Lipid	**1.12**	0.0164	0.0773	**1.47**	0.0001	0.0008	**1.31**	0.0064	0.0129
behenoyl dihydrosphingomyelin (d18:0/22:0)	Dihydrosphingomyelins	Lipid	**1.12**	0.0192	0.0868						
pseudouridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.11**	0.0001	0.0031	**1.28**	0.0000	0.0001	**1.16**	0.0002	0.0018
21-hydroxypregnenolone disulfate	Pregnenolone Steroids	Lipid	**1.11**	0.0121	0.0669	**1.71**	0.0008	0.0025	**1.54**	0.0028	0.0074

2-hydroxynervonate	Fatty Acid, Monohydroxy	Lipid	**1.11**	0.0353	0.1206	**1.47**	0.0001	0.0005	**1.32**	0.0001	0.0010
choline	Phospholipid Metabolism	Lipid	**1.10**	0.0026	0.0223						
carotene diol (1)	Vitamin A Metabolism	Cofactors and Vitamins	**1.10**	0.0046	0.0339	**1.24**	0.0002	0.0009	**1.13**	0.0056	0.0119
glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	Hexosylceramides (HCER)	Lipid	**1.10**	0.0223	0.0955	**1.15**	0.0414	0.0449			
gamma-carboxyglutamate	Glutamate Metabolism	Amino Acid	**1.10**	0.0310	0.1130	**1.24**	0.0002	0.0011	**1.13**	0.0123	0.0191
7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	Sterol	Lipid	**1.09**	0.0000	0.0012	**1.28**	0.0001	0.0005	**1.18**	0.0077	0.0141
(S)-3-hydroxybutyrylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**1.09**	0.0164	0.0773	**2.26**	0.0000	0.0005	**2.06**	0.0038	0.0089
flavin adenine dinucleotide (FAD)	Riboflavin Metabolism	Cofactors and Vitamins	**1.09**	0.0193	0.0868						
myo-inositol	Inositol Metabolism	Lipid	**1.09**	0.0248	0.1018	**1.24**	0.0013	0.0034	**1.14**	0.0029	0.0075
N-acetylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.09**	0.0368	0.1238	**1.17**	0.0137	0.0192			
4-hydroxychlorothalonil	Chemical	Xenobiotics	**1.08**	0.0084	0.0524	**1.16**	0.0049	0.0086	**1.07**	0.0231	0.0290
5,6-dihydrouridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.08**	0.0105	0.0604	**1.22**	0.0000	0.0003	**1.12**	0.0012	0.0049
cis-4-decenoate (10:1n6)	Medium Chain Fatty Acid	Lipid	**1.08**	0.0186	0.0859	**1.66**	0.0005	0.0018	**1.53**	0.0438	0.0442
1-methyl-4-imidazoleacetate	Histidine Metabolism	Amino Acid	**1.08**	0.0288	0.1082	**1.30**	0.0000	0.0002	**1.20**	0.0006	0.0034
succinylcarnitine (C4-DC)	TCA Cycle	Energy	**1.08**	0.0341	0.1199	**1.27**	0.0001	0.0005	**1.18**	0.0002	0.0018
1-stearoyl-2-linoleoyl-GPI (18:0/18:2)	Phosphatidylinositol (PI)	Lipid	**1.07**	0.0161	0.0773	**1.13**	0.0122	0.0178			

5-hydroxylysine	Lysine Metabolism	Amino Acid	**1.07**	0.0262	0.1027						
carotene diol (2)	Vitamin A Metabolism	Cofactors and Vitamins	**1.07**	0.0320	0.1158	**1.19**	0.0002	0.0009	**1.11**	0.0067	0.0132
citrate	TCA Cycle	Energy	**1.07**	0.0456	0.1408	**1.17**	0.0004	0.0015			
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.06**	0.0126	0.0673	**1.36**	0.0000	0.0001	**1.28**	0.0000	0.0002
N-acetylserine	Glycine, Serine and Threonine Metabolism	Amino Acid	**1.06**	0.0139	0.0707	**1.21**	0.0010	0.0029	**1.14**	0.0018	0.0055
dimethylarginine (SDMA + ADMA)	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**1.06**	0.0213	0.0925						
1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	Phosphatidylcholine (PC)	Lipid	**1.06**	0.0237	0.0996	**1.16**	0.0016	0.0040	**1.09**	0.0015	0.0052
N6,N6-dimethyllysine	Lysine Metabolism	Amino Acid	**1.05**	0.0032	0.0261						
N-acetylalanine	Alanine and Aspartate Metabolism	Amino Acid	**1.05**	0.0098	0.0587	**1.20**	0.0001	0.0008	**1.14**	0.0003	0.0023
alpha-tocopherol	Tocopherol Metabolism	Cofactors and Vitamins	**1.05**	0.0153	0.0756	**1.10**	0.0082	0.0129			
2-O-methylascorbic acid	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**1.05**	0.0423	0.1346	**1.27**	0.0001	0.0007	**1.20**	0.0004	0.0028
glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	Hexosylceramides (HCER)	Lipid	**1.02**	0.0322	0.1159						
glutamine conjugate of C6H10O2 (2)	Partially Characterized Molecules	Partially Characterized Molecules	**0.99**	0.0132	0.0680	**3.99**	0.0000	0.0004	**4.03**	0.0009	0.0039
carnitine	Carnitine Metabolism	Lipid	**0.93**	0.0017	0.0158	**0.84**	0.0002	0.0010	**0.90**	0.0211	0.0274

1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)	Plasmalogen	Lipid	**0.92**	0.0035	0.0280				**1.15**	0.0181	0.0249
sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)	Sphingomyelins	Lipid	**0.92**	0.0126	0.0673	**0.88**	0.0000	0.0003			
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)	Phosphatidylcholine (PC)	Lipid	**0.92**	0.0162	0.0773	**0.92**	0.0405	0.0442			
propionylcarnitine (C3)	Fatty Acid Metabolism (also BCAA Metabolism)	Lipid	**0.92**	0.0254	0.1018						
tryptophan	Tryptophan Metabolism	Amino Acid	**0.91**	0.0027	0.0230	**0.82**	0.0081	0.0129			
3,5-dichloro-2,6-dihydroxybenzoic acid	Chemical	Xenobiotics	**0.90**	0.0002	0.0034	**0.84**	0.0001	0.0005	**0.93**	0.0013	0.0050
1,5-anhydroglucitol (1,5-AG)	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.90**	0.0010	0.0108						
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)	Plasmalogen	Lipid	**0.90**	0.0145	0.0724						
sphingomyelin (d18:2/14:0, d18:1/14:1)	Sphingomyelins	Lipid	**0.90**	0.0189	0.0865				**1.10**	0.0268	0.0315
orotate	Pyrimidine Metabolism, Orotate containing	Nucleotide	**0.90**	0.0295	0.1091						
perfluorooctanoate (PFOA)	Chemical	Xenobiotics	**0.89**	0.0001	0.0031	**0.73**	0.0000	0.0004	**0.82**	0.0024	0.0066
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P-16:0/20:4)	Plasmalogen	Lipid	**0.89**	0.0053	0.0375						
N-formylanthranilic acid	Tryptophan Metabolism	Amino Acid	**0.89**	0.0200	0.0892	**0.84**	0.0298	0.0346			
1-palmitoyl-GPC (16:0)	Lysophospholipid	Lipid	**0.89**	0.0252	0.1018	1.04	0.8760	0.3978			
retinol (Vitamin A)	Vitamin A Metabolism	Cofactors and Vitamins	**0.88**	0.0002	0.0035	**0.80**	0.0007	0.0023			

perfluorooctanesulfonate (PFOS)	Chemical	Xenobiotics	**0.88**	0.0045	0.0339	**0.79**	0.0000	0.0001	**0.90**	0.0083	0.0147
sphingomyelin (d18:2/24:2)	Sphingomyelins	Lipid	**0.88**	0.0085	0.0528	**0.84**	0.0000	0.0001			
1,2-dilinoleoyl-GPC (18:2/18:2)	Phosphatidylcholine (PC)	Lipid	**0.88**	0.0284	0.1072				**1.12**	0.0402	0.0416
pregnenediol sulfate (C21H34O5S)	Pregnenolone Steroids	Lipid	**0.87**	0.0049	0.0357						
urea	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.87**	0.0355	0.1206				**1.36**	0.0001	0.0010
androsterone sulfate	Androgenic Steroids	Lipid	**0.86**	0.0046	0.0339	**0.85**	0.0082	0.0129			
1-lignoceroyl-GPC (24:0)	Lysophospholipid	Lipid	**0.86**	0.0075	0.0493	**0.84**	0.0085	0.0132			
dehydroepiandrosterone sulfate (DHEA-S)	Androgenic Steroids	Lipid	**0.84**	0.0001	0.0027	**0.91**	0.0192	0.0248			
1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)	Plasmalogen	Lipid	**0.84**	0.0006	0.0076				**1.13**	0.0057	0.0119
1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1)	Plasmalogen	Lipid	**0.84**	0.0018	0.0166						
1-(1-enyl-palmitoyl)-GPC (P-16:0)	Lysoplasmalogen	Lipid	**0.84**	0.0397	0.1291						
androstenediol (3alpha, 17alpha) monosulfate (3)	Androgenic Steroids	Lipid	**0.83**	0.0054	0.0376				**1.27**	0.0160	0.0229
3-phenylpropionate (hydrocinnamate)	Benzoate Metabolism	Xenobiotics	**0.83**	0.0203	0.0892	**0.49**	0.0141	0.0196			
2-palmitoleoyl-GPC (16:1)	Lysophospholipid	Lipid	**0.83**	0.0447	0.1390				**1.42**	0.0122	0.0191
1-cerotoyl-GPC (26:0)	Lysophospholipid	Lipid	**0.82**	0.0427	0.1351	**0.70**	0.0010	0.0028	**0.85**	0.0078	0.0141
ceramide (d18:1/14:0, d16:1/16:0)	Ceramides	Lipid	**0.81**	0.0395	0.1291				**1.29**	0.0217	0.0280
androstenediol (3beta,17beta) monosulfate (1)	Androgenic Steroids	Lipid	**0.80**	0.0001	0.0024						

epiandrosterone sulfate	Androgenic Steroids	Lipid	**0.78**	0.0003	0.0050	**0.76**	0.0033	0.0067			
homocitrulline	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.77**	0.0082	0.0524						
indolepropionate	Tryptophan Metabolism	Amino Acid	**0.76**	0.0140	0.0707	**0.73**	0.0165	0.0219			
androstenediol (3beta,17beta) monosulfate (2)	Androgenic Steroids	Lipid	**0.68**	0.0280	0.1071				**2.57**	0.0020	0.0059
4-methylbenzenesulfonate	Chemical	Xenobiotics	**0.67**	0.0406	0.1313						
o-cresol sulfate	Benzoate Metabolism	Xenobiotics	**0.63**	0.0448	0.1390						
4-acetamidophenol	Drug - Analgesics, Anesthetics	Xenobiotics				**260.84**	0.0004	0.0017	**46.73**	0.0019	0.0057
2-methoxyacetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics				**119.11**	0.0003	0.0014	**39.44**	0.0015	0.0052
2-methoxyacetaminophen glucuronide	Drug - Analgesics, Anesthetics	Xenobiotics				**110.11**	0.0004	0.0015	**19.80**	0.0015	0.0052
3-(cystein-S-yl)acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics				**89.58**	0.0003	0.0014	**27.52**	0.0017	0.0053
3-(N-acetyl-L-cystein-S-yl) acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics				**76.33**	0.0003	0.0013	**20.98**	0.0015	0.0052
lidocaine	Drug - Analgesics, Anesthetics	Xenobiotics				**66.54**	0.0358	0.0399	**66.54**	0.0364	0.0388
3-(methylthio)acetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics				**18.41**	0.0007	0.0024	**10.73**	0.0013	0.0049
3-hydroxyoleate	Fatty Acid, Monohydroxy	Lipid				**6.59**	0.0214	0.0268	**7.44**	0.0101	0.0167
propyl 4-hydroxybenzoate sulfate	Benzoate Metabolism	Xenobiotics				**6.12**	0.0030	0.0064	**19.51**	0.0003	0.0023
docosatrienoate (22:3n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid				**6.05**	0.0024	0.0053	**11.34**	0.0016	0.0053

glutamine conjugate of C7H12O2	Partially Characterized Molecules	Partially Characterized Molecules	**4.70**	0.0005	0.0017	**5.48**	0.0015	0.0052
methyl-4-hydroxybenzoate sulfate	Benzoate Metabolism	Xenobiotics	**4.39**	0.0188	0.0244	**64.62**	0.0173	0.0243
3-hydroxybutyrate (BHBA)	Ketone Bodies	Lipid	**4.28**	0.0002	0.0011	**4.03**	0.0008	0.0038
taurocholate	Primary Bile Acid Metabolism	Lipid	**3.95**	0.0106	0.0160			
tyramine O-sulfate	Tyrosine Metabolism	Amino Acid	**3.46**	0.0423	0.0456			
palmitoleate (16:1n7)	Long Chain Monounsaturated Fatty Acid	Lipid	**3.42**	0.0007	0.0023	**3.33**	0.0061	0.0124
laurate (12:0)	Medium Chain Fatty Acid	Lipid	**3.25**	0.0011	0.0033	**2.30**	0.0122	0.0191
hexadecadienoate (16:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**3.24**	0.0005	0.0018	**2.88**	0.0073	0.0138
10-heptadecenoate (17:1n7)	Long Chain Monounsaturated Fatty Acid	Lipid	**3.19**	0.0003	0.0014	**3.24**	0.0021	0.0060
stearidonate (18:4n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**3.05**	0.0034	0.0068	**3.07**	0.0067	0.0132
myristoleate (14:1n5)	Long Chain Monounsaturated Fatty Acid	Lipid	**2.91**	0.0018	0.0044	**2.92**	0.0088	0.0150
linolenate [alpha or gamma; (18:3n3 or 6)]	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.90**	0.0009	0.0028	**2.49**	0.0086	0.0148
eicosenoate (20:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**2.87**	0.0001	0.0006	**2.51**	0.0007	0.0036

oleate/vaccenate (18:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**2.79**	0.0002	0.0009	**2.62**	0.0024	0.0066
hexanoylglutamine	Fatty Acid Metabolism (Acyl Glutamine)	Lipid	**2.77**	0.0003	0.0014	**3.32**	0.0004	0.0028
myristate (14:0)	Long Chain Saturated Fatty Acid	Lipid	**2.69**	0.0003	0.0013	**2.73**	0.0017	0.0053
branched-chain, straight-chain, or cyclopropyl 12:1 fatty acid	Partially Characterized Molecules	Partially Characterized Molecules	**2.62**	0.0013	0.0034			
3-hydroxyoleoylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**2.60**	0.0022	0.0050	**3.25**	0.0061	0.0124
linoleate (18:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.59**	0.0004	0.0017	**2.31**	0.0073	0.0138
10-nonadecenoate (19:1n9)	Long Chain Monounsaturated Fatty Acid	Lipid	**2.56**	0.0004	0.0015	**2.67**	0.0009	0.0039
(R)-3-hydroxybutyrylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**2.49**	0.0006	0.0019	**2.67**	0.0006	0.0034
2-butenoylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**2.44**	0.0003	0.0014	**2.36**	0.0004	0.0028
erucate (22:1n9)	Long Chain Monounsaturated Fatty Acid	Lipid	**2.44**	0.0004	0.0016	**1.68**	0.0019	0.0057
9-hydroxystearate	Fatty Acid, Monohydroxy	Lipid	**2.38**	0.0219	0.0273	**2.04**	0.0376	0.0395

(12 or 13)-methylmyristate (a15:0 or i15:0)	Fatty Acid, Branched	Lipid	**2.33**	0.0012	0.0034	**2.76**	0.0004	0.0028
5-dodecenoate (12:1n7)	Medium Chain Fatty Acid	Lipid	**2.33**	0.0013	0.0035	**2.30**	0.0097	0.0162
palmitate (16:0)	Long Chain Saturated Fatty Acid	Lipid	**2.32**	0.0002	0.0008	**2.27**	0.0012	0.0049
sucrose	Disaccharides and Oligosaccharides	Carbohydrate	**2.27**	0.0203	0.0257	**1.92**	0.0328	0.0361
17alpha-hydroxypregnenolone 3-sulfate	Pregnenolone Steroids	Lipid	**2.27**	0.0321	0.0363			
docosatrienoate (22:3n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.26**	0.0221	0.0274	**2.87**	0.0146	0.0213
(14 or 15)-methylpalmitate (a17:0 or i17:0)	Fatty Acid, Branched	Lipid	**2.20**	0.0004	0.0015	**2.27**	0.0004	0.0027
1-palmitoleoylglycerol (16:1)	Monoacylglycerol	Lipid	**2.17**	0.0085	0.0132	**2.06**	0.0029	0.0074
3-methylhistidine	Histidine Metabolism	Amino Acid	**2.17**	0.0366	0.0405			
5alpha-androstan-3beta,17beta-diol disulfate	Androgenic Steroids	Lipid	**2.15**	0.0183	0.0239	**2.12**	0.0025	0.0067
inosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**2.14**	0.0133	0.0189	**1.37**	0.0494	0.0476
margarate (17:0)	Long Chain Saturated Fatty Acid	Lipid	**2.11**	0.0001	0.0008	**2.06**	0.0007	0.0036
dihomo-linoleate (20:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.11**	0.0012	0.0034	**2.16**	0.0032	0.0081
N-acetyl-1-methylhistidine	Histidine Metabolism	Amino Acid	**2.10**	0.0007	0.0023	**1.68**	0.0016	0.0053

3-hydroxylaurate	Fatty Acid, Monohydroxy	Lipid	**2.09**	0.0001	0.0005	**2.10**	0.0042	0.0097
docosadienoate (22:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.08**	0.0006	0.0022	**1.97**	0.0008	0.0038
N2,N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**2.08**	0.0075	0.0122	**1.98**	0.0218	0.0280
palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]	Diacylglycerol	Lipid	**2.07**	0.0024	0.0052	**1.59**	0.0355	0.0382
2-oleoylglycerol (18:1)	Monoacylglycerol	Lipid	**2.07**	0.0064	0.0108			
hypoxanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**2.06**	0.0001	0.0005	**2.12**	0.0000	0.0001
linoleoyl ethanolamide	Endocannabinoid	Lipid	**2.06**	0.0002	0.0008	**2.01**	0.0035	0.0086
succinoyltaurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**2.05**	0.0023	0.0051	**1.98**	0.0015	0.0052
8-methoxykynurenate	Tryptophan Metabolism	Amino Acid	**2.04**	0.0036	0.0070			
docosapentaenoate (n3 DPA; 22:5n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.03**	0.0041	0.0078	**2.19**	0.0055	0.0118
cysteine s-sulfate	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**2.02**	0.0001	0.0007	**1.85**	0.0002	0.0019
4-methylhexanoylglutamine	Fatty Acid Metabolism (Acyl Glutamine)	Lipid	**2.00**	0.0139	0.0194	**2.24**	0.0070	0.0135

glycerol	Glycerolipid Metabolism	Lipid	**1.98**	0.0001	0.0005	**1.75**	0.0130	0.0196
(16 or 17)-methylstearate (a19:0 or i19:0)	Fatty Acid, Branched	Lipid	**1.98**	0.0009	0.0028	**1.93**	0.0004	0.0028
3-hydroxykynurenine	Tryptophan Metabolism	Amino Acid	**1.98**	0.0290	0.0339			
corticosterone	Corticosteroids	Lipid	**1.97**	0.0267	0.0316			
adrenate (22:4n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.95**	0.0088	0.0135	**1.67**	0.0406	0.0418
decadienedioic acid (C10:2-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.94**	0.0058	0.0099	**2.28**	0.0292	0.0334
mead acid (20:3n9)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.93**	0.0008	0.0026	**1.89**	0.0025	0.0068
formiminoglutamate	Histidine Metabolism	Amino Acid	**1.93**	0.0011	0.0031	**2.33**	0.0000	0.0003
nicotinamide riboside	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**1.91**	0.0045	0.0081			
pentose acid	Partially Characterized Molecules	Partially Characterized Molecules	**1.86**	0.0196	0.0251			
16-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid	**1.84**	0.0003	0.0013	**1.75**	0.0018	0.0056
1-methylguanidine	Guanidino and Acetamido Metabolism	Amino Acid	**1.84**	0.0131	0.0189	**1.61**	0.0429	0.0435
1-linoleoyl-GPA (18:2)	Lysophospholipid	Lipid	**1.82**	0.0018	0.0044	**1.30**	0.0045	0.0100
3-hydroxymyristate	Fatty Acid, Monohydroxy	Lipid	**1.80**	0.0083	0.0129	**1.86**	0.0036	0.0086

5alpha-androstan-3alpha,17alpha-diol monosulfate	Androgenic Steroids	Lipid	**1.80**	0.0203	0.0257	**1.60**	0.0038	0.0090
theophylline	Xanthine Metabolism	Xenobiotics	**1.79**	0.0009	0.0027			
androstenediol (3alpha, 17alpha) monosulfate (2)	Androgenic Steroids	Lipid	**1.77**	0.0089	0.0136	**1.47**	0.0061	0.0124
androstenediol (3beta,17beta) disulfate (1)	Androgenic Steroids	Lipid	**1.76**	0.0012	0.0034	**1.82**	0.0001	0.0014
arachidate (20:0)	Long Chain Saturated Fatty Acid	Lipid	**1.75**	0.0000	0.0003	**1.61**	0.0001	0.0010
5-dodecenoylcarnitine (C12:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.75**	0.0015	0.0039	**1.90**	0.0123	0.0191
lignoceroylcarnitine (C24)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.74**	0.0001	0.0006	**1.69**	0.0004	0.0028
octadecenedioylcarnitine (C18:1-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	**1.74**	0.0008	0.0025	**1.60**	0.0022	0.0062
caprate (10:0)	Medium Chain Fatty Acid	Lipid	**1.74**	0.0055	0.0094	**2.21**	0.0009	0.0039
nonadecanoate (19:0)	Long Chain Saturated Fatty Acid	Lipid	**1.73**	0.0001	0.0005	**1.66**	0.0001	0.0013
stearate (18:0)	Long Chain Saturated Fatty Acid	Lipid	**1.72**	0.0001	0.0008	**1.64**	0.0003	0.0023
myristoleoylcarnitine (C14:1)	Fatty Acid Metabolism (Acyl Carnitine,	Lipid	**1.72**	0.0022	0.0049	**1.98**	0.0359	0.0385

	Monounsaturated)							
3-hydroxyhexanoylcarnitine (1)	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**1.72**	0.0034	0.0068	**1.61**	0.0323	0.0357
lactose	Disaccharides and Oligosaccharides	Carbohydrate	**1.71**	0.0025	0.0054	**1.53**	0.0001	0.0013
13-HODE + 9-HODE	Fatty Acid, Monohydroxy	Lipid	**1.71**	0.0308	0.0352	**1.83**	0.0197	0.0263
oleoyl-arachidonoyl-glycerol (18:1/20:4) [1]	Diacylglycerol	Lipid	**1.70**	0.0011	0.0031	**1.45**	0.0126	0.0193
(2 or 3)-decenoate (10:1n7 or n8)	Medium Chain Fatty Acid	Lipid	**1.70**	0.0291	0.0339			
1,3,7-trimethylurate	Xanthine Metabolism	Xenobiotics	**1.69**	0.0002	0.0011			
pentadecanoate (15:0)	Long Chain Saturated Fatty Acid	Lipid	**1.69**	0.0002	0.0011	**1.78**	0.0006	0.0035
nisinate (24:6n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.66**	0.0043	0.0078	**1.81**	0.0303	0.0341
21-hydroxypregnenolone monosulfate (1)	Pregnenolone Steroids	Lipid	**1.66**	0.0084	0.0131	**1.50**	0.0130	0.0196
eicosapentaenoate (EPA; 20:5n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.64**	0.0013	0.0035	**1.83**	0.0057	0.0119
1-oleoylglycerol (18:1)	Monoacylglycerol	Lipid	**1.64**	0.0024	0.0052			
palmitoleoylcarnitine (C16:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.63**	0.0012	0.0034	**1.84**	0.0024	0.0066

tetradecadienedioate (C14:2-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.63**	0.0021	0.0048	**1.60**	0.0341	0.0372
undecanedioate (C11-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.63**	0.0432	0.0463			
gulonate	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**1.62**	0.0005	0.0018	**1.36**	0.0006	0.0034
hexanoylcarnitine (C6)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	**1.62**	0.0008	0.0025	**1.63**	0.0076	0.0140
N6-succinyladenosine	Purine Metabolism, Adenine containing	Nucleotide	**1.62**	0.0017	0.0041	**1.51**	0.0421	0.0428
dihomo-linolenate (20:3n3 or n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.62**	0.0041	0.0077	**1.68**	0.0070	0.0135
1,7-dimethylurate	Xanthine Metabolism	Xenobiotics	**1.61**	0.0166	0.0220			
docosapentaenoate (n6 DPA; 22:5n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.59**	0.0031	0.0064	**1.66**	0.0084	0.0147
dodecenedioate (C12:1-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.57**	0.0016	0.0040			
2-hydroxybehenate	Fatty Acid, Monohydroxy	Lipid	**1.56**	0.0001	0.0007	**1.41**	0.0001	0.0014
cyclo(phe-pro)	Dipeptide	Peptide	**1.56**	0.0034	0.0068	**1.14**	0.0152	0.0220
1-methylhistidine	Histidine Metabolism	Amino Acid	**1.56**	0.0062	0.0104	**1.35**	0.0461	0.0460
3-hydroxyhexanoate	Fatty Acid, Monohydroxy	Lipid	**1.55**	0.0031	0.0064	**1.57**	0.0318	0.0356
cysteinylglycine	Glutathione Metabolism	Amino Acid	**1.55**	0.0035	0.0068	**1.37**	0.0080	0.0144

3-methylxanthine	Xanthine Metabolism	Xenobiotics	**1.55**	0.0062	0.0105			
1-palmitoyl-GPA (16:0)	Lysophospholipid	Lipid	**1.55**	0.0082	0.0129	**1.26**	0.0042	0.0097
3-methylglutarylcarnitine (2)	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.53**	0.0018	0.0044	**1.20**	0.0283	0.0327
3-hydroxyisobutyrate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.53**	0.0063	0.0106	**1.59**	0.0009	0.0039
pregnenetriol disulfate	Pregnenolone Steroids	Lipid	**1.53**	0.0360	0.0399	**1.35**	0.0162	0.0229
cystine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.53**	0.0488	0.0510	**1.38**	0.0191	0.0259
palmitoyl ethanolamide	Endocannabinoid	Lipid	**1.52**	0.0007	0.0023	**1.42**	0.0020	0.0059
caffeine	Xanthine Metabolism	Xenobiotics	**1.52**	0.0054	0.0094			
behenoylcarnitine (C22)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.52**	0.0078	0.0126	**1.54**	0.0028	0.0073
3-hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid	**1.51**	0.0001	0.0005	**1.98**	0.0013	0.0049
ethylmalonate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.49**	0.0001	0.0006	**1.36**	0.0003	0.0025
glucuronate	Aminosugar Metabolism	Carbohydrate	**1.49**	0.0008	0.0025	**1.40**	0.0006	0.0034
2-hydroxybutyrate/2-hydroxyisobutyrate	Glutathione Metabolism	Amino Acid	**1.49**	0.0023	0.0051	**1.40**	0.0076	0.0140

suberate (C8-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.49**	0.0035	0.0068	**1.56**	0.0013	0.0049
1,2-dipalmitoyl-GPE (16:0/16:0)	Phosphatidylethanolamine (PE)	Lipid	**1.49**	0.0066	0.0110	**1.70**	0.0009	0.0039
N-oleoyltaurine	Endocannabinoid	Lipid	**1.48**	0.0160	0.0215			
octadecanedioate (C18-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.47**	0.0487	0.0510			
pregnenolone sulfate	Pregnenolone Steroids	Lipid	**1.45**	0.0015	0.0039	**1.37**	0.0235	0.0290
1-oleoyl-GPI (18:1)	Lysophospholipid	Lipid	**1.45**	0.0016	0.0040	**1.25**	0.0492	0.0476
N-acetyltheanine	Food Component/Plant	Xenobiotics	**1.45**	0.0296	0.0345	**1.35**	0.0480	0.0470
7-methylxanthine	Xanthine Metabolism	Xenobiotics	**1.45**	0.0435	0.0465			
N-carbamoylaspartate	Pyrimidine Metabolism, Orotate containing	Nucleotide	**1.44**	0.0065	0.0109			
laurylcarnitine (C12)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	**1.44**	0.0240	0.0292			
ximenoylcarnitine (C26:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.43**	0.0007	0.0024	**1.37**	0.0094	0.0157
gamma-glutamylglutamate	Gamma-glutamyl Amino Acid	Peptide	**1.43**	0.0020	0.0047	**1.16**	0.0153	0.0221
arabonate/xylonate	Pentose Metabolism	Carbohydrate	**1.42**	0.0306	0.0351	**1.38**	0.0010	0.0042
nonanoylcarnitine (C9)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	**1.42**	0.0416	0.0450			

docosahexaenoate (DHA; 22:6n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.41**	0.0039	0.0074	**1.45**	0.0183	0.0251
oleoyl ethanolamide	Endocannabinoid	Lipid	**1.41**	0.0053	0.0092	**1.33**	0.0057	0.0119
lanthionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.41**	0.0138	0.0193	**1.51**	0.0042	0.0097
N-stearoyltaurine	Endocannabinoid	Lipid	**1.40**	0.0032	0.0066			
myristoylcarnitine (C14)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.40**	0.0067	0.0110	**1.59**	0.0054	0.0118
N-acetyl-4-chlorophenylalanine	Chemical	Xenobiotics	**1.40**	0.0095	0.0145			
hexadecanedioate (C16-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.40**	0.0107	0.0160	**1.31**	0.0158	0.0227
sphinganine-1-phosphate	Sphingolipid Synthesis	Lipid	**1.39**	0.0274	0.0324			
fumarate	TCA Cycle	Energy	**1.38**	0.0028	0.0059	**1.27**	0.0161	0.0229
1-ribosyl-imidazoleacetate	Histidine Metabolism	Amino Acid	**1.38**	0.0050	0.0087	**1.33**	0.0001	0.0014
pyruvate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**1.38**	0.0117	0.0171			
2-ketocaprylate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.38**	0.0131	0.0189			
N-palmitoylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**1.38**	0.0152	0.0207	**1.39**	0.0085	0.0148

1-carboxyethylphenylalanine	Phenylalanine Metabolism	Amino Acid	**1.38**	0.0159	0.0215			
margaroylcarnitine (C17)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.38**	0.0305	0.0351	**1.44**	0.0381	0.0398
N-acetylleucine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.38**	0.0319	0.0361	**1.45**	0.0235	0.0290
eicosenoylcarnitine (C20:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.37**	0.0014	0.0037	**1.55**	0.0023	0.0065
1-carboxyethyltyrosine	Tyrosine Metabolism	Amino Acid	**1.37**	0.0182	0.0239			
cytosine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**1.37**	0.0454	0.0483	**1.56**	0.0236	0.0290
N-acetylproline	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**1.36**	0.0001	0.0007			
oleoylcarnitine (C18:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.36**	0.0019	0.0044	**1.52**	0.0011	0.0047
octadecanedioylcarnitine (C18-DC)	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	**1.36**	0.0039	0.0074	**1.30**	0.0475	0.0468
1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)	Phosphatidylcholine (PC)	Lipid	**1.35**	0.0016	0.0040	**1.30**	0.0034	0.0082

1-linoleoylglycerol (18:2)	Monoacylglycerol	Lipid	**1.35**	0.0193	0.0248			
hydroxy-N6,N6,N6-trimethyllysine	Lysine Metabolism	Amino Acid	**1.34**	0.0039	0.0074	**1.25**	0.0003	0.0022
N-stearoyl-sphingosine (d18:1/18:0)	Ceramides	Lipid	**1.33**	0.0001	0.0005	**1.14**	0.0142	0.0210
arabitol/xylitol	Pentose Metabolism	Carbohydrate	**1.33**	0.0003	0.0014	**1.19**	0.0124	0.0191
branched-chain, straight-chain, or cyclopropyl 10:1 fatty acid (1)	Partially Characterized Molecules	Partially Characterized Molecules	**1.33**	0.0288	0.0339	**2.25**	0.0005	0.0033
malonate	Fatty Acid Synthesis	Lipid	**1.32**	0.0004	0.0015	**1.17**	0.0108	0.0176
arachidonate (20:4n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**1.32**	0.0132	0.0189			
1-linoleoyl-GPI (18:2)	Lysophospholipid	Lipid	**1.32**	0.0193	0.0248			
4-hydroxyphenylpyruvate	Tyrosine Metabolism	Amino Acid	**1.32**	0.0256	0.0308	**1.40**	0.0352	0.0380
valylglycine	Dipeptide	Peptide	**1.32**	0.0438	0.0467	**1.33**	0.0123	0.0191
N-acetylphenylalanine	Phenylalanine Metabolism	Amino Acid	**1.31**	0.0006	0.0019	**1.20**	0.0083	0.0147
lactate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**1.31**	0.0107	0.0160			
N-acetylcarnosine	Histidine Metabolism	Amino Acid	**1.31**	0.0177	0.0233	**1.24**	0.0179	0.0248
alpha-ketoglutarate	TCA Cycle	Energy	**1.30**	0.0012	0.0034	**1.27**	0.0002	0.0017
heptenedioate (C7:1-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.30**	0.0039	0.0074	**1.48**	0.0069	0.0135
3-dehydrocholate	Secondary Bile Acid Metabolism	Lipid	**1.30**	0.0147	0.0202			
2,4-di-tert-butylphenol	Chemical	Xenobiotics	**1.29**	0.0057	0.0098	**1.34**	0.0026	0.0070

butyrylcarnitine (C4)	Fatty Acid Metabolism (also BCAA Metabolism)	Lipid	**1.29**	0.0079	0.0126	**1.16**	0.0113	0.0182
2-hydroxyphenylacetate	Phenylalanine Metabolism	Amino Acid	**1.29**	0.0234	0.0286	**1.26**	0.0007	0.0036
N-stearoyl-sphingadienine (d18:2/18:0)	Ceramides	Lipid	**1.28**	0.0019	0.0044	**1.20**	0.0083	0.0147
malate	TCA Cycle	Energy	**1.28**	0.0037	0.0070			
3-hydroxysebacate	Fatty Acid, Monohydroxy	Lipid	**1.28**	0.0068	0.0111	**1.89**	0.0044	0.0100
linoleoylcarnitine (C18:2)	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	Lipid	**1.28**	0.0212	0.0265	**1.51**	0.0013	0.0049
3-hydroxyoctanoate	Fatty Acid, Monohydroxy	Lipid	**1.27**	0.0047	0.0084	**2.02**	0.0092	0.0154
mannose	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**1.27**	0.0066	0.0110			
undecenoylcarnitine (C11:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.27**	0.0158	0.0214	**1.37**	0.0092	0.0154
biliverdin	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	**1.27**	0.0177	0.0233			
kynurenate	Tryptophan Metabolism	Amino Acid	**1.26**	0.0042	0.0078	**1.19**	0.0306	0.0343
3-amino-2-piperidone	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**1.25**	0.0048	0.0085	**1.13**	0.0246	0.0297

10-undecenoate (11:1n1)	Medium Chain Fatty Acid	Lipid	**1.25**	0.0183	0.0239			
tetradecanedioate (C14-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.25**	0.0251	0.0304	**1.22**	0.0060	0.0123
gamma-glutamylphenylalanine	Gamma-glutamyl Amino Acid	Peptide	**1.24**	0.0020	0.0047	**1.21**	0.0013	0.0049
3-hydroxyadipate	Fatty Acid, Dicarboxylate	Lipid	**1.24**	0.0078	0.0125			
2,6-dihydroxybenzoic acid	Drug - Topical Agents	Xenobiotics	**1.23**	0.0377	0.0415			
N-palmitoyl-sphingosine (d18:1/16:0)	Ceramides	Lipid	**1.22**	0.0027	0.0058	**1.17**	0.0005	0.0030
3-hydroxydodecanedioate	Fatty Acid, Dicarboxylate	Lipid	**1.21**	0.0132	0.0189	**1.44**	0.0127	0.0193
homovanillate (HVA)	Tyrosine Metabolism	Amino Acid	**1.21**	0.0229	0.0281			
N,N,N-trimethyl-5-aminovalerate	Lysine Metabolism	Amino Acid	**1.20**	0.0207	0.0261	**1.10**	0.0255	0.0305
xanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**1.20**	0.0411	0.0447			
palmitoylcarnitine (C16)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.20**	0.0462	0.0489	**1.39**	0.0017	0.0053
glutarylcarnitine (C5-DC)	Lysine Metabolism	Amino Acid	**1.20**	0.0485	0.0510	**1.19**	0.0361	0.0386
2-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid	**1.19**	0.0077	0.0125			
N-palmitoyl-heptadecasphingosine (d17:1/16:0)	Ceramides	Lipid	**1.19**	0.0113	0.0166	**1.17**	0.0019	0.0057
N-acetylvaline	Leucine, Isoleucine and	Amino Acid	**1.19**	0.0136	0.0191	**1.24**	0.0033	0.0082

	Valine Metabolism							
tridecenedioate (C13:1-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.19**	0.0350	0.0391	**1.76**	0.0236	0.0290
1-stearoyl-GPG (18:0)	Lysophospholipid	Lipid	**1.18**	0.0233	0.0286			
heptanoate (7:0)	Medium Chain Fatty Acid	Lipid	**1.18**	0.0251	0.0304			
3-hydroxy-2-ethylpropionate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.18**	0.0488	0.0510			
N1-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide	**1.17**	0.0043	0.0078	**1.12**	0.0136	0.0202
N-acetyl-beta-alanine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.17**	0.0318	0.0361			
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.16**	0.0010	0.0029	**1.13**	0.0022	0.0063
cholesterol	Sterol	Lipid	**1.15**	0.0052	0.0091			
caproate (6:0)	Medium Chain Fatty Acid	Lipid	**1.15**	0.0163	0.0217			
3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-CMPFP)	Fatty Acid, Dicarboxylate	Lipid	**1.14**	0.0163	0.0217	**1.21**	0.0146	0.0213
5-oxoproline	Glutathione Metabolism	Amino Acid	**1.13**	0.0035	0.0068	**1.21**	0.0008	0.0039
sphingomyelin (d17:2/16:0, d18:2/15:0)	Sphingomyelins	Lipid	**1.12**	0.0162	0.0217	**1.13**	0.0007	0.0036
1-palmitoyl-2-dihomo-linolenoyl-GPC (16:0/20:3n3 or 6)	Phosphatidylcholine (PC)	Lipid	**1.11**	0.0021	0.0048	**1.10**	0.0000	0.0001

creatinine	Creatine Metabolism	Amino Acid	**1.11**	0.0029	0.0061	**1.09**	0.0220	0.0282
vanillylmandelate (VMA)	Tyrosine Metabolism	Amino Acid	**1.11**	0.0303	0.0351			
pantothenate	Pantothenate and CoA Metabolism	Cofactors and Vitamins	**1.10**	0.0384	0.0420	**1.06**	0.0492	0.0476
sphingomyelin (d18:2/16:0, d18:1/16:1)	Sphingomyelins	Lipid	**1.08**	0.0033	0.0067	**1.07**	0.0066	0.0131
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	Phosphatidylcholine (PC)	Lipid	**1.08**	0.0108	0.0160	**1.04**	0.0175	0.0244
behenoyl sphingomyelin (d18:1/22:0)	Sphingomyelins	Lipid	**1.07**	0.0133	0.0189	**1.04**	0.0395	0.0410
1-stearoyl-2-linoleoyl-GPC (18:0/18:2)	Phosphatidylcholine (PC)	Lipid	**1.06**	0.0149	0.0204	**1.02**	0.0149	0.0217
stearoyl sphingomyelin (d18:1/18:0)	Sphingomyelins	Lipid	**1.06**	0.0310	0.0354			
sphingomyelin (d18:2/18:1)	Sphingomyelins	Lipid	**1.05**	0.0008	0.0025	**1.04**	0.0210	0.0273
sphingomyelin (d18:1/14:0, d16:1/16:0)	Sphingomyelins	Lipid	**1.04**	0.0425	0.0457	**1.04**	0.0044	0.0100
palmitoyl dihydrosphingomyelin (d18:0/16:0)	Dihydrosphingomyelins	Lipid	**1.03**	0.0142	0.0197			
deoxycarnitine	Carnitine Metabolism	Lipid	**0.89**	0.0088	0.0136	**0.86**	0.0120	0.0191
2'-O-methylcytidine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**0.88**	0.0306	0.0351			
1-oleoyl-2-linoleoyl-GPC (18:1/18:2)	Phosphatidylcholine (PC)	Lipid	**0.87**	0.0492	0.0513	**0.88**	0.0259	0.0307
asparagine	Alanine and Aspartate Metabolism	Amino Acid	**0.86**	0.0262	0.0314	**0.86**	0.0194	0.0260
2'-O-methyluridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.86**	0.0338	0.0380	**0.80**	0.0351	0.0380

6-bromotryptophan	Tryptophan Metabolism	Amino Acid	**0.85**	0.0014	0.0037	**0.90**	0.0075	0.0140
5-methyluridine (ribothymidine)	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.85**	0.0143	0.0198	**0.83**	0.0144	0.0213
sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2)	Sphingomyelins	Lipid	**0.84**	0.0109	0.0162			
oxalate (ethanedioate)	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**0.84**	0.0241	0.0294			
homoarginine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.82**	0.0056	0.0095			
ascorbic acid 3-sulfate	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**0.81**	0.0335	0.0378			
glycocholenate sulfate	Secondary Bile Acid Metabolism	Lipid	**0.79**	0.0114	0.0167			
2'-deoxyuridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.79**	0.0379	0.0416	**0.71**	0.0009	0.0039
isobutyrylcarnitine (C4)	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.76**	0.0496	0.0515			
argininate	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.75**	0.0227	0.0280			
glycerate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.74**	0.0036	0.0069	**0.80**	0.0175	0.0244
hydroxy-CMPF	Fatty Acid, Dicarboxylate	Lipid	**0.74**	0.0036	0.0070	**0.83**	0.0056	0.0119

3-indoleglyoxylic acid	Food Component/Plant	Xenobiotics	**0.72**	0.0157	0.0214	**0.77**	0.0009	0.0039
propionylglycine	Fatty Acid Metabolism (also BCAA Metabolism)	Lipid	**0.72**	0.0343	0.0385			
sphingadienine	Sphingolipid Synthesis	Lipid	**0.70**	0.0048	0.0085	**0.58**	0.0064	0.0129
ergothioneine	Food Component/Plant	Xenobiotics	**0.70**	0.0111	0.0164			
N-palmitoylserine	Endocannabinoid	Lipid	**0.69**	0.0110	0.0162			
tartronate (hydroxymalonate)	Food Component/Plant	Xenobiotics	**0.67**	0.0290	0.0339	**0.64**	0.0320	0.0356
N-acetylkynurenine (2)	Tryptophan Metabolism	Amino Acid	**0.65**	0.0030	0.0062	**0.72**	0.0395	0.0410
N1-Methyl-2-pyridone-5-carboxamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.57**	0.0012	0.0034	**0.66**	0.0034	0.0082
4-allylcatechol sulfate	Benzoate Metabolism	Xenobiotics	**0.57**	0.0227	0.0280			
N1-Methyl-4-pyridone-3-carboxamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.56**	0.0033	0.0066	**0.68**	0.0037	0.0089
S-1-pyrroline-5-carboxylate	Glutamate Metabolism	Amino Acid	**0.49**	0.0000	0.0003	**0.52**	0.0000	0.0006
phenylacetate	Phenylalanine Metabolism	Amino Acid	**0.47**	0.0073	0.0119	**0.52**	0.0264	0.0310
1-methylnicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.45**	0.0000	0.0003	**0.40**	0.0001	0.0011
isoursodeoxycholate	Secondary Bile Acid Metabolism	Lipid	**0.40**	0.0020	0.0047	**0.57**	0.0046	0.0102
4-vinylguaiacol sulfate	Food Component/Plant	Xenobiotics	**0.40**	0.0353	0.0393			
ferulic acid 4-sulfate	Food Component/Plant	Xenobiotics	**0.39**	0.0193	0.0248	**0.48**	0.0241	0.0294

glycerophosphoinositol	Phospholipid Metabolism	Lipid	**0.37**	0.0463	0.0489	**0.21**	0.0469	0.0465
ursodeoxycholate	Secondary Bile Acid Metabolism	Lipid	**0.33**	0.0009	0.0027	**0.63**	0.0101	0.0167
eugenol sulfate	Food Component/Plant	Xenobiotics	**0.32**	0.0146	0.0201			
salicylate	Drug - Topical Agents	Xenobiotics	**0.09**	0.0455	0.0483	**0.58**	0.0232	0.0290
glucuronide of C10H18O2 (7)	Partially Characterized Molecules	Partially Characterized Molecules				**10.30**	0.0009	0.0039
glucuronide of C10H18O2 (8)	Partially Characterized Molecules	Partially Characterized Molecules				**8.15**	0.0067	0.0132
saccharin	Food Component/Plant	Xenobiotics				**5.77**	0.0194	0.0260
4-hydroxyhippurate	Benzoate Metabolism	Xenobiotics				**4.91**	0.0254	0.0304
1,2,3-benzenetriol sulfate (2)	Chemical	Xenobiotics				**4.54**	0.0271	0.0315
glucuronide of C10H18O2 (1)	Partially Characterized Molecules	Partially Characterized Molecules				**3.84**	0.0238	0.0291
HWESASLLR	Polypeptide	Peptide				**3.80**	0.0070	0.0135
bradykinin	Polypeptide	Peptide				**3.68**	0.0446	0.0446
XHWESASXXR	Polypeptide	Peptide				**2.88**	0.0475	0.0468
glycine conjugate of C10H14O2 (1)	Partially Characterized Molecules	Partially Characterized Molecules				**2.27**	0.0473	0.0468
N-methylhydroxyproline	Urea cycle; Arginine and Proline Metabolism	Amino Acid				**2.18**	0.0205	0.0269
ethyl alpha-glucopyranoside	Food Component/Plant	Xenobiotics				**2.18**	0.0491	0.0476

5-hydroxymethyl-2-furoylcarnitine	Food Component/Plant	Xenobiotics	**1.92**	0.0072	0.0138
acesulfame	Food Component/Plant	Xenobiotics	**1.90**	0.0300	0.0340
phytanate	Food Component/Plant	Xenobiotics	**1.73**	0.0086	0.0148
dihomo-linolenoylcarnitine (C20:3n3 or 6)	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	Lipid	**1.66**	0.0015	0.0052
hydantoin-5-propionate	Histidine Metabolism	Amino Acid	**1.49**	0.0271	0.0315
linolenoylcarnitine (C18:3)	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	Lipid	**1.45**	0.0193	0.0260
sebacate (C10-DC)	Fatty Acid, Dicarboxylate	Lipid	**1.45**	0.0232	0.0290
2-hydroxyarachidate	Fatty Acid, Monohydroxy	Lipid	**1.44**	0.0121	0.0191
stachydrine	Food Component/Plant	Xenobiotics	**1.40**	0.0342	0.0372
cystathionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.37**	0.0000	0.0002
N-acetylcitrulline	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**1.35**	0.0437	0.0441
stearoylcarnitine (C18)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.30**	0.0221	0.0282
phenyllactate (PLA)	Phenylalanine Metabolism	Amino Acid	**1.30**	0.0419	0.0427

2-hydroxy-4-(methylthio)butanoic acid	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.28**	0.0200	0.0265
cys-gly, oxidized	Glutathione Metabolism	Amino Acid	**1.28**	0.0233	0.0290
3,4-dihydroxybutyrate	Fatty Acid, Dihydroxy	Lipid	**1.26**	0.0000	0.0001
cysteinylglycine disulfide	Glutathione Metabolism	Amino Acid	**1.26**	0.0111	0.0179
N-acetylglycine	Glycine, Serine and Threonine Metabolism	Amino Acid	**1.24**	0.0322	0.0357
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	Phosphatidylinositol (PI)	Lipid	**1.23**	0.0263	0.0310
gamma-glutamyltyrosine	Gamma-glutamyl Amino Acid	Peptide	**1.22**	0.0222	0.0282
alpha-ketobutyrate	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.22**	0.0339	0.0371
tiglylcarnitine (C5:1-DC)	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.19**	0.0298	0.0338
nervonoylcarnitine (C24:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.19**	0.0374	0.0395
gamma-glutamyltryptophan	Gamma-glutamyl Amino Acid	Peptide	**1.17**	0.0320	0.0356
gamma-glutamylhistidine	Gamma-glutamyl Amino Acid	Peptide	**1.15**	0.0149	0.0217
N-acetylglutamate	Glutamate Metabolism	Amino Acid	**1.15**	0.0466	0.0464

1,2-dipalmitoyl-GPC (16:0/16:0)	Phosphatidylcholine (PC)	Lipid	**1.13**	0.0005	0.0031
ribonate	Pentose Metabolism	Carbohydrate	**1.13**	0.0258	0.0307
beta-hydroxyisovaleroylcarnitine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.10**	0.0132	0.0197
N-acetyl-aspartyl-glutamate (NAAG)	Glutamate Metabolism	Amino Acid	**1.09**	0.0129	0.0196
tricosanoyl sphingomyelin (d18:1/23:0)	Sphingomyelins	Lipid	**1.06**	0.0294	0.0335
sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)	Sphingomyelins	Lipid	**1.05**	0.0444	0.0446
alanine	Alanine and Aspartate Metabolism	Amino Acid	**0.86**	0.0284	0.0327
uridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.81**	0.0057	0.0119
uracil	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.81**	0.0111	0.0179
choline phosphate	Phospholipid Metabolism	Lipid	**0.81**	0.0376	0.0395
1-stearoyl-2-oleoyl-GPS (18:0/18:1)	Phosphatidylserine (PS)	Lipid	**0.81**	0.0493	0.0476
4-hydroxy-2-oxoglutaric acid	Fatty Acid, Dicarboxylate	Lipid	**0.77**	0.0194	0.0260
1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	Phosphatidylserine (PS)	Lipid	**0.75**	0.0201	0.0265
guanine	Purine Metabolism, Guanine containing	Nucleotide	**0.73**	0.0209	0.0273

alpha-hydroxycaproate	Fatty Acid, Monohydroxy	Lipid	**0.70**	0.0232	0.0290
N6-carboxymethyllysine	Advanced Glycation End-product	Carbohydrate	**0.69**	0.0078	0.0141
nicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.67**	0.0089	0.0151
adenosine 5'-diphosphate (ADP)	Purine Metabolism, Adenine containing	Nucleotide	**0.66**	0.0191	0.0259
serotonin	Tryptophan Metabolism	Amino Acid	**0.66**	0.0381	0.0398
phenylacetylglutamine	Acetylated Peptides	Peptide	**0.64**	0.0370	0.0393
p-cresol sulfate	Benzoate Metabolism	Xenobiotics	**0.63**	0.0480	0.0470
deoxycholic acid 12-sulfate	Secondary Bile Acid Metabolism	Lipid	**0.59**	0.0358	0.0384
1-stearoyl-GPS (18:0)	Lysophospholipid	Lipid	**0.58**	0.0283	0.0327
lithocholate sulfate (1)	Secondary Bile Acid Metabolism	Lipid	**0.56**	0.0234	0.0290

APPENDIX 3A4: Table presenting metabolites for which the fold-change (FC) is significant (Welch's two-sample t-test significant when p≤0.05) in the cord artery between the different mode of labour onset groups. Corresponding p- and q-values are shown.

Biochemical name	Sub Pathway	Super Pathway	CAIOL CAS (FC)	p-value	q-value	CACS CAS (FC)	p-value	q-value	CACS CAIOL (FC)	p-value	q-value
Ranitidine	Drug - Gastrointestinal	Xenobiotics	**959.17**	0.0012	0.0951	**1199.67**	0.0000	0.0022			
Fibrinopeptide B	Fibrinogen Cleavage Peptide	Peptide	**142.41**	0.0013	0.0951	**105.63**	0.0254	0.0703			
Fibrinopeptide B (1-13)	Fibrinogen Cleavage Peptide	Peptide	**101.32**	0.0014	0.0951	**31.58**	0.0314	0.0787			
Fibrinopeptide A	Fibrinogen Cleavage Peptide	Peptide	**76.31**	0.0003	0.0731	**43.27**	0.0022	0.0240			
Fibrinopeptide A, phosphono-ser(3)	Fibrinogen Cleavage Peptide	Peptide	**32.93**	0.0000	0.0181	**26.70**	0.0093	0.0394			
Fibrinopeptide A, des-ala(1)	Fibrinogen Cleavage Peptide	Peptide	**29.86**	0.0001	0.0181	**18.76**	0.0198	0.0611			
Adenosine 5'-monophosphate (AMP)	Purine Metabolism, Adenine containing	Nucleotide	**3.44**	0.0214	0.2667						
2'-deoxyuridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.90**	0.0017	0.0951	**1.96**	0.0015	0.0233			
Edta	Chemical	Xenobiotics	**1.79**	0.0328	0.3059	**1.98**	0.0193	0.0606			
Butyrylglycine	Fatty Acid Metabolism (also BCAA Metabolism)	Lipid	**1.75**	0.0156	0.2619				**0.56**	0.0084	0.4340
Sarcosine	Glycine, Serine and Threonine Metabolism	Amino Acid	**1.66**	0.0006	0.0951						
Eicosenoylcarnitine (C20:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.66**	0.0403	0.3059				**0.54**	0.0340	0.4506
4-hydroxyhippurate	Benzoate Metabolism	Xenobiotics	**1.58**	0.0195	0.2619				**0.56**	0.0391	0.4506
3-formylindole	Food Component/Plant	Xenobiotics	**1.52**	0.0094	0.2465						
N-acetylaspartate (NAA)	Alanine and Aspartate Metabolism	Amino Acid	**1.32**	0.0465	0.3062						
Palmitoylcarnitine (C16)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.30**	0.0386	0.3059						

Lysine	Lysine Metabolism	Amino Acid	**0.83**	0.0191	0.2619						
Asparagine	Alanine and Aspartate Metabolism	Amino Acid	**0.80**	0.0338	0.3059						
1-palmitoyl-GPA (16:0)	Lysophospholipid	Lipid	**0.78**	0.0357	0.3059						
Citrate	TCA Cycle	Energy	**0.76**	0.0051	0.1773	**0.41**	0.0110	0.0439	**0.53**	0.0291	0.4506
1-methylhistidine	Histidine Metabolism	Amino Acid	**0.76**	0.0294	0.3041						
Tryptophan	Tryptophan Metabolism	Amino Acid	**0.75**	0.0190	0.2619	**0.81**	0.0476	0.0978			
5-oxoproline	Glutathione Metabolism	Amino Acid	**0.75**	0.0439	0.3059	**0.54**	0.0000	0.0054	**0.72**	0.0436	0.4506
2-hydroxyhippurate (salicylurate)	Benzoate Metabolism	Xenobiotics	**0.73**	0.0117	0.2508						
Arabitol/xylitol	Pentose Metabolism	Carbohydrate	**0.73**	0.0432	0.3059	**0.67**	0.0154	0.0531			
Arabonate/xylonate	Pentose Metabolism	Carbohydrate	**0.72**	0.0011	0.0951						
N1-methylinosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.71**	0.0377	0.3059						
Erythronate	Aminosugar Metabolism	Carbohydrate	**0.69**	0.0019	0.0951	**0.59**	0.0031	0.0272			
4-hydroxy-2-oxoglutaric acid	Fatty Acid, Dicarboxylate	Lipid	**0.69**	0.0346	0.3059	**0.44**	0.0160	0.0531			
N-acetyltryptophan	Tryptophan Metabolism	Amino Acid	**0.69**	0.0494	0.3186						
Alanine	Alanine and Aspartate Metabolism	Amino Acid	**0.68**	0.0007	0.0951	**0.53**	0.0020	0.0240	**0.78**	0.0383	0.4506
Beta-alanine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.66**	0.0369	0.3059						
N-oxalyl glycine (NOG)	Food Component/Plant	Xenobiotics	**0.66**	0.0475	0.3102	**0.53**	0.0097	0.0401			
S-1-pyrroline-5-carboxylate	Glutamate Metabolism	Amino Acid	**0.65**	0.0162	0.2619						
N-stearoyltaurine	Endocannabinoid	Lipid	**0.65**	0.0442	0.3059	**0.64**	0.0233	0.0680			
3-methyl-2-oxobutyrate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.62**	0.0410	0.3059						
Gamma-glutamylglutamine	Gamma-glutamyl Amino Acid	Peptide	**0.62**	0.0426	0.3059						
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)	Plasmalogen	Lipid	**0.61**	0.0103	0.2465						
Pelargonate (9:0)	Medium Chain Fatty Acid	Lipid	**0.61**	0.0436	0.3059						

3-hydroxy-2-ethylpropionate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.60**	0.0414	0.3059						
Sulfate	Chemical	Xenobiotics	**0.60**	0.0456	0.3059	**0.49**	0.0248	0.0694			
Ascorbic acid 2-sulfate	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**0.59**	0.0042	0.1656	**0.45**	0.0136	0.0502			
N-acetyl-4-chlorophenylalanine	Chemical	Xenobiotics	**0.59**	0.0153	0.2619	**0.46**	0.0034	0.0283			
1-(1-enyl-palmitoyl)-GPE (P-16:0)	Lysoplasmalogen	Lipid	**0.59**	0.0417	0.3059	**0.59**	0.0439	0.0940			
Bilirubin (Z,Z)	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	**0.58**	0.0145	0.2619						
Glycerophosphoglycerol	Glycerolipid Metabolism	Lipid	**0.58**	0.0286	0.3041	**0.41**	0.0235	0.0682			
Gluconate	Food Component/Plant	Xenobiotics	**0.58**	0.0390	0.3059	**0.32**	0.0046	0.0329			
N-acetyltaurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.57**	0.0047	0.1756	**0.42**	0.0082	0.0382			
Guanidinoacetate	Creatine Metabolism	Amino Acid	**0.57**	0.0411	0.3059						
Fructose	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**0.56**	0.0172	0.2619	**0.35**	0.0006	0.0224	**0.62**	0.0431	0.4506
Aconitate [cis or trans]	TCA Cycle	Energy	**0.56**	0.0173	0.2619	**0.42**	0.0027	0.0256			
3-hydroxyisobutyrate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.56**	0.0319	0.3059						
Dihomo-linoleate (20:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.56**	0.0396	0.3059	**0.32**	0.0017	0.0233	**0.57**	0.0295	0.4506
Glycerol	Glycerolipid Metabolism	Lipid	**0.54**	0.0053	0.1773	**0.23**	0.0004	0.0224	**0.43**	0.0045	0.3385
Docosahexaenoate (DHA; 22:6n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.53**	0.0418	0.3059	**0.43**	0.0116	0.0459			
Butyrate/isobutyrate (4:0)	Short Chain Fatty Acid	Lipid	**0.52**	0.0199	0.2619	**0.47**	0.0052	0.0339			
1-carboxyethylvaline	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.52**	0.0266	0.3028	**0.27**	0.0014	0.0233	**0.53**	0.0133	0.4422
Androstenediol (3alpha, 17alpha) monosulfate (2)	Androgenic Steroids	Lipid	**0.52**	0.0394	0.3059				**1.91**	0.0380	0.4506
Dodecadienoate (12:2)	Fatty Acid, Dicarboxylate	Lipid	**0.51**	0.0406	0.3059	**0.29**	0.0008	0.0228			
Phytanate	Food Component/Plant	Xenobiotics	**0.49**	0.0096	0.2465	**0.39**	0.0018	0.0233			
Salicylate	Drug - Topical Agents	Xenobiotics	**0.49**	0.0103	0.2465				**2.12**	0.0355	0.4506

Sphinganine-1-phosphate	Sphingolipid Synthesis	Lipid	**0.49**	0.0129	0.2594	**0.32**	0.0017	0.0233			
Flavin adenine dinucleotide (FAD)	Riboflavin Metabolism	Cofactors and Vitamins	**0.47**	0.0064	0.1953	**0.34**	0.0035	0.0285			
3-hydroxylaurate	Fatty Acid, Monohydroxy	Lipid	**0.47**	0.0425	0.3059	**0.19**	0.0036	0.0287	**0.40**	0.0306	0.4506
1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)	Plasmalogen	Lipid	**0.46**	0.0111	0.2508	**0.50**	0.0269	0.0715			
Methylmalonate (MMA)	Fatty Acid Metabolism (also BCAA Metabolism)	Lipid	**0.46**	0.0283	0.3041						
10-nonadecenoate (19:1n9)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.46**	0.0421	0.3059	**0.27**	0.0049	0.0329			
Tartronate (hydroxymalonate)	Food Component/Plant	Xenobiotics	**0.45**	0.0195	0.2619	**0.27**	0.0387	0.0867			
Nisinate (24:6n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.45**	0.0210	0.2656	**0.36**	0.0079	0.0382			
5-methylthioadenosine (MTA)	Polyamine Metabolism	Amino Acid	**0.45**	0.0284	0.3041	**0.41**	0.0131	0.0498			
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	Phosphatidylethanolamine (PE)	Lipid	**0.41**	0.0080	0.2326	**0.33**	0.0060	0.0354			
Docosapentaenoate (n3 DPA; 22:5n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.41**	0.0223	0.2729	**0.27**	0.0082	0.0382	**0.66**	0.0450	0.4506
Malonate	Fatty Acid Synthesis	Lipid	**0.41**	0.0358	0.3059	**0.31**	0.0016	0.0233			
Eicosapentaenoate (EPA; 20:5n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.40**	0.0339	0.3059	**0.32**	0.0147	0.0518			
(12 or 13)-methylmyristate (a15:0 or i15:0)	Fatty Acid, Branched	Lipid	**0.40**	0.0460	0.3059	**0.24**	0.0101	0.0411			
Linoleate (18:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.39**	0.0450	0.3059	**0.19**	0.0012	0.0233			
Docosatrienoate (22:3n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.38**	0.0288	0.3041	**0.26**	0.0089	0.0394			
Hexadecadienoate (16:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.37**	0.0209	0.2656	**0.12**	0.0007	0.0224	**0.33**	0.0290	0.4506
Myristate (14:0)	Long Chain Saturated Fatty Acid	Lipid	**0.37**	0.0329	0.3059	**0.22**	0.0077	0.0378	**0.59**	0.0449	0.4506
(2 or 3)-decenoate (10:1n7 or n8)	Medium Chain Fatty Acid	Lipid	**0.37**	0.0434	0.3059	**0.19**	0.0047	0.0329			

Isovalerate (i5:0)	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.36**	0.0083	0.2326	**0.33**	0.0089	0.0394			
1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	Phosphatidylserine (PS)	Lipid	**0.36**	0.0114	0.2508	**0.47**	0.0280	0.0728			
Androsterone sulfate	Androgenic Steroids	Lipid	**0.35**	0.0131	0.2594	**0.31**	0.0069	0.0363			
Threonate	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**0.35**	0.0290	0.3041	**0.35**	0.0266	0.0715			
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	Phosphatidylethanolamine (PE)	Lipid	**0.34**	0.0140	0.2619	**0.36**	0.0162	0.0531			
12,13-dihome	Fatty Acid, Dihydroxy	Lipid	**0.34**	0.0191	0.2619	**0.25**	0.0012	0.0233			
Fructosyllysine	Lysine Metabolism	Amino Acid	**0.34**	0.0227	0.2729	**0.25**	0.0102	0.0414	**0.74**	0.0083	0.4340
Caprylate (8:0)	Medium Chain Fatty Acid	Lipid	**0.34**	0.0453	0.3059	**0.23**	0.0146	0.0518			
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)	Plasmalogen	Lipid	**0.33**	0.0019	0.0951	**0.30**	0.0019	0.0234			
9,10-dihome	Fatty Acid, Dihydroxy	Lipid	**0.32**	0.0257	0.2975	**0.33**	0.0393	0.0870			
Linolenate [alpha or gamma; (18:3n3 or 6)]	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.32**	0.0403	0.3059	**0.13**	0.0007	0.0224			
Oxalate (ethanedioate)	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**0.29**	0.0332	0.3059	**0.18**	0.0076	0.0378			
Caprate (10:0)	Medium Chain Fatty Acid	Lipid	**0.28**	0.0193	0.2619	**0.18**	0.0080	0.0382			
Bilirubin (E,E)	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	**0.27**	0.0119	0.2508	**0.27**	0.0073	0.0371			
1-palmitoyl-2-stearoyl-GPE (16:0/18:0)	Phosphatidylethanolamine (PE)	Lipid	**0.27**	0.0441	0.3059						
Gamma-glutamylalanine	Gamma-glutamyl Amino Acid	Peptide	**0.26**	0.0020	0.0951	**0.12**	0.0029	0.0271			
Laurate (12:0)	Medium Chain Fatty Acid	Lipid	**0.26**	0.0059	0.1901	**0.17**	0.0017	0.0233			
Bilirubin (E,Z or Z,E)	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	**0.24**	0.0168	0.2619	**0.21**	0.0323	0.0799			
1-stearoyl-2-oleoyl-GPS (18:0/18:1)	Phosphatidylserine (PS)	Lipid	**0.21**	0.0024	0.0996	**0.18**	0.0022	0.0240			

Inosine 5'-monophosphate (IMP)	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.18**	0.0009	0.0951	**0.05**	0.0006	0.0224	**0.29**	0.0164	0.4422
Caffeine	Xanthine Metabolism	Xenobiotics	**0.18**	0.0247	0.2907						
Trans-3,4-methyleneheptanoate	Food Component/Plant	Xenobiotics	**0.17**	0.0156	0.2619	**0.20**	0.0270	0.0715			
1-stearoyl-GPS (18:0)	Lysophospholipid	Lipid	**0.10**	0.0021	0.0951	**0.07**	0.0040	0.0307			
Tartarate	Food Component/Plant	Xenobiotics	**0.09**	0.0183	0.2619	**0.05**	0.0161	0.0531			
Metronidazole	Drug - Antibiotic	Xenobiotics				**3583.00**	0.0000	0.0003	**826.85**	0.0016	0.1868
Ranitidine N-oxide	Drug - Gastrointestinal	Xenobiotics				**11.45**	0.0049	0.0329			
Heme	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins				**9.30**	0.0448	0.0943			
N-ethylglycinexylidide	Drug - Analgesics, Anesthetics	Xenobiotics				**6.72**	0.0049	0.0329			
Heptadecenamide (17:1)	Fatty Acid, Amide	Lipid				**5.90**	0.0064	0.0354			
Valylglycine	Dipeptide	Peptide				**3.39**	0.0006	0.0224			
N6-carboxymethyllysine	Advanced Glycation End-product	Carbohydrate				**2.81**	0.0011	0.0233			
Carnosine	Histidine Metabolism	Amino Acid				**1.95**	0.0263	0.0711	**2.01**	0.0155	0.4422
3-ureidopropionate	Pyrimidine Metabolism, Uracil containing	Nucleotide				**1.56**	0.0428	0.0929			
Metoclopramide	Drug - Gastrointestinal	Xenobiotics				**1.32**	0.0289	0.0743	**1.32**	0.0289	0.4506
Citrulline	Urea cycle; Arginine and Proline Metabolism	Amino Acid				**1.26**	0.0127	0.0489			
Gamma-glutamyltryptophan	Gamma-glutamyl Amino Acid	Peptide				**1.18**	0.0044	0.0325	**1.28**	0.0097	0.4422
Palmitoyl sphingomyelin (d18:1/16:0)	Sphingomyelins	Lipid				**0.87**	0.0093	0.0394			
Proline	Urea cycle; Arginine and Proline Metabolism	Amino Acid				**0.80**	0.0139	0.0505			
N-acetylserine	Glycine, Serine and Threonine Metabolism	Amino Acid				**0.78**	0.0346	0.0822			
Indolelactate	Tryptophan Metabolism	Amino Acid				**0.76**	0.0159	0.0531			

N-acetylvaline	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.75**	0.0376	0.0859			
Creatinine	Creatine Metabolism	Amino Acid	**0.73**	0.0063	0.0354	**0.82**	0.0270	0.4506
Behenoyl sphingomyelin (d18:1/22:0)	Sphingomyelins	Lipid	**0.73**	0.0208	0.0628			
Sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)	Sphingomyelins	Lipid	**0.72**	0.0348	0.0822			
1-oleoyl-GPG (18:1)	Lysophospholipid	Lipid	**0.71**	0.0179	0.0579			
Glycerophosphoethanolamine	Phospholipid Metabolism	Lipid	**0.71**	0.0360	0.0834			
Acetylcarnitine (C2)	Fatty Acid Metabolism (Acyl Carnitine, Short Chain)	Lipid	**0.70**	0.0211	0.0633			
2-O-methylascorbic acid	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**0.69**	0.0086	0.0390			
1-palmitoyl-GPG (16:0)	Lysophospholipid	Lipid	**0.69**	0.0187	0.0594			
1-linoleoyl-GPG (18:2)	Lysophospholipid	Lipid	**0.68**	0.0355	0.0831			
2-ketocaprylate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.67**	0.0346	0.0822			
Glycerophosphorylcholine (GPC)	Phospholipid Metabolism	Lipid	**0.67**	0.0456	0.0954			
Myristoylcarnitine (C14)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**0.66**	0.0138	0.0505	**0.67**	0.0218	0.4506
Decanoylcarnitine (C10)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	**0.66**	0.0260	0.0710			
Alpha-tocopherol	Tocopherol Metabolism	Cofactors and Vitamins	**0.65**	0.0277	0.0728	**0.63**	0.0493	0.4533
Cysteine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.65**	0.0309	0.0783			
N-palmitoyltaurine	Endocannabinoid	Lipid	**0.63**	0.0032	0.0272			
Margarate (17:0)	Long Chain Saturated Fatty Acid	Lipid	**0.63**	0.0373	0.0858			
N-palmitoylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**0.62**	0.0288	0.0743			
Ethylmalonate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.62**	0.0359	0.0834			

2-hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid	**0.62**	0.0443	0.0943			
1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	Phosphatidylinositol (PI)	Lipid	**0.61**	0.0063	0.0354			
Homoarginine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.61**	0.0314	0.0787			
Allantoin	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.60**	0.0049	0.0329			
Laurylcarnitine (C12)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	**0.59**	0.0012	0.0233			
1-palmitoyl-2-oleoyl-GPI (16:0/18:1)	Phosphatidylinositol (PI)	Lipid	**0.58**	0.0012	0.0233			
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	Phosphatidylethanolamine (PE)	Lipid	**0.58**	0.0031	0.0272			
Palmitoleoylcarnitine (C16:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**0.58**	0.0040	0.0307	**0.55**	0.0008	0.1868
Myristoleoylcarnitine (C14:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**0.58**	0.0124	0.0480	**0.57**	0.0168	0.4422
16-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid	**0.58**	0.0241	0.0686			
Pentadecanoate (15:0)	Long Chain Saturated Fatty Acid	Lipid	**0.58**	0.0335	0.0809			
Orotate	Pyrimidine Metabolism, Orotate containing	Nucleotide	**0.57**	0.0317	0.0788			
Glycochenodeoxycholate 3-sulfate	Primary Bile Acid Metabolism	Lipid	**0.56**	0.0019	0.0234			
Benzoate	Benzoate Metabolism	Xenobiotics	**0.56**	0.0163	0.0533			
Pyruvate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.56**	0.0334	0.0809	**0.71**	0.0359	0.4506
Mannose	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**0.54**	0.0094	0.0394			
3-hydroxyhexanoate	Fatty Acid, Monohydroxy	Lipid	**0.52**	0.0214	0.0636	**0.63**	0.0438	0.4506
N-oleoyltaurine	Endocannabinoid	Lipid	**0.52**	0.0251	0.0697			
Octadecenedioate (C18:1-DC)	Fatty Acid, Dicarboxylate	Lipid	**0.52**	0.0369	0.0852			

Indolebutyrate	Tryptophan Metabolism	Amino Acid	**0.52**	0.0381	0.0859			
Tetradecadienedioate (C14:2-DC)	Fatty Acid, Dicarboxylate	Lipid	**0.51**	0.0471	0.0977			
Glycocholenate sulfate	Secondary Bile Acid Metabolism	Lipid	**0.49**	0.0024	0.0247	**0.56**	0.0432	0.4506
Sphingosine	Sphingosines	Lipid	**0.49**	0.0161	0.0531	**0.42**	0.0172	0.4422
Taurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.48**	0.0064	0.0354	**0.61**	0.0471	0.4533
(S)-3-hydroxybutyrylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**0.46**	0.0262	0.0711	**0.58**	0.0304	0.4506
(14 or 15)-methylpalmitate (a17:0 or i17:0)	Fatty Acid, Branched	Lipid	**0.46**	0.0307	0.0781			
3-methylglutarate/2-methylglutarate	Fatty Acid, Dicarboxylate	Lipid	**0.45**	0.0025	0.0250			
Palmitate (16:0)	Long Chain Saturated Fatty Acid	Lipid	**0.45**	0.0133	0.0500			
Adrenate (22:4n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.45**	0.0199	0.0611			
N-acetylglutamine	Glutamate Metabolism	Amino Acid	**0.45**	0.0222	0.0652			
Glucose	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.44**	0.0004	0.0224			
Erucate (22:1n9)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.44**	0.0022	0.0240			
N-acetylneuraminate	Aminosugar Metabolism	Carbohydrate	**0.44**	0.0036	0.0287			
N-acetylglutamate	Glutamate Metabolism	Amino Acid	**0.44**	0.0062	0.0354			
1-arachidonoyl-GPA (20:4)	Lysophospholipid	Lipid	**0.44**	0.0120	0.0467			
Methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.44**	0.0215	0.0636			
Maleate	Fatty Acid, Dicarboxylate	Lipid	**0.44**	0.0410	0.0902			
Sphingosine 1-phosphate	Sphingosines	Lipid	**0.43**	0.0014	0.0233	**0.61**	0.0430	0.4506
Mannitol/sorbitol	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**0.43**	0.0240	0.0686			
Cortolone glucuronide (1)	Corticosteroids	Lipid	**0.43**	0.0390	0.0870	**0.39**	0.0259	0.4506

N6-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide	**0.43**	0.0463	0.0965			
Hypoxanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.42**	0.0014	0.0233	**0.42**	0.0361	0.4506
4-hydroxychlorothalonil	Chemical	Xenobiotics	**0.42**	0.0015	0.0233			
Lactate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.42**	0.0032	0.0272	**0.53**	0.0104	0.4422
1-methylguanidine	Guanidino and Acetamido Metabolism	Amino Acid	**0.42**	0.0085	0.0388			
3-hydroxyoctanoate	Fatty Acid, Monohydroxy	Lipid	**0.42**	0.0098	0.0402	**0.57**	0.0339	0.4506
Cis-urocanate	Histidine Metabolism	Amino Acid	**0.42**	0.0194	0.0606			
Estrone 3-sulfate	Estrogenic Steroids	Lipid	**0.42**	0.0294	0.0751	**0.35**	0.0132	0.4422
Heptenedioate (C7:1-DC)	Fatty Acid, Dicarboxylate	Lipid	**0.42**	0.0446	0.0943			
Corticosterone	Corticosteroids	Lipid	**0.41**	0.0394	0.0870	**0.39**	0.0377	0.4506
3-hydroxybutyroylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**0.41**	0.0433	0.0936			
2-hydroxybutyrate/2-hydroxyisobutyrate	Glutathione Metabolism	Amino Acid	**0.40**	0.0044	0.0325	**0.52**	0.0120	0.4422
Mead acid (20:3n9)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.40**	0.0146	0.0518			
3-hydroxydecanoylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**0.40**	0.0424	0.0924	**0.47**	0.0211	0.4506
Fumarate	TCA Cycle	Energy	**0.39**	0.0157	0.0531	**0.61**	0.0416	0.4506
Glucuronate	Aminosugar Metabolism	Carbohydrate	**0.38**	0.0052	0.0338			
Pregnanediol-3-glucuronide	Progestin Steroids	Lipid	**0.38**	0.0328	0.0804	**0.57**	0.0497	0.4533
3-methylglutaconate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.38**	0.0437	0.0939			
11beta-hydroxyandrosterone glucuronide	Androgenic Steroids	Lipid	**0.37**	0.0349	0.0822			
Docosadienoate (22:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.36**	0.0007	0.0224			
Malate	TCA Cycle	Energy	**0.36**	0.0064	0.0354	**0.52**	0.0309	0.4506

Cytosine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**0.36**	0.0279	0.0728			
5,6-dihydrouracil	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.36**	0.0445	0.0943	**0.38**	0.0182	0.4506
2-isopropylmalate	Food Component/Plant	Xenobiotics	**0.35**	0.0082	0.0382			
3-bromo-5-chloro-2,6-dihydroxybenzoic acid	Chemical	Xenobiotics	**0.35**	0.0475	0.0978			
17alpha-hydroxyprogesterone	Progestin Steroids	Lipid	**0.34**	0.0207	0.0627	**0.47**	0.0144	0.4422
Dihomo-linolenate (20:3n3 or n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.34**	0.0244	0.0688			
1,3-dimethylurate	Xanthine Metabolism	Xenobiotics	**0.34**	0.0490	0.1004			
Citramalate	Glutamate Metabolism	Amino Acid	**0.33**	0.0271	0.0715			
Caproate (6:0)	Medium Chain Fatty Acid	Lipid	**0.32**	0.0034	0.0283			
Undecanedioate (C11-DC)	Fatty Acid, Dicarboxylate	Lipid	**0.32**	0.0056	0.0341	**0.34**	0.0010	0.1868
Androsterone glucuronide	Androgenic Steroids	Lipid	**0.32**	0.0076	0.0378			
1-carboxyethyltyrosine	Tyrosine Metabolism	Amino Acid	**0.32**	0.0159	0.0531	**0.52**	0.0262	0.4506
10-undecenoate (11:1n1)	Medium Chain Fatty Acid	Lipid	**0.31**	0.0119	0.0467			
Cortisol	Corticosteroids	Lipid	**0.31**	0.0131	0.0498			
3-hydroxyoleoylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**0.31**	0.0182	0.0587	**0.31**	0.0246	0.4506
Cortisone	Corticosteroids	Lipid	**0.30**	0.0003	0.0224	**0.38**	0.0016	0.1868
5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	Progestin Steroids	Lipid	**0.30**	0.0241	0.0686	**0.33**	0.0221	0.4506
14-hdohe/17-hdohe	Docosanoid	Lipid	**0.30**	0.0325	0.0801			
N-formylphenylalanine	Tyrosine Metabolism	Amino Acid	**0.30**	0.0495	0.1009			
Eicosenoate (20:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.29**	0.0027	0.0256			
Myo-inositol	Inositol Metabolism	Lipid	**0.28**	0.0018	0.0233			
3-hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid	**0.28**	0.0021	0.0240	**0.43**	0.0124	0.4422
1-carboxyethylphenylalanine	Phenylalanine Metabolism	Amino Acid	**0.28**	0.0094	0.0394	**0.47**	0.0133	0.4422

Decadienedioic acid (C10:2-DC)	Fatty Acid, Dicarboxylate	Lipid	**0.28**	0.0330	0.0806			
Isocaproate (i6:0)	Fatty Acid, Branched	Lipid	**0.27**	0.0011	0.0233			
Trans-urocanate	Histidine Metabolism	Amino Acid	**0.27**	0.0017	0.0233			
Xanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.26**	0.0055	0.0340	**0.38**	0.0066	0.3925
Progesterone	Progestin Steroids	Lipid	**0.26**	0.0082	0.0382			
9-hydroxystearate	Fatty Acid, Monohydroxy	Lipid	**0.26**	0.0260	0.0710			
3-hydroxymyristate	Fatty Acid, Monohydroxy	Lipid	**0.25**	0.0086	0.0390			
Maltose	Glycogen Metabolism	Carbohydrate	**0.25**	0.0195	0.0608	**0.37**	0.0381	0.4506
Beta-citrylglutamate	Glutamate Metabolism	Amino Acid	**0.25**	0.0202	0.0617			
1-carboxyethylisoleucine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.24**	0.0025	0.0250	**0.41**	0.0168	0.4422
Cis-4-decenoate (10:1n6)	Medium Chain Fatty Acid	Lipid	**0.24**	0.0140	0.0506			
Oleate/vaccenate (18:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.23**	0.0061	0.0354			
Succinoyltaurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.23**	0.0242	0.0686			
Tetradecadienoate (14:2)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.22**	0.0073	0.0371	**0.41**	0.0345	0.4506
10-heptadecenoate (17:1n7)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.22**	0.0161	0.0531			
13-hode + 9-hode	Fatty Acid, Monohydroxy	Lipid	**0.21**	0.0378	0.0859			
1-carboxyethylleucine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.19**	0.0017	0.0233	**0.33**	0.0030	0.2907
Cortisone 21-sulfate	Corticosteroids	Lipid	**0.19**	0.0091	0.0394	**0.28**	0.0048	0.3385
Estriol	Estrogenic Steroids	Lipid	**0.18**	0.0412	0.0902			
Palmitoleate (16:1n7)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.16**	0.0093	0.0394			
5-dodecenoate (12:1n7)	Medium Chain Fatty Acid	Lipid	**0.15**	0.0067	0.0362			
Testosterone sulfate	Androgenic Steroids	Lipid	**0.15**	0.0187	0.0594			
1,3,7-trimethylurate	Xanthine Metabolism	Xenobiotics	**0.15**	0.0380	0.0859			

Branched-chain, straight-chain, or cyclopropyl 12:1 fatty acid	Partially Characterized Molecules	Partially Characterized Molecules	**0.13**	0.0053	0.0340			
Branched-chain, straight-chain, or cyclopropyl 10:1 fatty acid (1)	Partially Characterized Molecules	Partially Characterized Molecules	**0.13**	0.0056	0.0341			
Myristoleate (14:1n5)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.09**	0.0067	0.0362	**0.32**	0.0411	0.4506
Glucuronide of C10H18O2 (8)	Partially Characterized Molecules	Partially Characterized Molecules	**0.08**	0.0340	0.0817	**0.07**	0.0007	0.1868
Stearidonate (18:4n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.07**	0.0072	0.0371			
3-(methylthio)acetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**0.06**	0.0008	0.0224			
3-hydroxyoleate	Fatty Acid, Monohydroxy	Lipid	**0.06**	0.0047	0.0329			
Glucuronide of C10H18O2 (7)	Partially Characterized Molecules	Partially Characterized Molecules	**0.03**	0.0023	0.0246	**0.04**	0.0035	0.3005
2-hydroxyacetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**0.03**	0.0069	0.0363			
2-methoxyacetaminophen glucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**0.02**	0.0002	0.0195			
2-methoxyacetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**0.02**	0.0006	0.0224			
3-(N-acetyl-L-cystein-S-yl) acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics	**0.02**	0.0054	0.0340			
4-acetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics	**0.02**	0.0134	0.0500			
4-acetamidophenylglucuronide	Drug - Analgesics, Anesthetics	Xenobiotics	**0.02**	0.0150	0.0524			
3-(cystein-S-yl)acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics	**0.01**	0.0000	0.0054			
4-acetamidophenol	Drug - Analgesics, Anesthetics	Xenobiotics	**0.01**	0.0009	0.0233			

Saccharin	Food Component/Plant	Xenobiotics	**7.37**	0.0404	0.4506
1-palmitoyl-2-docosahexaenoyl-GPC (16:0/22:6)	Phosphatidylcholine (PC)	Lipid	**0.85**	0.0279	0.4506
1-stearoyl-2-docosahexaenoyl-GPC (18:0/22:6)	Phosphatidylcholine (PC)	Lipid	**0.77**	0.0497	0.4533
Linolenoylcarnitine (C18:3)	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	Lipid	**0.73**	0.0288	0.4506
Lignoceroylcarnitine (C24)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**0.72**	0.0003	0.1868
3-hydroxyhexanoylcarnitine (1)	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**0.69**	0.0360	0.4506
Vanillactate	Tyrosine Metabolism	Amino Acid	**0.61**	0.0155	0.4422
Uridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.58**	0.0118	0.4422
Cis-4-decenoylcarnitine (C10:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**0.58**	0.0460	0.4533
5-dodecenoylcarnitine (C12:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**0.57**	0.0329	0.4506
Succinate	TCA Cycle	Energy	**0.56**	0.0017	0.1868
5alpha-pregnan-3beta,20beta-diol monosulfate (1)	Progestin Steroids	Lipid	**0.48**	0.0195	0.4506
Margaroylcarnitine (C17)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**0.46**	0.0308	0.4506
Undecenoylcarnitine (C11:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**0.44**	0.0236	0.4506
(R)-3-hydroxybutyrylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**0.42**	0.0226	0.4506

3-hydroxydodecanedioate	Fatty Acid, Dicarboxylate	Lipid	**0.38**	0.0402	0.4506
N,N-dimethylalanine	Alanine and Aspartate Metabolism	Amino Acid	**0.30**	0.0419	0.4506
3-methoxycatechol sulfate (1)	Benzoate Metabolism	Xenobiotics	**0.16**	0.0328	0.4506
Glucuronide of C10H18O2 (1)	Partially Characterized Molecules	Partially Characterized Molecules	**0.10**	0.0053	0.3398

Cord artery of the induction of labour group (CAIOL) and cord artery of the spontaneously labouring group (CAS) (CAIOL/CAS); cord artery of the elective caesarean section group (CACS) and cord artery of the spontaneous labour group (CAS) (CACS/CAS); and cord artery of the elective caesarean section group (CACS) and cord artery of the induction of labour group (CAIOL) (CACS/CAIOL). Corresponding p-values are shown.

APPENDIX 3A5: Table presenting metabolites for which the fold-change (FC) is significant (Welch's two-sample t-test significant when p≤0.05) in the cord vein between the different mode of labour onset groups. Corresponding p- and q-values are shown.

Biochemical name	Sub Pathway	Super Pathway	CVIOL CVS (FC)	p-value	q-value	CVCS CVS (FC)	p-value	q-value	CVIOL CVS (FC)	p-value	q-value
Ranitidine	Drug - Gastrointestinal	Xenobiotics	**1013.92**	0.0010	0.0927	**988.83**	0.0004	0.0312			
Ranitidine N-oxide	Drug - Gastrointestinal	Xenobiotics	**34.42**	0.0291	0.2247	**19.15**	0.0011	0.0509			
Cis-4-decenoate (10:1n6)	Medium Chain Fatty Acid	Lipid	**10.10**	0.0002	0.0541	**4.19**	0.0068	0.1430	**2.41**	0.0356	0.6531
Stearidonate (18:4n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**9.05**	0.0175	0.1917						
Estriol	Estrogenic Steroids	Lipid	**6.45**	0.0352	0.2355						
(2 or 3)-decenoate (10:1n7 or n8)	Medium Chain Fatty Acid	Lipid	**5.47**	0.0066	0.1588						
Linolenate [alpha or gamma; (18:3n3 or 6)]	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**5.30**	0.0154	0.1766						
Oxalate (ethanedioate)	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**5.22**	0.0275	0.2242	**5.05**	0.0244	0.2071			
Caprylate (8:0)	Medium Chain Fatty Acid	Lipid	**4.52**	0.0007	0.0927	**2.63**	0.0335	0.2182			
Isocaproate (i6:0)	Fatty Acid, Branched	Lipid	**4.47**	0.0275	0.2242						
Linoleate (18:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**4.39**	0.0089	0.1588						
Branched-chain, straight-chain, or cyclopropyl 10:1 fatty acid (1)	Partially Characterized Molecules	Partially Characterized Molecules	**4.34**	0.0472	0.2520						
Hexadecadienoate (16:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**4.31**	0.0060	0.1588				**2.48**	0.0392	0.6800
Progesterone	Progestin Steroids	Lipid	**4.27**	0.0105	0.1614				**2.28**	0.0431	0.6800
N-acetyltheanine	Food Component/Plant	Xenobiotics	**4.24**	0.0440	0.2520	**7.60**	0.0415	0.2357			
Cys-gly, oxidized	Glutathione Metabolism	Amino Acid	**4.07**	0.0089	0.1588	**5.66**	0.0044	0.1313			
Isovalerate (i5:0)	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**4.01**	0.0079	0.1588	**3.39**	0.0186	0.1915			
Caproate (6:0)	Medium Chain Fatty Acid	Lipid	**3.83**	0.0008	0.0927	**3.00**	0.0280	0.2182			
N-formylphenylalanine	Tyrosine Metabolism	Amino Acid	**3.82**	0.0014	0.0927						
Palmitoleate (16:1n7)	Long Chain Monounsaturated Fatty Acid	Lipid	**3.73**	0.0378	0.2424						

17alpha-hydroxyprogesterone	Progestin Steroids	Lipid	**3.50**	0.0357	0.2355				**2.29**	0.0461	0.6800
3-indoleglyoxylic acid	Food Component/Plant	Xenobiotics	**3.40**	0.0034	0.1411	**3.35**	0.0118	0.1724			
Nisinate (24:6n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**3.32**	0.0076	0.1588	**2.53**	0.0200	0.1926			
N6-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide	**3.26**	0.0016	0.0927						
Eicosapentaenoate (EPA; 20:5n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**3.24**	0.0003	0.0541	**3.45**	0.0002	0.0244			
Oleate/vaccenate (18:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**3.22**	0.0289	0.2247						
Gamma-glutamyl-2-aminobutyrate	Gamma-glutamyl Amino Acid	Peptide	**3.07**	0.0126	0.1687	**3.24**	0.0095	0.1488			
Tetradecadienoate (14:2)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.96**	0.0380	0.2424						
Adrenate (22:4n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.93**	0.0329	0.2320						
Docosapentaenoate (n3 DPA; 22:5n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.83**	0.0002	0.0541	**2.01**	0.0307	0.2182			
Docosahexaenoate (DHA; 22:6n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.82**	0.0099	0.1596	**2.42**	0.0326	0.2182			
Fructosyllysine	Lysine Metabolism	Amino Acid	**2.78**	0.0263	0.2242						
3-hydroxyoleoylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**2.69**	0.0157	0.1766				**2.44**	0.0148	0.6390
Arachidonate (20:4n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.46**	0.0049	0.1588						
Butyrate/isobutyrate (4:0)	Short Chain Fatty Acid	Lipid	**2.45**	0.0136	0.1688	**2.34**	0.0147	0.1760			
Mannitol/sorbitol	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**2.44**	0.0074	0.1588	**2.22**	0.0228	0.2011			
Docosadienoate (22:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.44**	0.0138	0.1688				**2.01**	0.0337	0.6531
Valerate (5:0)	Short Chain Fatty Acid	Lipid	**2.39**	0.0378	0.2424						
Docosapentaenoate (n6 DPA; 22:5n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.38**	0.0206	0.1999						
Hexanoylcarnitine (C6)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	**2.36**	0.0181	0.1921						
Docosatrienoate (22:3n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.34**	0.0134	0.1688						
Eicosenoate (20:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**2.32**	0.0208	0.1999						
Taurochenodeoxycholic acid 3-sulfate	Secondary Bile Acid Metabolism	Lipid	**2.27**	0.0244	0.2134						
Dihomo-linoleate (20:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.26**	0.0074	0.1588						

Dihomo-linolenate (20:3n3 or n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**2.26**	0.0137	0.1688						
1-arachidonoyl-GPA (20:4)	Lysophospholipid	Lipid	**2.23**	0.0153	0.1766						
Methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**2.19**	0.0332	0.2320	**2.19**	0.0329	0.2182			
Erucate (22:1n9)	Long Chain Monounsaturated Fatty Acid	Lipid	**2.16**	0.0268	0.2242						
Maleate	Fatty Acid, Dicarboxylate	Lipid	**2.10**	0.0088	0.1588						
Mannose	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**2.05**	0.0062	0.1588				**1.60**	0.0297	0.6390
1-linoleoyl-GPI (18:2)	Lysophospholipid	Lipid	**2.03**	0.0410	0.2455						
Dodecenedioate (C12:1-DC)	Fatty Acid, Dicarboxylate	Lipid	**2.02**	0.0409	0.2455						
Octanoylcarnitine (C8)	Fatty Acid Metabolism (Acyl Carnitine, Medium Chain)	Lipid	**1.99**	0.0399	0.2455						
Glycosyl-N-behenoyl-sphingadienine (d18:2/22:0)	Hexosylceramides (HCER)	Lipid	**1.98**	0.0099	0.1596						
1-arachidonoyl-GPC (20:4n6)	Lysophospholipid	Lipid	**1.87**	0.0061	0.1588	**1.96**	0.0009	0.0507			
Lignoceroylcarnitine (C24)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.86**	0.0208	0.1999						
Palmitate (16:0)	Long Chain Saturated Fatty Acid	Lipid	**1.85**	0.0305	0.2268						
1-linoleoyl-GPC (18:2)	Lysophospholipid	Lipid	**1.82**	0.0493	0.2520	**2.04**	0.0072	0.1430			
Aconitate [cis or trans]	TCA Cycle	Energy	**1.76**	0.0183	0.1921						
Fructose	Fructose, Mannose and Galactose Metabolism	Carbohydrate	**1.71**	0.0223	0.2017						
2-hydroxynervonate	Fatty Acid, Monohydroxy	Lipid	**1.71**	0.0458	0.2520						
2-hydroxybutyrate/2-hydroxyisobutyrate	Glutathione Metabolism	Amino Acid	**1.69**	0.0476	0.2520						
2-hydroxystearate	Fatty Acid, Monohydroxy	Lipid	**1.68**	0.0064	0.1588						
Glycosyl ceramide (d18:1/20:0, d16:1/22:0)	Hexosylceramides (HCER)	Lipid	**1.66**	0.0076	0.1588						
N-stearoyltaurine	Endocannabinoid	Lipid	**1.56**	0.0305	0.2268						
Glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	Hexosylceramides (HCER)	Lipid	**1.54**	0.0048	0.1588						
Allantoin	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**1.50**	0.0176	0.1917	**1.42**	0.0328	0.2182			
Cortisone	Corticosteroids	Lipid	**1.49**	0.0416	0.2466	**0.52**	0.0086	0.1485	**2.85**	0.0004	0.1042
1-stearoyl-GPC (18:0)	Lysophospholipid	Lipid	**1.47**	0.0469	0.2520	**1.40**	0.0450	0.2428			
Tryptophan	Tryptophan Metabolism	Amino Acid	**1.29**	0.0114	0.1678	**1.36**	0.0055	0.1430			

2-aminobutyrate	Glutathione Metabolism	Amino Acid	**1.20**	0.0114	0.1678						
N2,N2-dimethylguanosine	Purine Metabolism, Guanine containing	Nucleotide	**0.78**	0.0429	0.2520						
2-O-methylascorbic acid	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	**0.78**	0.0482	0.2520	**0.66**	0.0202	0.1926			
7-methylguanine	Purine Metabolism, Guanine containing	Nucleotide	**0.77**	0.0015	0.0927						
N1-methyladenosine	Purine Metabolism, Adenine containing	Nucleotide	**0.77**	0.0155	0.1766						
Acetylcarnitine (C2)	Fatty Acid Metabolism (Acyl Carnitine, Short Chain)	Lipid	**0.69**	0.0301	0.2268	**0.56**	0.0060	0.1430			
N1-methylinosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.67**	0.0273	0.2242				**0.68**	0.0191	0.6390
2-hydroxyhippurate (salicylurate)	Benzoate Metabolism	Xenobiotics	**0.56**	0.0022	0.1055						
Iminodiacetate (IDA)	Chemical	Xenobiotics	**0.56**	0.0122	0.1687	**0.56**	0.0275	0.2182			
Hydroxy-N6,N6,N6-trimethyllysine	Lysine Metabolism	Amino Acid	**0.56**	0.0314	0.2272	**0.64**	0.0483	0.2507			
1-methylguanidine	Guanidino and Acetamido Metabolism	Amino Acid	**0.52**	0.0318	0.2273	**0.40**	0.0304	0.2182			
Glutarate (C5-DC)	Fatty Acid, Dicarboxylate	Lipid	**0.49**	0.0473	0.2520	**0.44**	0.0319	0.2182			
Corticosterone	Corticosteroids	Lipid	**0.48**	0.0220	0.2017	**0.19**	0.0004	0.0312	**2.46**	0.0064	0.6373
Deoxycarnitine	Carnitine Metabolism	Lipid	**0.48**	0.0237	0.2110	**0.47**	0.0212	0.1949			
Glutamate	Glutamate Metabolism	Amino Acid	**0.44**	0.0405	0.2455						
Maltose	Glycogen Metabolism	Carbohydrate	**0.41**	0.0198	0.1999	**0.16**	0.0017	0.0663	**2.51**	0.0235	0.6390
Cortisol	Corticosteroids	Lipid	**0.41**	0.0280	0.2247	**0.17**	0.0000	0.0066	**2.45**	0.0456	0.6800
3-hydroxyphenylacetoylglutamine	Acetylated Peptides	Peptide	**0.40**	0.0048	0.1588						
N-acetylglucosaminylasparagine	Aminosugar Metabolism	Carbohydrate	**0.38**	0.0071	0.1588	**0.42**	0.0123	0.1724			
Biliverdin	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	**0.37**	0.0451	0.2520						
Cytosine	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**0.37**	0.0494	0.2520	**0.32**	0.0383	0.2249			
Edta	Chemical	Xenobiotics	**0.36**	0.0019	0.1009	**0.42**	0.0133	0.1748			
2-keto-3-deoxy-gluconate	Food Component/Plant	Xenobiotics	**0.36**	0.0388	0.2445	**0.39**	0.0389	0.2249			
Vanillic acid glycine	Food Component/Plant	Xenobiotics	**0.33**	0.0014	0.0927						
Cysteinylglycine	Glutathione Metabolism	Amino Acid	**0.33**	0.0341	0.2327	**0.35**	0.0376	0.2249			
Glutamate, gamma-methyl ester	Glutamate Metabolism	Amino Acid	**0.30**	0.0291	0.2247	**0.33**	0.0332	0.2182			
4-chlorobenzoic acid	Chemical	Xenobiotics	**0.30**	0.0354	0.2355						

Sphingosine	Sphingosines	Lipid	**0.28**	0.0489	0.2520	**0.22**	0.0340	0.2189			
Adenosine 3',5'-cyclic monophosphate (camp)	Purine Metabolism, Adenine containing	Nucleotide	**0.22**	0.0336	0.2320	**0.11**	0.0091	0.1488			
S-adenosylhomocysteine (SAH)	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.22**	0.0494	0.2520						
Sarcosine	Glycine, Serine and Threonine Metabolism	Amino Acid	**0.20**	0.0126	0.1687	**0.23**	0.0122	0.1724			
Sphinganine	Sphingolipid Synthesis	Lipid	**0.19**	0.0218	0.2017	**0.12**	0.0142	0.1760			
Maltotriose	Glycogen Metabolism	Carbohydrate	**0.16**	0.0204	0.1999	**0.17**	0.0167	0.1839			
1-oleoyl-2-arachidonoyl-GPE (18:1/20:4)	Phosphatidylethanolamine (PE)	Lipid	**0.15**	0.0311	0.2272	**0.07**	0.0195	0.1926			
Cytidine 5'-monophosphate (5'-CMP)	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**0.13**	0.0087	0.1588	**0.24**	0.0201	0.1926			
N2,N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.12**	0.0029	0.1329	**0.12**	0.0082	0.1485			
Cysteine-glutathione disulfide	Glutathione Metabolism	Amino Acid	**0.11**	0.0042	0.1588						
Heptadecenamide (17:1)	Fatty Acid, Amide	Lipid	**0.11**	0.0096	0.1596						
Maltotetraose	Glycogen Metabolism	Carbohydrate	**0.11**	0.0101	0.1596	**0.16**	0.0193	0.1926			
1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)	Phosphatidylethanolamine (PE)	Lipid	**0.10**	0.0404	0.2455	**0.04**	0.0216	0.1949			
Spermidine	Polyamine Metabolism	Amino Acid	**0.03**	0.0220	0.2017	**0.08**	0.0288	0.2182			
Adenosine 5'-diphosphoribose (ADP-ribose)	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.02**	0.0120	0.1687	**0.07**	0.0186	0.1915			
Glutathione, oxidized (GSSG)	Glutathione Metabolism	Amino Acid	**0.00**	0.0477	0.2520						
Metronidazole	Drug - Antibiotic	Xenobiotics				**4274.67**	0.0000	0.0011	**0.00**	0.0000	0.0054
N-ethylglycinexylidide	Drug - Analgesics, Anesthetics	Xenobiotics				**6.70**	0.0002	0.0244			
Gamma-glutamylcitrulline	Gamma-glutamyl Amino Acid	Peptide				**3.79**	0.0062	0.1430			
Trimethylamine N-oxide	Phospholipid Metabolism	Lipid				**3.04**	0.0299	0.2182			
Gamma-glutamylmethionine	Gamma-glutamyl Amino Acid	Peptide				**2.27**	0.0426	0.2357			
Gamma-glutamylhistidine	Gamma-glutamyl Amino Acid	Peptide				**2.16**	0.0463	0.2437			
1-arachidonoyl-GPI (20:4)	Lysophospholipid	Lipid				**2.05**	0.0251	0.2091			
Phenylalanylhydroxyproline	Dipeptide	Peptide				**2.03**	0.0266	0.2179			
Gamma-glutamylglutamine	Gamma-glutamyl Amino Acid	Peptide				**1.90**	0.0235	0.2035			
Gamma-glutamyl-alpha-lysine	Gamma-glutamyl Amino Acid	Peptide				**1.82**	0.0268	0.2179			
1-linolenoyl-GPC (18:3)	Lysophospholipid	Lipid				**1.79**	0.0166	0.1839			

1-linoleoyl-GPE (18:2)	Lysophospholipid	Lipid	**1.78**	0.0422	0.2357				
1-arachidonoyl-GPE (20:4n6)	Lysophospholipid	Lipid	**1.71**	0.0151	0.1781				
1-oleoyl-GPC (18:1)	Lysophospholipid	Lipid	**1.66**	0.0019	0.0688				
Gamma-glutamylisoleucine	Gamma-glutamyl Amino Acid	Peptide	**1.65**	0.0066	0.1430				
Gamma-glutamylleucine	Gamma-glutamyl Amino Acid	Peptide	**1.58**	0.0067	0.1430				
Gamma-glutamyltyrosine	Gamma-glutamyl Amino Acid	Peptide	**1.58**	0.0288	0.2182				
Beta-hydroxyisovalerate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.57**	0.0205	0.1931				
1-(1-enyl-palmitoyl)-GPC (P-16:0)	Lysoplasmalogen	Lipid	**1.55**	0.0362	0.2189				
1-palmitoleoyl-GPC (16:1)	Lysophospholipid	Lipid	**1.54**	0.0424	0.2357				
Gamma-glutamylvaline	Gamma-glutamyl Amino Acid	Peptide	**1.44**	0.0388	0.2249				
1-lignoceroyl-GPC (24:0)	Lysophospholipid	Lipid	**1.40**	0.0435	0.2368				
3-methyl-2-oxobutyrate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.39**	0.0245	0.2071				
1-oleoyl-GPE (18:1)	Lysophospholipid	Lipid	**1.36**	0.0359	0.2189				
1-stearoyl-GPE (18:0)	Lysophospholipid	Lipid	**1.34**	0.0355	0.2189				
Citrulline	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**1.28**	0.0459	0.2437				
Valine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.24**	0.0298	0.2182				
Creatinine	Creatine Metabolism	Amino Acid	**0.71**	0.0216	0.1949				
N,N,N-trimethyl-5-aminovalerate	Lysine Metabolism	Amino Acid	**0.70**	0.0185	0.1915				
1-stearoyl-2-linoleoyl-GPI (18:0/18:2)	Phosphatidylinositol (PI)	Lipid	**0.70**	0.0352	0.2189	**1.59**	0.0252	0.6390	
4-hydroxychlorothalonil	Chemical	Xenobiotics	**0.67**	0.0229	0.2011				
1-palmitoyl-2-oleoyl-GPI (16:0/18:1)	Phosphatidylinositol (PI)	Lipid	**0.67**	0.0403	0.2309				
Carboxyethyl-GABA	Glutamate Metabolism	Amino Acid	**0.66**	0.0358	0.2189				
Taurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.64**	0.0433	0.2368				
N-acetyltaurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.63**	0.0309	0.2182				
Nicotinamide riboside	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.62**	0.0008	0.0507				
N,N,N-trimethyl-alanylproline betaine (TMAP)	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.62**	0.0334	0.2182				
3-amino-2-piperidone	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.58**	0.0077	0.1485				

Palmitoleoylcarnitine (C16:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**0.57**	0.0352	0.2189	**1.88**	0.0025	0.4356
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	Phosphatidylethanolamine (PE)	Lipid	**0.55**	0.0021	0.0688			
Phytanate	Food Component/Plant	Xenobiotics	**0.50**	0.0094	0.1488	**2.38**	0.0298	0.6390
1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6)	Phosphatidylethanolamine (PE)	Lipid	**0.47**	0.0070	0.1430			
Succinylcarnitine (C4-DC)	TCA Cycle	Energy	**0.47**	0.0090	0.1488			
Lactate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.45**	0.0156	0.1806	**1.97**	0.0156	0.6390
Inosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.44**	0.0500	0.2551	**3.45**	0.0421	0.6800
Androsterone glucuronide	Androgenic Steroids	Lipid	**0.43**	0.0386	0.2249			
1-carboxyethylvaline	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.42**	0.0021	0.0688	**2.17**	0.0015	0.3332
Glycerol	Glycerolipid Metabolism	Lipid	**0.41**	0.0107	0.1601	**2.20**	0.0092	0.6373
1-carboxyethylisoleucine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.38**	0.0318	0.2182			
Hypoxanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.37**	0.0144	0.1760			
(S)-3-hydroxybutyrylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**0.36**	0.0066	0.1430			
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)	Phosphatidylethanolamine (PE)	Lipid	**0.35**	0.0349	0.2189			
1-carboxyethyltyrosine	Tyrosine Metabolism	Amino Acid	**0.34**	0.0012	0.0515	**2.21**	0.0030	0.4356
Estrone 3-sulfate	Estrogenic Steroids	Lipid	**0.34**	0.0129	0.1730	**2.22**	0.0240	0.6390
1-carboxyethylleucine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.33**	0.0049	0.1356	**2.53**	0.0081	0.6373
1-carboxyethylphenylalanine	Phenylalanine Metabolism	Amino Acid	**0.33**	0.0159	0.1810	**2.29**	0.0107	0.6390
Xanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.29**	0.0178	0.1915	**1.73**	0.0450	0.6800
Oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]	Diacylglycerol	Lipid	**0.28**	0.0084	0.1485			
1,3-dimethylurate	Xanthine Metabolism	Xenobiotics	**0.28**	0.0097	0.1492			
Flavin adenine dinucleotide (FAD)	Riboflavin Metabolism	Cofactors and Vitamins	**0.28**	0.0322	0.2182			
2-hydroxyglutarate	Fatty Acid, Dicarboxylate	Lipid	**0.23**	0.0297	0.2182			
1,3,7-trimethylurate	Xanthine Metabolism	Xenobiotics	**0.21**	0.0328	0.2182			
Cortisone 21-sulfate	Corticosteroids	Lipid	**0.20**	0.0146	0.1760	**3.32**	0.0160	0.6390

1-stearoyl-2-oleoyl-GPE (18:0/18:1)	Phosphatidylethanolamine (PE)	Lipid				**0.20**	0.0473	0.2472			
1-palmitoyl-2-stearoyl-GPE (16:0/18:0)	Phosphatidylethanolamine (PE)	Lipid				**0.20**	0.0498	0.2551			
Trans-urocanate	Histidine Metabolism	Amino Acid				**0.16**	0.0010	0.0507			
1,2-dipalmitoyl-GPE (16:0/16:0)	Phosphatidylethanolamine (PE)	Lipid				**0.11**	0.0462	0.2437			
3-(methylthio)acetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics				**0.07**	0.0009	0.0507			
Glucuronide of C10H18O2 (7)	Partially Characterized Molecules	Partially Characterized Molecules				**0.04**	0.0048	0.1356	**22.28**	0.0046	0.5794
2-hydroxyacetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics				**0.03**	0.0085	0.1485			
3-(cystein-S-yl)acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics				**0.02**	0.0000	0.0065			
2-methoxyacetaminophen glucuronide	Drug - Analgesics, Anesthetics	Xenobiotics				**0.02**	0.0002	0.0244			
2-methoxyacetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics				**0.02**	0.0003	0.0305			
3-(N-acetyl-L-cystein-S-yl) acetaminophen	Drug - Analgesics, Anesthetics	Xenobiotics				**0.02**	0.0024	0.0762			
4-acetaminophen sulfate	Drug - Analgesics, Anesthetics	Xenobiotics				**0.02**	0.0127	0.1730			
4-acetamidophenylglucuronide	Drug - Analgesics, Anesthetics	Xenobiotics				**0.02**	0.0139	0.1760			
4-acetamidophenol	Drug - Analgesics, Anesthetics	Xenobiotics				**0.01**	0.0013	0.0546			
Glucuronide of C10H18O2 (8)	Partially Characterized Molecules	Partially Characterized Molecules							**15.58**	0.0002	0.0732
Glucuronide of C10H18O2 (1)	Partially Characterized Molecules	Partially Characterized Molecules							**10.56**	0.0065	0.6373
3-methoxycatechol sulfate (1)	Benzoate Metabolism	Xenobiotics							**6.70**	0.0191	0.6390
N,N-dimethylalanine	Alanine and Aspartate Metabolism	Amino Acid							**3.26**	0.0228	0.6390
5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	Progestin Steroids	Lipid							**3.09**	0.0281	0.6390
Cortolone glucuronide (1)	Corticosteroids	Lipid							**2.56**	0.0197	0.6390
5alpha-pregnan-3beta,20beta-diol monosulfate (1)	Progestin Steroids	Lipid							**2.29**	0.0262	0.6390
3-hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid							**2.20**	0.0309	0.6428
Undecenoylcarnitine (C11:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid							**2.01**	0.0137	0.6390
5,6-dihydrouracil	Pyrimidine Metabolism, Uracil containing	Nucleotide							**1.83**	0.0337	0.6531
3-hydroxydecanoylcarnitine	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid							**1.77**	0.0276	0.6390

20a-dihydroprogesterone	Progestin Steroids	Lipid	**1.72**	0.0457	0.6800
5-dodecenoylcarnitine (C12:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.70**	0.0224	0.6390
Cis-4-decenoylcarnitine (C10:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.63**	0.0314	0.6428
Oleoylcarnitine (C18:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.57**	0.0233	0.6390
Myristoleoylcarnitine (C14:1)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**1.54**	0.0152	0.6390
(14 or 15)-methylpalmitate (a17:0 or i17:0)	Fatty Acid, Branched	Lipid	**1.52**	0.0479	0.6800
Glucose	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**1.44**	0.0348	0.6531
Linoleoylcarnitine (C18:2)	Fatty Acid Metabolism (Acyl Carnitine, Polyunsaturated)	Lipid	**1.43**	0.0094	0.6373
Margarate (17:0)	Long Chain Saturated Fatty Acid	Lipid	**1.41**	0.0431	0.6800
Myristoylcarnitine (C14)	Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	Lipid	**1.29**	0.0349	0.6531
Alanine	Alanine and Aspartate Metabolism	Amino Acid	**1.28**	0.0297	0.6390
Cysteine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**1.27**	0.0408	0.6800
Citrate	TCA Cycle	Energy	**1.23**	0.0193	0.6390
Histidine	Histidine Metabolism	Amino Acid	**0.81**	0.0142	0.6390
(N(1) + N(8))-acetylspermidine	Polyamine Metabolism	Amino Acid	**0.77**	0.0086	0.6373
N(1)-acetylspermine	Polyamine Metabolism	Amino Acid	**0.54**	0.0221	0.6390
3-dehydrocholate	Secondary Bile Acid Metabolism	Lipid	**0.53**	0.0262	0.6390
Salicylate	Drug - Topical Agents	Xenobiotics	**0.50**	0.0460	0.6800
2-butenoylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**0.49**	0.0201	0.6390
Valylglycine	Dipeptide	Peptide	**0.49**	0.0282	0.6390
Carnosine	Histidine Metabolism	Amino Acid	**0.41**	0.0153	0.6390
Saccharin	Food Component/Plant	Xenobiotics	**0.13**	0.0491	0.6800

Cord vein of the induction of labour group (CVIOL) and cord vein of the spontaneously labouring group (CVS) (CVIOL/CVS); cord vein of the elective caesarean section group (CVCS) and cord vein of the spontaneous labour group (CVS) (CVCS/CVS); and cord vein of the elective caesarean section group (CVCS) and cord vein of the induction of labour group (CVIOL) (CVCS/CVIOL). Corresponding p-values are shown.

APPENDIX 3A6: Table presenting metabolites for which the fold-change (FC) is significant (Welch's two-sample t-test significant when p≤0.05) between latent stage/early IOL/pre-operative samples between the different modes of labour onset and delivery. Corresponding p- and q-values are shown.

Biochemical name	Sub Pathway	Super Pathway	LIOL LS (FC)	p-value	q-value	LCS LS (FC)	p-value	q-value	LCS LIOL (FC)	p-value	q-value
Propyl 4-hydroxybenzoate sulfate	Benzoate Metabolism	Xenobiotics	**7.95**	0.0054	0.9742				**0.08**	0.0013	1.0000
Methyl-4-hydroxybenzoate sulfate	Benzoate Metabolism	Xenobiotics	**7.68**	0.0470	0.9742				**0.16**	0.0393	1.0000
Hyocholate	Secondary Bile Acid Metabolism	Lipid	**4.42**	0.0248	0.9742						
Stearoylcholine	Fatty Acid Metabolism (Acyl Choline)	Lipid	**3.94**	0.0465	0.9742						
Arachidonoylcholine	Fatty Acid Metabolism (Acyl Choline)	Lipid	**3.76**	0.0408	0.9742						
1-arachidonoyl-GPA (20:4)	Lysophospholipid	Lipid	**3.66**	0.0377	0.9742				**0.40**	0.0500	1.0000
Ribulonate/xylulonate/lyxonate	Pentose Metabolism	Carbohydrate	**3.37**	0.0332	0.9742						
1-arachidonylglycerol (20:4)	Monoacylglycerol	Lipid	**2.54**	0.0282	0.9742						
3-ureidopropionate	Pyrimidine Metabolism, Uracil containing	Nucleotide	**2.38**	0.0324	0.9742						
2'-O-methyluridine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.77**	0.0221	0.9742				**0.61**	0.0318	1.0000
1-stearoyl-GPI (18:0)	Lysophospholipid	Lipid	**1.69**	0.0162	0.9742	**1.47**	0.0411	0.4355			
N-acetyl-aspartyl-glutamate (NAAG)	Glutamate Metabolism	Amino Acid	**1.50**	0.0310	0.9742						
5-methyluridine (ribothymidine)	Pyrimidine Metabolism, Uracil containing	Nucleotide	**1.47**	0.0498	0.9742				**0.76**	0.0373	1.0000
N-acetylglutamate	Glutamate Metabolism	Amino Acid	**0.73**	0.0281	0.9742						
Cysteinylglycine disulfide	Glutathione Metabolism	Amino Acid	**0.69**	0.0081	0.9742	**0.77**	0.0499	0.4546			
3-hydroxy-2-ethylpropionate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.62**	0.0205	0.9742						

N-palmitoyl-heptadecasphingosine (d17:1/16:0)	Ceramides	Lipid	**0.61**	0.0068	0.9742	**0.63**	0.0088	0.3140
Valylglycine	Dipeptide	Peptide	**0.52**	0.0278	0.9742			
3-hydroxyisobutyrate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.46**	0.0041	0.9742	**0.57**	0.0114	0.3140
Nisinate (24:6n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.44**	0.0480	0.9742	**0.36**	0.0287	0.3865
21-hydroxypregnenolone monosulfate (2)	Pregnenolone Steroids	Lipid	**0.42**	0.0261	0.9742			
Androsterone glucuronide	Androgenic Steroids	Lipid	**0.42**	0.0448	0.9742			
13-hode + 9-hode	Fatty Acid, Monohydroxy	Lipid	**0.39**	0.0353	0.9742			
Androstenediol (3alpha, 17alpha) monosulfate (2)	Androgenic Steroids	Lipid	**0.35**	0.0025	0.9742	**0.24**	0.0008	0.1776
Corticosterone	Corticosteroids	Lipid	**0.35**	0.0299	0.9742	**0.19**	0.0009	0.1776
(12 or 13)-methylmyristate (a15:0 or i15:0)	Fatty Acid, Branched	Lipid	**0.34**	0.0360	0.9742	**0.40**	0.0164	0.3567
21-hydroxypregnenolone monosulfate (1)	Pregnenolone Steroids	Lipid	**0.30**	0.0060	0.9742	**0.45**	0.0324	0.3908
Phytanate	Food Component/Plant	Xenobiotics	**0.30**	0.0140	0.9742	**0.43**	0.0458	0.4499
Glutamine conjugate of C6H10O2 (1)	Partially Characterized Molecules	Partially Characterized Molecules	**0.30**	0.0191	0.9742	**0.32**	0.0314	0.3908
Epiandrosterone sulfate	Androgenic Steroids	Lipid	**0.30**	0.0237	0.9742			
Laurate (12:0)	Medium Chain Fatty Acid	Lipid	**0.25**	0.0245	0.9742			
Leucylglycine	Dipeptide	Peptide	**0.12**	0.0219	0.9742			
Fructose	Fructose, Mannose and Galactose Metabolism	Carbohydrate				**2.78**	0.0298	0.3865
N-palmitoylserine	Endocannabinoid	Lipid				**2.73**	0.0044	0.2967
1-carboxyethyltyrosine	Tyrosine Metabolism	Amino Acid				**1.98**	0.0121	0.3140
Behenoyl dihydrosphingomyelin (d18:0/22:0)	Dihydrosphingomyelins	Lipid				**1.69**	0.0250	0.3865
1-stearoyl-GPG (18:0)	Lysophospholipid	Lipid				**1.59**	0.0039	0.2967

1-palmitoyl-GPG (16:0)	Lysophospholipid	Lipid	**1.52**	0.0257	0.3865				
1-carboxyethylvaline	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**1.44**	0.0337	0.3939				
1-oleoyl-2-linoleoyl-GPC (18:1/18:2)	Phosphatidylcholine (PC)	Lipid	**1.27**	0.0083	0.3140				
1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6)	Phosphatidylethanolamine (PE)	Lipid	**1.23**	0.0029	0.2967				
1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)	Plasmalogen	Lipid	**1.18**	0.0300	0.3865				
Malonate	Fatty Acid Synthesis	Lipid	**0.92**	0.0407	0.4355				
N-acetyl-beta-alanine	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.81**	0.0332	0.3938				
1-methyl-4-imidazoleacetate	Histidine Metabolism	Amino Acid	**0.79**	0.0444	0.4462				
Ribitol	Pentose Metabolism	Carbohydrate	**0.78**	0.0069	0.3140				
Cysteine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.78**	0.0388	0.4273				
N-acetylputrescine	Polyamine Metabolism	Amino Acid	**0.77**	0.0486	0.4546	**0.72**	0.0411	1.0000	
2-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid	**0.75**	0.0274	0.3865				
Lactate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.74**	0.0472	0.4525				
4-hydroxychlorothalonil	Chemical	Xenobiotics	**0.71**	0.0237	0.3846				
Alpha-ketoglutarate	TCA Cycle	Energy	**0.71**	0.0466	0.4525				
Adenosine 3',5'-cyclic monophosphate (camp)	Purine Metabolism, Adenine containing	Nucleotide	**0.69**	0.0302	0.3865				
Cortisone	Corticosteroids	Lipid	**0.64**	0.0283	0.3865	**0.67**	0.0352	1.0000	
S-methylcysteine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.61**	0.0429	0.4432				
Stearate (18:0)	Long Chain Saturated Fatty Acid	Lipid	**0.59**	0.0182	0.3766				
Palmitoyl ethanolamide	Endocannabinoid	Lipid	**0.56**	0.0008	0.1776				
N-palmitoylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**0.54**	0.0053	0.3106				
Arachidonate (20:4n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.54**	0.0182	0.3766				

3-hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid	**0.54**	0.0356	0.4040				
Nonadecanoate (19:0)	Long Chain Saturated Fatty Acid	Lipid	**0.53**	0.0058	0.3106				
3-hydroxymyristate	Fatty Acid, Monohydroxy	Lipid	**0.52**	0.0125	0.3140				
1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)	Phosphatidylinositol (PI)	Lipid	**0.52**	0.0271	0.3865	**0.43**	0.0205	1.0000	
Glycerol	Glycerolipid Metabolism	Lipid	**0.51**	0.0221	0.3846				
Pentadecanoate (15:0)	Long Chain Saturated Fatty Acid	Lipid	**0.50**	0.0145	0.3253				
Ceramide (d18:1/14:0, d16:1/16:0)	Ceramides	Lipid	**0.50**	0.0278	0.3865				
Cortisol	Corticosteroids	Lipid	**0.49**	0.0007	0.1776				
Dihomo-linolenate (20:3n3 or n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.49**	0.0295	0.3865				
16-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid	**0.48**	0.0114	0.3140				
Octadecanedioate (C18-DC)	Fatty Acid, Dicarboxylate	Lipid	**0.48**	0.0123	0.3140				
Erucate (22:1n9)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.48**	0.0141	0.3240				
10-undecenoate (11:1n1)	Medium Chain Fatty Acid	Lipid	**0.47**	0.0263	0.3865				
Docosapentaenoate (n6 DPA; 22:5n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.46**	0.0229	0.3846				
Lactose	Disaccharides and Oligosaccharides	Carbohydrate	**0.46**	0.0491	0.4546				
Linoleoyl ethanolamide	Endocannabinoid	Lipid	**0.44**	0.0239	0.3846				
9-hydroxystearate	Fatty Acid, Monohydroxy	Lipid	**0.43**	0.0028	0.2967				
Docosadienoate (22:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.43**	0.0110	0.3140				
Palmitate (16:0)	Long Chain Saturated Fatty Acid	Lipid	**0.41**	0.0026	0.2967				
Margarate (17:0)	Long Chain Saturated Fatty Acid	Lipid	**0.41**	0.0073	0.3140				
Mead acid (20:3n9)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.41**	0.0324	0.3908				

Tetradecadienoate (14:2)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.39**	0.0235	0.3846
Eicosenoate (20:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.38**	0.0134	0.3240
Tridecenedioate (C13:1-DC)	Fatty Acid, Dicarboxylate	Lipid	**0.38**	0.0389	0.4273
(16 or 17)-methylstearate (a19:0 or i19:0)	Fatty Acid, Branched	Lipid	**0.38**	0.0443	0.4462
Myristate (14:0)	Long Chain Saturated Fatty Acid	Lipid	**0.37**	0.0105	0.3140
Docosapentaenoate (n3 DPA; 22:5n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.37**	0.0209	0.3846
Dihomo-linoleate (20:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.36**	0.0044	0.2967
Oleate/vaccenate (18:1)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.36**	0.0081	0.3140
(14 or 15)-methylpalmitate (a17:0 or i17:0)	Fatty Acid, Branched	Lipid	**0.36**	0.0225	0.3846
5-dodecenoate (12:1n7)	Medium Chain Fatty Acid	Lipid	**0.36**	0.0289	0.3865
N-palmitoyl-sphingadienine (d18:2/16:0)	Ceramides	Lipid	**0.35**	0.0037	0.2967
Linoleate (18:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.35**	0.0082	0.3140
3-hydroxybutyroylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**0.34**	0.0190	0.3785
Linolenate [alpha or gamma; (18:3n3 or 6)]	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.34**	0.0205	0.3846
Tetradecadienedioate (C14:2-DC)	Fatty Acid, Dicarboxylate	Lipid	**0.34**	0.0423	0.4425
Adrenate (22:4n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.33**	0.0115	0.3140
3-hydroxybutyrate (BHBA)	Ketone Bodies	Lipid	**0.32**	0.0220	0.3846
Docosatrienoate (22:3n3)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.31**	0.0097	0.3140
10-nonadecenoate (19:1n9)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.30**	0.0193	0.3785

2-butenoylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**0.25**	0.0056	0.3106			
Myristoleate (14:1n5)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.25**	0.0100	0.3140			
3-hydroxykynurenine	Tryptophan Metabolism	Amino Acid	**0.25**	0.0353	0.4040			
10-heptadecenoate (17:1n7)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.24**	0.0137	0.3240			
Testosterone sulfate	Androgenic Steroids	Lipid	**0.24**	0.0392	0.4273			
Hexadecadienoate (16:2n6)	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.21**	0.0039	0.2967			
Palmitoleate (16:1n7)	Long Chain Monounsaturated Fatty Acid	Lipid	**0.21**	0.0064	0.3140			
Trans-2-hexenoylglycine	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**0.21**	0.0449	0.4462			
Glutamine conjugate of C7H12O2	Partially Characterized Molecules	Partially Characterized Molecules	**0.09**	0.0325	0.3908			
Phenylacetylglycine	Acetylated Peptides	Peptide				**4.55**	0.0105	1.0000
N2,N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	Amino Acid				**2.43**	0.0446	1.0000
1,3-dimethylurate	Xanthine Metabolism	Xenobiotics				**2.37**	0.0265	1.0000
Homocitrulline	Urea cycle; Arginine and Proline Metabolism	Amino Acid				**1.48**	0.0158	1.0000
Isoleucine	Leucine, Isoleucine and Valine Metabolism	Amino Acid				**1.40**	0.0085	1.0000
Methylmalonate (MMA)	Fatty Acid Metabolism (also BCAA Metabolism)	Lipid				**1.28**	0.0472	1.0000
1-palmitoyl-2-dihomo-linolenoyl-GPC (16:0/20:3n3 or 6)	Phosphatidylcholine (PC)	Lipid				**1.20**	0.0344	1.0000
2-O-methylascorbic acid	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins				**0.70**	0.0143	1.0000
N,N,N-trimethyl-alanylproline betaine (TMAP)	Urea cycle; Arginine and Proline Metabolism	Amino Acid				**0.67**	0.0092	1.0000

Butyrylcarnitine (C4)	Fatty Acid Metabolism (also BCAA Metabolism)	Lipid	**0.63**	0.0412	1.0000
Ethylmalonate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.59**	0.0326	1.0000
20a-dihydroprogesterone	Progestin Steroids	Lipid	**0.58**	0.0253	1.0000

Early stage of induction of labour (LIOL) of the induction of labour (LIOL) and latent stage of spontaneous labour group (LS) (LIOL/LS); pre-operative sample of the elective caesarean section group (LCS) and latent stage of the spontaneous labour group (LS) (LCS/LS); and pre-operative sample of the elective caesarean section group (LCS) and early stage of induction of labour of the induction of labour group (LIOL) (LCS/LIOL).

APPENDIX 3A7: Table presenting metabolites for which the fold-change (FC) is significant (Welch's two-sample t-test significant when p≤0.05) in the intervillous blood between the different mode of labour onset groups. Corresponding p- and q-values are shown.

Biochemical name	Sub Pathway	Super Pathway	IVIOL IVS (FC)	p-value	q-value	IVCS IVS (FC)	p-value	q-value	IVIOL IVCS (FC)	p-value	q-value
Ranitidine	Drug - Gastrointestinal	Xenobiotics	**1547.50**	0.0002	0.1451	**1273.00**	0.0009	0.0801			
Phenylalanylhydroxyproline	Drug - Gastrointestinal	Xenobiotics	**25.61**	0.0049	0.7798	**13.70**	0.0393	0.3281			
Glutarylcarnitine (C5-DC)	Glycine, Serine and Threonine Metabolism	Amino Acid	**0.71**	0.0062	0.7798						
Ranitidine N-oxide	Glycerolipid Metabolism	Lipid	**0.70**	0.0379	0.7798	**0.64**	0.0145	0.2507			
N-acetylthreonine	Fatty Acid, Dihydroxy	Lipid	**0.70**	0.0448	0.7798						
Glucuronide of C12H22O4 (1)	Histidine Metabolism	Amino Acid	**0.67**	0.0252	0.7798	**0.56**	0.0052	0.1713			
Maltol sulfate	Pyrimidine Metabolism, Orotate containing	Nucleotide	**0.67**	0.0324	0.7798						
8-methoxykynurenate	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**0.67**	0.0348	0.7798						
Isobutyrylglycine	Pentose Metabolism	Carbohydrate	**0.62**	0.0181	0.7798						
Arabitol/xylitol	Gamma-glutamyl Amino Acid	Peptide	**0.62**	0.0210	0.7798				**0.59**	0.0196	0.4939
1-methylguanidine	Aminosugar Metabolism	Carbohydrate	**0.62**	0.0228	0.7798						
Gamma-glutamylhistidine	Tryptophan Metabolism	Amino Acid	**0.61**	0.0295	0.7798	**0.50**	0.0070	0.1860			
Cytosine	Sterol	Lipid	**0.61**	0.0486	0.7798						
Erythronate	Lysine Metabolism	Amino Acid	**0.60**	0.0044	0.7798	**0.55**	0.0224	0.2735			
Malonylcarnitine	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.56**	0.0339	0.7798						
Heptadecenamide (17:1)	Dipeptide	Peptide	**0.52**	0.0041	0.7798						
1-methylhistidine	Fatty Acid Synthesis	Lipid	**0.44**	0.0230	0.7798						
Glycerophosphoglycerol	Fatty Acid Metabolism (Acyl Glycine)	Lipid	**0.44**	0.0444	0.7798						
Glycerophosphoethanolamine	Guanidino and Acetamido Metabolism	Amino Acid	**0.42**	0.0197	0.7798	**0.48**	0.0381	0.3281			

Kynurenate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.41**	0.0172	0.7798				**0.46**	0.0275	0.4939
Glycine conjugate of C10H14O2 (1)	Partially Characterized Molecules	Partially Characterized Molecules	**0.39**	0.0303	0.7798	**0.26**	0.0364	0.3281			
Orotidine	Phospholipid Metabolism	Lipid	**0.38**	0.0488	0.7798				**0.31**	0.0064	0.4497
Quinolinate	Fatty Acid, Amide	Lipid	**0.34**	0.0238	0.7798						
3-methylcytidine	Glycerolipid Metabolism	Lipid	**0.34**	0.0270	0.7798						
Glycerol	Partially Characterized Molecules	Partially Characterized Molecules	**0.33**	0.0118	0.7798	**0.28**	0.0056	0.1713			
7-methylurate	Tryptophan Metabolism	Amino Acid	**0.33**	0.0154	0.7798	**0.38**	0.0198	0.2735			
Picolinoylglycine	Food Component/Plant	Xenobiotics	**0.30**	0.0122	0.7798	**0.40**	0.0407	0.3281			
3,4-dihydroxybutyrate	Pyrimidine Metabolism, Cytidine containing	Nucleotide	**0.27**	0.0212	0.7798	**0.35**	0.0465	0.3488			
7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	Phospholipid Metabolism	Lipid	**0.22**	0.0293	0.7798				**0.23**	0.0015	0.2208
Glycerophosphorylcholine (GPC)	Xanthine Metabolism	Xenobiotics	**0.08**	0.0414	0.7798						
Ribitol	Drug - Antibiotic	Xenobiotics				**4018.33**	0.0000	0.0000	**0.00**	0.0004	0.1197
Isovalerylglycine	Fatty Acid Metabolism (Acyl Choline)	Lipid				**22.21**	0.0009	0.0801			
2-keto-3-deoxy-gluconate	Fatty Acid Metabolism (Acyl Choline)	Lipid				**21.86**	0.0029	0.1599			
Salicylate	Fatty Acid Metabolism (Acyl Choline)	Lipid				**18.24**	0.0037	0.1651			
12,13-dihome	Fatty Acid Metabolism (Acyl Choline)	Lipid				**17.41**	0.0079	0.1869			
2,3-diphosphoglycerate	Fatty Acid Metabolism (Acyl Choline)	Lipid				**15.25**	0.0150	0.2507			
Caffeic acid sulfate	Fatty Acid Metabolism (Acyl Choline)	Lipid				**13.74**	0.0004	0.0618			
N2,N5-diacetylornithine	Fatty Acid Metabolism (Acyl Choline)	Lipid				**13.55**	0.0070	0.1860			
Gamma-carboxyglutamate	Monoacylglycerol	Lipid				**4.56**	0.0168	0.2598			

Azelate (C9-DC)	Drug - Gastrointestinal	Xenobiotics	**2.86**	0.0022	0.1592	**0.35**	0.0022	0.2544
Cortisol 21-sulfate	Primary Bile Acid Metabolism	Lipid	**2.47**	0.0201	0.2735	**0.40**	0.0201	0.4939
Androsterone glucuronide	Food Component/Plant	Xenobiotics	**2.13**	0.0463	0.3488			
Maltotetraose	Androgenic Steroids	Lipid	**1.74**	0.0207	0.2735			
Nicotinamide adenine dinucleotide (NAD+)	Sterol	Lipid	**1.53**	0.0457	0.3488	**0.72**	0.0411	0.4939
N-acetylcarnosine	Medium Chain Fatty Acid	Lipid	**1.51**	0.0488	0.3557	**0.57**	0.0155	0.4939
Vanillic acid glycine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**1.40**	0.0110	0.2206			
Suberate (C8-DC)	Lysine Metabolism	Amino Acid	**0.76**	0.0200	0.2735			
Hexadecasphingosine (d16:1)	Ceramide PEs	Lipid	**0.75**	0.0347	0.3281			
N-acetyltaurine	TCA Cycle	Energy	**0.74**	0.0046	0.1712			
N-acetylglutamate	Lysophospholipid	Lipid	**0.72**	0.0344	0.3281			
Caffeine	Fatty Acid, Dicarboxylate	Lipid	**0.71**	0.0054	0.1713			
Erythritol	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.71**	0.0105	0.2169	**1.56**	0.0374	0.4939
2-hydroxyhippurate (salicylurate)	Alanine and Aspartate Metabolism	Amino Acid	**0.71**	0.0413	0.3281			
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.70**	0.0122	0.2337			
1-carboxyethyltyrosine	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.69**	0.0231	0.2735			
5-oxoproline	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid	**0.68**	0.0234	0.2735	**1.51**	0.0104	0.4842
N-stearoyl-sphinganine (d18:0/18:0)	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.68**	0.0303	0.3249			
Theophylline	Sphingomyelins	Lipid	**0.68**	0.0322	0.3249			
1-carboxyethylisoleucine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.67**	0.0030	0.1599			
7-methylguanine	Chemical	Xenobiotics	**0.65**	0.0397	0.3281			
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid	**0.64**	0.0142	0.2507			

1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	Glutamate Metabolism	Amino Acid	**0.63**	0.0026	0.1599			
N-acetylglucosaminylasparagine	Fatty Acid, Monohydroxy	Lipid	**0.63**	0.0049	0.1713			
1,3,7-trimethylurate	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.63**	0.0197	0.2735			
Succinylcarnitine (C4-DC)	Lysine Metabolism	Amino Acid	**0.61**	0.0227	0.2735			
Sedoheptulose	Pyrimidine Metabolism, Uracil containing	Nucleotide	**0.61**	0.0346	0.3281			
1-stearoyl-2-oleoyl-GPS (18:0/18:1)	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.61**	0.0391	0.3281			
1-linoleoyl-GPA (18:2)	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.60**	0.0216	0.2735			
Tyramine O-sulfate	Fatty Acid, Dicarboxylate	Lipid	**0.60**	0.0235	0.2735			
Homocitrulline	Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	Lipid	**0.60**	0.0406	0.3281			
N-acetyl-2-aminooctanoate	Creatine Metabolism	Amino Acid	**0.60**	0.0425	0.3346			
Xanthosine	Pyrimidine Metabolism, Orotate containing	Nucleotide	**0.59**	0.0073	0.1860			
Gulonate	Fatty Acid, Dicarboxylate	Lipid	**0.59**	0.0378	0.3281	**1.54**	0.0277	0.4939
Sphingosine 1-phosphate	Docosanoid	Lipid	**0.58**	0.0472	0.3506			
N1-methyladenosine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.57**	0.0231	0.2735			
Lactose	Lysine Metabolism	Amino Acid	**0.57**	0.0296	0.3249			
1-arachidonoyl-GPA (20:4)	Fatty Acid, Dicarboxylate	Lipid	**0.57**	0.0411	0.3281			
Guanidinoacetate	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.57**	0.0495	0.3581			
Pseudouridine	Phenylalanine Metabolism	Amino Acid	**0.56**	0.0047	0.1712			
Dihydrocaffeate sulfate (2)	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	**0.56**	0.0190	0.2735			
3-hydroxymyristate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.55**	0.0007	0.0801	**1.49**	0.0191	0.4939
Theobromine	Fatty Acid, Monohydroxy	Lipid	**0.55**	0.0043	0.1712			

Adenosine 5'-diphosphoribose (ADP-ribose)	Medium Chain Fatty Acid	Lipid	**0.55**	0.0255	0.2887				
Cystathionine	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	Lipid	**0.55**	0.0324	0.3249				
N,N-dimethylalanine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.52**	0.0384	0.3281				
5alpha-pregnan-3beta,20alpha-diol monosulfate (1)	Fatty Acid, Dicarboxylate	Lipid	**0.50**	0.0073	0.1860				
1,2-dipalmitoyl-GPE (16:0/16:0)	Sphingosines	Lipid	**0.50**	0.0154	0.2507				
Flavin adenine dinucleotide (FAD)	Pregnenolone Steroids	Lipid	**0.50**	0.0219	0.2735				
1-carboxyethylvaline	Fatty Acid, Amino	Lipid	**0.50**	0.0446	0.3442				
Orotate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	**0.48**	0.0025	0.1599	**2.18**	0.0052	0.4497	
11beta-hydroxyandrosterone glucuronide	Tyrosine Metabolism	Amino Acid	**0.48**	0.0093	0.2014				
Pentose acid	Fatty Acid, Monohydroxy	Lipid	**0.48**	0.0165	0.2590				
Eicosapentaenoate (EPA; 20:5n3)	Food Component/Plant	Xenobiotics	**0.47**	0.0388	0.3281				
Palmitoyl-sphingosine-phosphoethanolamine (d18:1/16:0)	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.46**	0.0371	0.3281				
3-phosphoglycerate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.43**	0.0083	0.1911				
3-hydroxyphenylacetoylglutamine	Fatty Acid, Monohydroxy	Lipid	**0.43**	0.0255	0.2887				
2-butenoylglycine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.43**	0.0321	0.3249	**2.24**	0.0387	0.4939	
Beta-citrylglutamate	Fatty Acid, Dicarboxylate	Lipid	**0.43**	0.0338	0.3281				
N1-methylinosine	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.42**	0.0095	0.2014				
Xanthurenate	Fatty Acid, Dicarboxylate	Lipid	**0.42**	0.0349	0.3281				
N-acetylleucine	Food Component/Plant	Xenobiotics	**0.38**	0.0311	0.3249				
Glutamine conjugate of C6H10O2 (1)	Phenylalanine Metabolism	Amino Acid	**0.36**	0.0004	0.0618	**2.10**	0.0253	0.4939	

N-acetylvaline	Partially Characterized Molecules	Partially Characterized Molecules	**0.35**	0.0133	0.2478			
Propionylglycine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	**0.35**	0.0315	0.3249			
1,3-dimethylurate	Tyrosine Metabolism	Amino Acid	**0.34**	0.0037	0.1651			
Creatinine	Corticosteroids	Lipid	**0.34**	0.0074	0.1860	**2.44**	0.0005	0.1197
Estriol-3-glucuronide	Androgenic Steroids	Lipid	**0.31**	0.0392	0.3281			
Spermine	Corticosteroids	Lipid	**0.31**	0.0476	0.3506			
Methyl indole-3-acetate	Androgenic Steroids	Lipid	**0.30**	0.0153	0.2507			
4-acetamidobutanoate	Histidine Metabolism	Amino Acid	**0.30**	0.0307	0.3249			
Maltotriose	Fatty Acid, Dicarboxylate	Lipid	**0.29**	0.0064	0.1860			
4-hydroxyphenylacetylglutamine	Pregnenolone Steroids	Lipid	**0.27**	0.0199	0.2735			
2-hydroxysebacate	Corticosteroids	Lipid	**0.25**	0.0156	0.2507			
Glycine	Fatty Acid, Dicarboxylate	Lipid	**0.24**	0.0121	0.2337			
(S)-3-hydroxybutyrylcarnitine	Pyrimidine Metabolism, Orotate containing	Nucleotide	**0.24**	0.0403	0.3281			
Glutarate (C5-DC)	Androgenic Steroids	Lipid	**0.21**	0.0215	0.2735			
Citraconate/glutaconate	Estrogenic Steroids	Lipid	**0.21**	0.0380	0.3281			
Cysteine	Histidine Metabolism	Amino Acid	**0.18**	0.0442	0.3442			
Mannitol/sorbitol	Corticosteroids	Lipid	**0.16**	0.0032	0.1599	**3.67**	0.0111	0.4842
Adenine	Drug - Analgesics, Anesthetics	Xenobiotics	**0.06**	0.0040	0.1670			
Lactate	Drug - Analgesics, Anesthetics	Xenobiotics	**0.03**	0.0093	0.2014			
Docosadioate (C22-DC)	Partially Characterized Molecules	Partially Characterized Molecules	**0.03**	0.0283	0.3158	**12.33**	0.0167	0.4939
Nicotinamide	Drug - Analgesics, Anesthetics	Xenobiotics	**0.02**	0.0008	0.0801			
Ectoine	Drug - Analgesics, Anesthetics	Xenobiotics	**0.02**	0.0077	0.1869			
14-hdohe/17-hdohe	Drug - Analgesics, Anesthetics	Xenobiotics	**0.02**	0.0141	0.2507			
1-palmitoyl-2-gamma-linolenoyl-GPC (16:0/18:3n6)	Drug - Analgesics, Anesthetics	Xenobiotics	**0.02**	0.0181	0.2734			
Gluconate	Drug - Analgesics, Anesthetics	Xenobiotics	**0.01**	0.0002	0.0618			

N-acetylneuraminate	Drug - Analgesics, Anesthetics	Xenobiotics	**0.01**	0.0003	0.0618			
N-ethylglycinexylidide	Drug - Analgesics, Anesthetics	Xenobiotics	**0.01**	0.0010	0.0801			
1,2-dioleoyl-GPC (18:1/18:1)	Partially Characterized Molecules	Partially Characterized Molecules				**18.15**	0.0005	0.1197
Aspartate	Partially Characterized Molecules	Partially Characterized Molecules				**10.90**	0.0016	0.2208
N-acetylglutamine	Benzoate Metabolism	Xenobiotics				**6.24**	0.0305	0.4939
Eicosanedioate (C20-DC)	Phosphatidylcholine (PC)	Lipid				**5.18**	0.0083	0.4497
3-hydroxy-2-ethylpropionate	Alanine and Aspartate Metabolism	Amino Acid				**3.72**	0.0439	0.4939
Acisoga	Pregnenolone Steroids	Lipid				**2.32**	0.0405	0.4939
5alpha-androstan-3beta,17beta-diol disulfate	Phenylalanine Metabolism	Amino Acid				**1.80**	0.0366	0.4939
Pro-hydroxy-pro	Dihydroceramides	Lipid				**1.66**	0.0457	0.4939
Alpha-ketoglutaramate	Fatty Acid, Branched	Lipid				**1.53**	0.0413	0.4939
4-methylbenzenesulfonate	Medium Chain Fatty Acid	Lipid				**1.51**	0.0393	0.4939
5,6-dihydrouridine	Fatty Acid Metabolism (Acyl Carnitine, Monounsaturated)	Lipid				**1.50**	0.0425	0.4939
Indoleacetylglutamine	Polyamine Metabolism	Amino Acid				**1.45**	0.0140	0.4939
Carnitine of C10H14O2 (5)	Tyrosine Metabolism	Amino Acid				**1.20**	0.0084	0.4497
Androstenediol (3alpha, 17alpha) monosulfate (2)	Sphingomyelins	Lipid				**0.79**	0.0078	0.4497
Hydroxy-N6,N6,N6-trimethyllysine	Phosphatidylcholine (PC)	Lipid				**0.79**	0.0433	0.4939
N-acetylhistidine	Phosphatidylcholine (PC)	Lipid				**0.77**	0.0470	0.4939
3-hydroxybutyroylglycine	Pyrimidine Metabolism, Uracil containing	Nucleotide				**0.69**	0.0457	0.4939
Taurine	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	Lipid				**0.64**	0.0365	0.4939
Malate	Glutamate Metabolism	Amino Acid				**0.53**	0.0267	0.4939
2-isopropylmalate	Phosphatidylserine (PS)	Lipid				**0.50**	0.0395	0.4939

Glutathione, oxidized (GSSG)	Tryptophan Metabolism	Amino Acid	**0.38**	0.0495	0.4939
Glucuronide of piperine metabolite C17H21NO3 (4)	Fatty Acid Metabolism (also BCAA Metabolism)	Lipid	**0.36**	0.0097	0.4830
2-O-methylascorbic acid	Leucine, Isoleucine and Valine Metabolism	Amino Acid	**0.36**	0.0355	0.4939
1,2-dipalmitoyl-GPC (16:0/16:0)	Gamma-glutamyl Amino Acid	Peptide	**0.34**	0.0419	0.4939
Ceramide (d18:1/14:0, d16:1/16:0)	Aminosugar Metabolism	Carbohydrate	**0.33**	0.0476	0.4939
1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	Alanine and Aspartate Metabolism	Amino Acid	**0.30**	0.0297	0.4939
Methylmalonate (MMA)	Phosphatidylcholine (PC)	Lipid	**0.27**	0.0425	0.4939
Pyrraline	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	**0.25**	0.0247	0.4939
Laurate (12:0)	Glycogen Metabolism	Carbohydrate	**0.24**	0.0287	0.4939
Fumarate	Urea cycle; Arginine and Proline Metabolism	Amino Acid	**0.16**	0.0072	0.4497
N-methylhydroxyproline	Polyamine Metabolism	Amino Acid	**0.11**	0.0032	0.3187

Intervillous blood of the induction of labour group (IVIOL) and intervillous blood of the spontaneously labouring group (IVS) (IVIOL/IVS); intervillous blood of the elective caesarean section group (IVCS) and intervillous blood of the spontaneous labour group (IVS) (IVCS/IVS); and intervillous blood of the elective caesarean section group (IVCS) and intervillous blood of the induction of labour group (IVIOL) (IVCS/IVIOL).

APPENDIX 4A1: Maternal pregnancy metabolites measured using NMR metabolomics among the women included in the analysis dataset with mean, standard deviation and number of missing/not measured values for those pregnancies included in the analysis sample (n=7440). The biochemical group of the metabolite and units the metabolite is measured in are also presented.

Metabolite	Group	Units	Mean	Standard Deviation	Missing
Alanine	Amino acids	mmol/l	0.418	0.048	32
Glutamine	Amino acids	mmol/l	0.460	0.046	9
Glycine	Amino acids	mmol/l	0.216	0.032	19
Histidine	Amino acids	mmol/l	0.061	0.010	10
Apolipoprotein A-I	Apolipoproteins	g/l	1.895	0.220	5
Apolipoprotein B	Apolipoproteins	g/l	1.026	0.228	5
apolipoprotein B:apolipoprotein A-I	Apolipoproteins	NA	0.545	0.121	5
Phenylalanine	Aromatic amino acids	mmol/l	0.080	0.011	4
Tyrosine	Aromatic amino acids	mmol/l	0.037	0.006	13
Isoleucine	Branched--chain amino acids	mmol/l	0.047	0.010	7
Leucine	Branched--chain amino acids	mmol/l	0.069	0.010	7
Valine	Branched--chain amino acids	mmol/l	0.112	0.018	14
Esterified cholesterol	Cholesterol	mmol/l	4.205	0.807	31
Free cholesterol	Cholesterol	mmol/l	1.592	0.334	31
Remnant cholesterol (non-HDL, non-LDL)	Cholesterol	mmol/l	1.684	0.467	5
Serum total cholesterol	Cholesterol	mmol/l	5.806	1.157	5
Total cholesterol in HDL	Cholesterol	mmol/l	1.993	0.370	5
Total cholesterol in HDL2	Cholesterol	mmol/l	1.415	0.335	5
Total cholesterol in HDL3	Cholesterol	mmol/l	0.578	0.044	5
Total cholesterol in LDL	Cholesterol	mmol/l	2.129	0.617	5
Total cholesterol in VLDL	Cholesterol	mmol/l	0.811	0.276	5
18:2 linoleic acid to total FAs	Fatty acids (%)	%	28.266	2.691	54

22:6 docosahexaenoic acid to total FAs	Fatty acids (%)	%	1.746	0.230	54
Monounsaturated FAs to total FAs	Fatty acids (%)	%	27.967	2.415	54
Omega-3 FAs to total FAs	Fatty acids (%)	%	3.832	0.493	54
Omega-6 FAs to total FAs	Fatty acids (%)	%	31.395	2.205	54
Polyunsaturated FAs to total FAs	Fatty acids (%)	%	35.227	2.386	54
Saturated FAs to total FAs	Fatty acids (%)	%	36.806	1.508	54
18:2, linoleic acid	Fatty acids and saturation measures	mmol/l	4.467	0.766	54
22:6, docosahexaenoic acid	Fatty acids and saturation measures	mmol/l	0.274	0.043	54
Estimated degree of unsaturation	Fatty acids and saturation measures	NA	1.149	0.043	54
Monounsaturated fatty acids; 16:1, 18:1	Fatty acids and saturation measures	mmol/l	4.467	0.997	54
Omega-3 fatty acids	Fatty acids and saturation measures	mmol/l	0.604	0.104	54
Omega-6 fatty acids	Fatty acids and saturation measures	mmol/l	4.970	0.840	54
Polyunsaturated fatty acids	Fatty acids and saturation measures	mmol/l	5.574	0.917	54
Saturated fatty acids	Fatty acids and saturation measures	mmol/l	5.846	1.044	54
Total fatty acids	Fatty acids and saturation measures	mmol/l	15.887	2.779	54
Albumin	Fluid balance and inflammation	Signal area	0.078	0.006	4
Creatinine	Fluid balance and inflammation	mmol/l	0.040	0.006	9
Glycoprotein acetyls (a1-acid glycoprotein)	Fluid balance and inflammation	mmol/l	1.744	0.198	4
Phosphatidylcholine and other cholines	Glycerides and phospholipids	mmol/l	2.515	0.459	33
Ratio of triglycerides to phosphoglycerides	Glycerides and phospholipids	NA	0.764	0.160	31
Serum total triglycerides	Glycerides and phospholipids	mmol/l	1.565	0.580	5
Sphingomyelins	Glycerides and phospholipids	mmol/l	0.526	0.086	33
Total cholines	Glycerides and phospholipids	mmol/l	3.124	0.485	33
Total phosphoglycerides	Glycerides and phospholipids	mmol/l	2.508	0.399	31
Triglycerides in HDL	Glycerides and phospholipids	mmol/l	0.205	0.042	5
Triglycerides in LDL	Glycerides and phospholipids	mmol/l	0.288	0.070	5
Triglycerides in VLDL	Glycerides and phospholipids	mmol/l	0.906	0.460	5
Citrate	Glycolysis related metabolites	mmol/l	0.110	0.017	132
Glucose	Glycolysis related metabolites	mmol/l	3.257	0.489	29
Glycerol	Glycolysis related metabolites	mmol/l	0.055	0.016	19

Lactate	Glycolysis related metabolites	mmol/l	1.642	0.493	16
Pyruvate	Glycolysis related metabolites	mmol/l	0.087	0.022	20
3-hydroxybutyrate	Ketone bodies	mmol/l	0.114	0.038	5
Acetate	Ketone bodies	mmol/l	0.035	0.006	11
Mean diameter for HDL particles	Lipoprotein particle size	nm	10.268	0.205	5
Mean diameter for LDL particles	Lipoprotein particle size	nm	23.528	0.062	5
Mean diameter for VLDL particles	Lipoprotein particle size	nm	36.368	1.009	5
Cholesterol esters in large HDL	Lipoprotein subclasses HDL	mmol/l	0.486	0.144	0
Cholesterol esters in medium HDL	Lipoprotein subclasses HDL	mmol/l	0.399	0.087	0
Cholesterol esters in small HDL	Lipoprotein subclasses HDL	mmol/l	0.416	0.059	0
Cholesterol esters in very large HDL	Lipoprotein subclasses HDL	mmol/l	0.245	0.074	0
Concentration of large HDL particles	Lipoprotein subclasses HDL	mol/l	2.1E-06	5.56E-07	0
Concentration of medium HDL particles	Lipoprotein subclasses HDL	mol/l	2.49E-06	4.49E-07	0
Concentration of small HDL particles	Lipoprotein subclasses HDL	mol/l	5.31E-06	6.38E-07	0
Concentration of very large HDL particles	Lipoprotein subclasses HDL	mol/l	7.46E-07	2.28E-07	0
Free cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	0.142	0.045	0
Free cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	0.102	0.023	0
Free cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	0.115	0.017	0
Free cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	0.087	0.030	0
Phospholipids in large HDL	Lipoprotein subclasses HDL	mmol/l	0.629	0.157	0
Phospholipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	0.504	0.087	0
Phospholipids in small HDL	Lipoprotein subclasses HDL	mmol/l	0.590	0.094	0
Phospholipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	0.384	0.123	0
Total cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	0.628	0.189	0
Total cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	0.501	0.109	0
Total cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	0.531	0.067	0
Total cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	0.333	0.104	0
Total lipids in large HDL	Lipoprotein subclasses HDL	mmol/l	1.320	0.356	0
Total lipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	1.057	0.195	0
Total lipids in small HDL	Lipoprotein subclasses HDL	mmol/l	1.179	0.142	0

Total lipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	0.748	0.231	0
Triglycerides in large HDL	Lipoprotein subclasses HDL	mmol/l	0.063	0.016	0
Triglycerides in medium HDL	Lipoprotein subclasses HDL	mmol/l	0.052	0.013	0
Triglycerides in small HDL	Lipoprotein subclasses HDL	mmol/l	0.058	0.016	0
Triglycerides in very large HDL	Lipoprotein subclasses HDL	mmol/l	0.032	0.010	0
Cholesterol esters in large LDL	Lipoprotein subclasses LDL	mmol/l	0.794	0.234	0
Cholesterol esters in LDL	Lipoprotein subclasses LDL	mmol/l	0.615	0.163	0
Cholesterol esters in medium LDL	Lipoprotein subclasses LDL	mmol/l	0.464	0.157	0
Cholesterol esters in small LDL	Lipoprotein subclasses LDL	mmol/l	0.285	0.095	0
Concentration of large LDL particles	Lipoprotein subclasses LDL	mol/l	2.33E-07	5.99E-08	0
Concentration of LDL particles	Lipoprotein subclasses LDL	mol/l	1.4E-07	3.42E-08	5
Concentration of medium LDL particles	Lipoprotein subclasses LDL	mol/l	0.000	0.000	0
Concentration of small LDL particles	Lipoprotein subclasses LDL	mol/l	2.22E-07	5.67E-08	0
Free cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	0.308	0.076	0
Free cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	0.257	0.067	0
Free cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	0.172	0.037	0
Free cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	0.106	0.023	0
Phospholipids in large LDL	Lipoprotein subclasses LDL	mmol/l	0.391	0.088	0
Phospholipids in LDL	Lipoprotein subclasses LDL	mmol/l	0.369	0.090	0
Phospholipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	0.241	0.050	0
Phospholipids in small LDL	Lipoprotein subclasses LDL	mmol/l	0.179	0.034	0
Total cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	1.101	0.310	0
Total cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	0.872	0.230	0
Total cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	0.636	0.193	0
Total cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	0.391	0.117	0
Total lipids in large LDL	Lipoprotein subclasses LDL	mmol/l	1.650	0.428	0
Total lipids in LDL	Lipoprotein subclasses LDL	mmol/l	1.409	0.349	5
Total lipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	0.957	0.258	0
Total lipids in small LDL	Lipoprotein subclasses LDL	mmol/l	0.618	0.159	0
Triglycerides in large LDL	Lipoprotein subclasses LDL	mmol/l	0.158	0.039	0

Triglycerides in LDL	Lipoprotein subclasses LDL	mmol/l	0.167	0.042	0
Triglycerides in medium LDL	Lipoprotein subclasses LDL	mmol/l	0.081	0.020	0
Triglycerides in small LDL	Lipoprotein subclasses LDL	mmol/l	0.049	0.013	0
Cholesterol esters in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.003	0.002	0
Cholesterol esters in large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.033	0.022	0
Cholesterol esters in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	0.107	0.043	0
Cholesterol esters in small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.161	0.054	0
Cholesterol esters in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.007	0.006	0
Cholesterol esters in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.191	0.053	0
Conc. of CMs & extremely large VLDL particles	Lipoprotein subclasses VLDL	mol/l	1.1E-10	8.42E-11	0
Concentration of large VLDL particles	Lipoprotein subclasses VLDL	mol/l	5.11E-09	3.42E-09	0
Concentration of medium VLDL particles	Lipoprotein subclasses VLDL	mol/l	1.77E-08	8.93E-09	0
Concentration of small VLDL particles	Lipoprotein subclasses VLDL	mol/l	3.24E-08	1.1E-08	0
Concentration of very large VLDL particles	Lipoprotein subclasses VLDL	mol/l	6.97E-10	5.67E-10	0
Concentration of very small VLDL particles	Lipoprotein subclasses VLDL	mol/l	4.85E-08	1.28E-08	0
Free cholesterol in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.002	0.002	0
Free cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.033	0.023	0
Free cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	0.074	0.039	0
Free cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.098	0.031	0
Free cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.006	0.005	0
Free cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.096	0.026	0
Phospholipids in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.003	0.002	0
Phospholipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.057	0.037	0
Phospholipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	0.122	0.058	0
Phospholipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.148	0.044	0
Phospholipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.012	0.009	0
Phospholipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.186	0.052	0
Total cholesterol in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.005	0.004	0
Total cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.066	0.046	0

Total cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	0.181	0.080	0
Total cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.258	0.082	0
Total cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.013	0.011	0
Total cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.286	0.078	0
Total lipids in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.024	0.018	0
Total lipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.296	0.199	0
Total lipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	0.598	0.298	0
Total lipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.647	0.215	0
Total lipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.068	0.055	0
Total lipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.611	0.161	0
Triglycerides in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.016	0.012	0
Triglycerides in large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.173	0.117	0
Triglycerides in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	0.294	0.163	0
Triglycerides in small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.240	0.097	0
Triglycerides in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.043	0.035	0
Triglycerides in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.139	0.042	0

VLDL= very low density lipoprotein; LDL= low density lipoprotein; HDL= high density lipoprotein; CM= chylomicron; mmol/l= millimole per litre; mol/l= mole per litre; nm= nanometre; %=percentage

APPENDIX 4A2: Maternal pregnancy metabolites measured using NMR metabolomics among the women included in the analysis dataset comparing median, interquartile range and number of missing/not measured values between those pregnancies that delivered preterm (n=399), at term (n=6994) and post-term (n=58). The biochemical group of the metabolite and units the metabolite is measured in are also presented.

			Pre-term (n=392)			Term (n=6994)			Post-term (n=54)		
Metabolite	**Group**	**Units**	**Median**	**IQR**	**Missing**	**Median**	**IQR**	**Missing**	**Median**	**IQR**	**Missing**
Alanine	**Amino acids**	**mmol/l**	**0.421**	**0.388 to 0.454**	**3**	**0.415**	**0.386 to 0.447**	**29**	**0.406**	**0.377 to 0.436**	**0**
Glutamine	**Amino acids**	**mmol/l**	**0.459**	**0.434 to 0.488**	**0**	**0.457**	**0.431 to 0.488**	**9**	**0.451**	**0.425 to 0.479**	**0**
Glycine	**Amino acids**	**mmol/l**	**0.215**	**0.195 to 0.236**	**4**	**0.213**	**0.194 to 0.235**	**15**	**0.220**	**0.196 to 0.237**	**0**
Histidine	**Amino acids**	**mmol/l**	**0.061**	**0.055 to 0.067**	**1**	**0.060**	**0.055 to 0.067**	**9**	**0.060**	**0.057 to 0.067**	**0**
Apolipoprotein A-I	**Apolipoproteins**	**g/l**	**1.891**	**1.734 to 2.027**	**0**	**1.885**	**1.748 to 2.027**	**5**	**1.826**	**1.715 to 1.994**	**0**
Apolipoprotein B	**Apolipoproteins**	**g/l**	**1.040**	**0.879 to 1.182**	**0**	**1.002**	**0.864 to 1.155**	**5**	**1.000**	**0.895 to 1.135**	**0**
Apolipoprotein B:apolipoprotein A-I	**Apolipoproteins**	**#N/A**	**0.545**	**0.467 to 0.635**	**0**	**0.531**	**0.461 to 0.615**	**5**	**0.526**	**0.46 to 0.631**	**0**
Phenylalanine	**Aromatic amino acids**	**mmol/l**	**0.080**	**0.074 to 0.089**	**0**	**0.078**	**0.072 to 0.086**	**4**	**0.079**	**0.074 to 0.088**	**0**

Tyrosine	Aromatic amino acids	mmol/l	0.038	0.035 to 0.042	0	0.037	0.034 to 0.041	13	0.038	0.035 to 0.041	0
Isoleucine	Branched--chain amino acids	mmol/l	0.047	0.041 to 0.055	1	0.045	0.04 to 0.053	6	0.045	0.039 to 0.052	0
Leucine	Branched--chain amino acids	mmol/l	0.069	0.064 to 0.077	1	0.068	0.063 to 0.075	6	0.067	0.063 to 0.073	0
Valine	Branched--chain amino acids	mmol/l	0.111	0.1 to 0.124	2	0.110	0.1 to 0.122	12	0.111	0.1 to 0.122	0
Esterified cholesterol	Cholesterol	mmol/l	4.212	3.683 to 4.723	2	4.151	3.648 to 4.69	29	4.100	3.686 to 4.721	0
Free cholesterol	Cholesterol	mmol/l	1.590	1.379 to 1.825	2	1.568	1.361 to 1.789	29	1.541	1.358 to 1.787	0
Remnant cholesterol (non-HDL, non-LDL)	Cholesterol	mmol/l	1.695	1.385 to 1.996	0	1.633	1.353 to 1.947	5	1.608	1.435 to 1.916	0
Serum total cholesterol	Cholesterol	mmol/l	5.795	5.092 to 6.578	0	5.722	5.019 to 6.48	5	5.627	5.044 to 6.515	0
Total cholesterol in HDL	Cholesterol	mmol/l	1.984	1.715 to 2.213	0	1.976	1.745 to 2.217	5	1.920	1.612 to 2.201	0
Total cholesterol in HDL2	Cholesterol	mmol/l	1.400	1.152 to 1.608	0	1.399	1.192 to 1.617	5	1.349	1.091 to 1.589	0
Total cholesterol in HDL3	Cholesterol	mmol/l	0.577	0.55 to 0.607	0	0.575	0.549 to 0.604	5	0.570	0.548 to 0.596	0

Total cholesterol in LDL	**Cholesterol**	**mmol/l**	**2.118**	**1.698 to 2.569**	**0**	**2.076**	**1.708 to 2.482**	**5**	**2.097**	**1.675 to 2.454**	**0**
Total cholesterol in VLDL	**Cholesterol**	**mmol/l**	**0.820**	**0.64 to 1.001**	**0**	**0.776**	**0.616 to 0.96**	**5**	**0.781**	**0.605 to 0.972**	**0**
18:2 linoleic acid to total fas	**Fatty acids (%)**	**%**	**27.885**	**26.08 to 29.46**	**6**	**28.220**	**26.48 to 30.03**	**48**	**27.535**	**26.26 to 29.17**	**0**
22:6 docosahexaenoic acid to total fas	**Fatty acids (%)**	**%**	**1.717**	**1.576 to 1.852**	**6**	**1.724**	**1.593 to 1.878**	**48**	**1.732**	**1.573 to 1.906**	**0**
Monounsaturated fas to total fas	**Fatty acids (%)**	**%**	**28.090**	**26.61 to 29.93**	**6**	**27.880**	**26.29 to 29.49**	**48**	**28.400**	**27.05 to 30.55**	**0**
Omega-3 fas to total fas	**Fatty acids (%)**	**%**	**3.771**	**3.489 to 4.116**	**6**	**3.806**	**3.518 to 4.111**	**48**	**3.841**	**3.408 to 4.125**	**0**
Omega-6 fas to total fas	**Fatty acids (%)**	**%**	**31.045**	**29.48 to 32.58**	**6**	**31.435**	**30 to 32.9**	**48**	**30.885**	**29.54 to 32.27**	**0**
Polyunsaturated fas to total fas	**Fatty acids (%)**	**%**	**34.870**	**33.16 to 36.6**	**6**	**35.270**	**33.71 to 36.87**	**48**	**35.030**	**32.82 to 36.22**	**0**
Saturated fas to total fas	**Fatty acids (%)**	**%**	**36.910**	**35.82 to 37.91**	**6**	**36.720**	**35.76 to 37.78**	**48**	**36.495**	**35.42 to 37.66**	**0**
18:2, linoleic acid	**Fatty acids and saturation measures**	**mmol/l**	**4.427**	**3.95 to 4.961**	**6**	**4.409**	**3.936 to 4.931**	**48**	**4.402**	**3.879 to 4.707**	**0**
22:6, docosahexaenoic acid	**Fatty acids and saturation measures**	**mmol/l**	**0.273**	**0.247 to 0.3**	**6**	**0.270**	**0.244 to 0.299**	**48**	**0.267**	**0.243 to 0.303**	**0**

Estimated degree of unsaturation	Fatty acids and saturation measures	#N/A	1.148	1.119 to 1.173	6	1.150	1.121 to 1.177	48	1.143	1.107 to 1.175	0
Monounsaturated fatty acids; 16:1, 18:1	Fatty acids and saturation measures	mmol/l	4.520	3.848 to 5.221	6	4.350	3.766 to 5.041	48	4.488	3.802 to 5.052	0
Omega-3 fatty acids	Fatty acids and saturation measures	mmol/l	0.604	0.534 to 0.672	6	0.593	0.533 to 0.664	48	0.583	0.517 to 0.644	0
Omega-6 fatty acids	Fatty acids and saturation measures	mmol/l	4.979	4.393 to 5.541	6	4.911	4.388 to 5.478	48	4.774	4.362 to 5.295	0
Polyunsaturated fatty acids	Fatty acids and saturation measures	mmol/l	5.586	4.934 to 6.225	6	5.514	4.936 to 6.125	48	5.370	4.879 to 5.922	0
Saturated fatty acids	Fatty acids and saturation measures	mmol/l	5.966	5.224 to 6.579	6	5.750	5.108 to 6.46	48	5.731	5.119 to 6.312	0
Total fatty acids	Fatty acids and saturation measures	mmol/l	16.150	14.06 to 17.83	6	15.650	13.92 to 17.57	48	15.595	14.22 to 17.05	0
Albumin	Fluid balance and inflammation	signal area	0.079	0.077 to 0.082	0	0.079	0.076 to 0.082	4	0.079	0.078 to 0.081	0
Creatinine	Fluid balance and inflammation	mmol/l	0.041	0.038 to 0.045	1	0.039	0.036 to 0.044	8	0.040	0.038 to 0.044	0
Glycoprotein acetyls (a1-acid glycoprotein)	Fluid balance and inflammation	mmol/l	1.755	1.633 to 1.9	0	1.724	1.609 to 1.851	4	1.709	1.651 to 1.851	0
Phosphatidylcholine and other cholines	Glycerides and phospholipids	mmol/l	2.547	2.198 to 2.853	2	2.485	2.202 to 2.797	31	2.447	2.173 to 2.777	0

Ratio of triglycerides to phosphoglycerides	Glycerides and phospholipids	#N/A	0.770	0.663 to 0.878	2	0.742	0.654 to 0.853	29	0.743	0.66 to 0.893	0
Serum total triglycerides	Glycerides and phospholipids	mmol/l	1.576	1.218 to 1.964	0	1.467	1.171 to 1.851	5	1.452	1.195 to 1.98	0
Sphingomyelins	Glycerides and phospholipids	mmol/l	0.520	0.462 to 0.591	2	0.522	0.469 to 0.58	31	0.499	0.46 to 0.584	0
Total cholines	Glycerides and phospholipids	mmol/l	3.140	2.795 to 3.492	2	3.097	2.791 to 3.417	31	3.020	2.737 to 3.394	0
Total phosphoglycerides	Glycerides and phospholipids	mmol/l	2.551	2.247 to 2.824	2	2.485	2.235 to 2.753	29	2.448	2.2 to 2.711	0
Triglycerides in HDL	Glycerides and phospholipids	mmol/l	0.206	0.179 to 0.234	0	0.200	0.177 to 0.229	5	0.204	0.173 to 0.231	0
Triglycerides in LDL	Glycerides and phospholipids	mmol/l	0.290	0.244 to 0.335	0	0.280	0.239 to 0.326	5	0.282	0.244 to 0.318	0
Triglycerides in VLDL	Glycerides and phospholipids	mmol/l	0.882	0.636 to 1.232	0	0.818	0.595 to 1.122	5	0.793	0.586 to 1.246	0
Citrate	Glycolysis related metabolites	mmol/l	0.108	0.099 to 0.123	9	0.108	0.099 to 0.12	123	0.109	0.097 to 0.118	0
Glucose	Glycolysis related metabolites	mmol/l	3.245	3.014 to 3.5	0	3.241	3.009 to 3.48	29	3.212	3.052 to 3.487	0
Glycerol	Glycolysis related metabolites	mmol/l	0.056	0.047 to 0.069	0	0.053	0.044 to 0.064	18	0.057	0.045 to 0.069	1

Lactate	**Glycolysis related metabolites**	**mmol/l**	**1.557**	**1.355 to 1.855**	**0**	**1.545**	**1.316 to 1.86**	**16**	**1.621**	**1.358 to 1.9**	**0**
Pyruvate	**Glycolysis related metabolites**	**mmol/l**	**0.085**	**0.074 to 0.102**	**0**	**0.083**	**0.072 to 0.099**	**20**	**0.085**	**0.07 to 0.108**	**0**
3-hydroxybutyrate	**Ketone bodies**	**mmol/l**	**0.106**	**0.094 to 0.128**	**1**	**0.107**	**0.093 to 0.126**	**4**	**0.110**	**0.097 to 0.122**	**0**
Acetate	**Ketone bodies**	**mmol/l**	**0.033**	**0.031 to 0.038**	**1**	**0.034**	**0.031 to 0.038**	**10**	**0.033**	**0.031 to 0.036**	**0**
Mean diameter for HDL particles	**Lipoprotein particle size**	**nm**	**10.230**	**10.08 to 10.375**	**0**	**10.270**	**10.13 to 10.4**	**5**	**10.225**	**10.1 to 10.43**	**0**
Mean diameter for LDL particles	**Lipoprotein particle size**	**nm**	**23.520**	**23.48 to 23.56**	**0**	**23.530**	**23.49 to 23.56**	**5**	**23.530**	**23.49 to 23.56**	**0**
Mean diameter for VLDL particles	**Lipoprotein particle size**	**nm**	**36.500**	**35.84 to 37.17**	**0**	**36.320**	**35.69 to 36.99**	**5**	**36.320**	**35.64 to 37**	**0**
Cholesterol esters in large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.465**	**0.366 to 0.569**	**0**	**0.479**	**0.39 to 0.576**	**0**	**0.452**	**0.367 to 0.549**	**0**
Cholesterol esters in medium HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.401**	**0.35 to 0.463**	**0**	**0.396**	**0.342 to 0.452**	**0**	**0.380**	**0.335 to 0.442**	**0**
Cholesterol esters in small HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.419**	**0.388 to 0.458**	**0**	**0.414**	**0.379 to 0.45**	**0**	**0.416**	**0.379 to 0.455**	**0**
Cholesterol esters in very large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.234**	**0.182 to 0.286**	**0**	**0.242**	**0.196 to 0.293**	**0**	**0.234**	**0.183 to 0.294**	**0**

Concentration of large HDL particles	**Lipoprotein subclasses HDL**	**mol/l**	**0.000**	**1.654e-06 to 2.433e-06**	**0**	**0.000**	**1.732e-06 to 2.440e-06**	**0**	**0.000**	**1.576e-06 to 2.381e-06**	**0**
Concentration of medium HDL particles	**Lipoprotein subclasses HDL**	**mol/l**	**0.000**	**2.233e-06 to 2.831e-06**	**0**	**0.000**	**2.191e-06 to 2.760e-06**	**0**	**0.000**	**2.159e-06 to 2.688e-06**	**0**
Concentration of small HDL particles	**Lipoprotein subclasses HDL**	**mol/l**	**0.000**	**5.029e-06 to 5.852e-06**	**0**	**0.000**	**4.884e-06 to 5.664e-06**	**0**	**0.000**	**4.958e-06 to 5.653e-06**	**0**
Concentration of very large HDL particles	**Lipoprotein subclasses HDL**	**mol/l**	**0.000**	**5.505e-07 to 8.7455e-07**	**0**	**0.000**	**5.903e-07 to 8.889e-07**	**0**	**0.000**	**5.490e-07 to 8.812e-07**	**0**
Free cholesterol in large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.135**	**0.105 to 0.17**	**0**	**0.140**	**0.112 to 0.17**	**0**	**0.130**	**0.104 to 0.166**	**0**
Free cholesterol in medium HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.102**	**0.089 to 0.121**	**0**	**0.100**	**0.087 to 0.116**	**0**	**0.097**	**0.085 to 0.112**	**0**
Free cholesterol in small HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.117**	**0.106 to 0.129**	**0**	**0.113**	**0.104 to 0.125**	**0**	**0.113**	**0.105 to 0.125**	**0**
Free cholesterol in very large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.083**	**0.061 to 0.104**	**0**	**0.086**	**0.067 to 0.107**	**0**	**0.079**	**0.061 to 0.108**	**0**
Phospholipids in large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.620**	**0.506 to 0.727**	**0**	**0.622**	**0.526 to 0.725**	**0**	**0.598**	**0.48 to 0.722**	**0**

Phospholipids in medium HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.506**	**0.453 to 0.573**	**0**	**0.497**	**0.447 to 0.555**	**0**	**0.485**	**0.44 to 0.542**	**0**
Phospholipids in small HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.606**	**0.541 to 0.667**	**0**	**0.583**	**0.528 to 0.645**	**0**	**0.580**	**0.527 to 0.65**	**0**
Phospholipids in very large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.370**	**0.28 to 0.452**	**0**	**0.380**	**0.301 to 0.461**	**0**	**0.366**	**0.284 to 0.458**	**0**
Total cholesterol in large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.600**	**0.469 to 0.736**	**0**	**0.619**	**0.502 to 0.745**	**0**	**0.583**	**0.471 to 0.715**	**0**
Total cholesterol in medium HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.502**	**0.438 to 0.582**	**0**	**0.495**	**0.429 to 0.567**	**0**	**0.474**	**0.415 to 0.55**	**0**
Total cholesterol in small HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.536**	**0.498 to 0.577**	**0**	**0.527**	**0.489 to 0.569**	**0**	**0.523**	**0.491 to 0.575**	**0**
Total cholesterol in very large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.318**	**0.245 to 0.389**	**0**	**0.329**	**0.263 to 0.398**	**0**	**0.312**	**0.245 to 0.398**	**0**
Total lipids in large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**1.278**	**1.037 to 1.538**	**0**	**1.303**	**1.086 to 1.538**	**0**	**1.226**	**0.989 to 1.502**	**0**
Total lipids in medium HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**1.063**	**0.942 to 1.209**	**0**	**1.045**	**0.926 to 1.174**	**0**	**1.007**	**0.909 to 1.142**	**0**
Total lipids in small HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**1.199**	**1.117 to 1.299**	**0**	**1.165**	**1.085 to 1.26**	**0**	**1.164**	**1.096 to 1.257**	**0**
Total lipids in very large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.716**	**0.549 to 0.878**	**0**	**0.738**	**0.591 to 0.893**	**0**	**0.709**	**0.549 to 0.886**	**0**

Triglycerides in large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.060**	**0.051 to 0.072**	**0**	**0.061**	**0.053 to 0.073**	**0**	**0.059**	**0.051 to 0.07**	**0**
Triglycerides in medium HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.053**	**0.046 to 0.064**	**0**	**0.051**	**0.044 to 0.06**	**0**	**0.053**	**0.044 to 0.062**	**0**
Triglycerides in small HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.059**	**0.049 to 0.072**	**0**	**0.056**	**0.048 to 0.067**	**0**	**0.056**	**0.05 to 0.071**	**0**
Triglycerides in very large HDL	**Lipoprotein subclasses HDL**	**mmol/l**	**0.030**	**0.025 to 0.038**	**0**	**0.031**	**0.025 to 0.038**	**0**	**0.029**	**0.024 to 0.04**	**0**
Cholesterol esters in large LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.791**	**0.635 to 0.96**	**0**	**0.773**	**0.633 to 0.927**	**0**	**0.776**	**0.644 to 0.92**	**0**
Cholesterol esters in LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.607**	**0.515 to 0.729**	**0**	**0.600**	**0.503 to 0.708**	**0**	**0.586**	**0.5 to 0.716**	**0**
Cholesterol esters in medium LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.464**	**0.363 to 0.577**	**0**	**0.452**	**0.358 to 0.555**	**0**	**0.458**	**0.358 to 0.528**	**0**
Cholesterol esters in small LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.283**	**0.224 to 0.354**	**0**	**0.277**	**0.221 to 0.341**	**0**	**0.283**	**0.221 to 0.328**	**0**
Concentration of large LDL particles	**Lipoprotein subclasses LDL**	**mol/l**	**0.000**	**1.930e-07 to 2.731e-07**	**0**	**0.000**	**1.919e-07 to 2.669e-07**	**0**	**0.000**	**1.954e-07 to 2.599e-07**	**0**
Concentration of LDL particles	**Lipoprotein subclasses LDL**	**mol/l**	**0.000**	**1.174e-07 to 1.622e-07**	**0**	**0.000**	**1.156e-07 to 1.588e-07**	**5**	**0.000**	**1.158e-07 to 1.565e-07**	**0**

Concentration of medium LDL particles	**Lipoprotein subclasses LDL**	**mol/l**	**0.000**	**1.567e-07 to 2.245e-07**	**0**	**0.000**	**1.546e-07 to 2.186e-07**	**0**	**0.000**	**1.569e-07 to 2.148e-07**	**0**
Concentration of small LDL particles	**Lipoprotein subclasses LDL**	**mol/l**	**0.000**	**1.874e-07 to 2.601e-07**	**0**	**0.000**	**1.828e-07 to 2.538e-07**	**0**	**0.000**	**1.839e-07 to 2.488e-07**	**0**
Free cholesterol in large LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.303**	**0.256 to 0.359**	**0**	**0.301**	**0.257 to 0.352**	**0**	**0.304**	**0.248 to 0.353**	**0**
Free cholesterol in LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.250**	**0.211 to 0.302**	**0**	**0.251**	**0.212 to 0.296**	**0**	**0.250**	**0.202 to 0.298**	**0**
Free cholesterol in medium LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.171**	**0.15 to 0.197**	**0**	**0.169**	**0.147 to 0.193**	**0**	**0.169**	**0.146 to 0.192**	**0**
Free cholesterol in small LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.106**	**0.093 to 0.122**	**0**	**0.105**	**0.091 to 0.119**	**0**	**0.105**	**0.091 to 0.12**	**0**
Phospholipids in large LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.388**	**0.333 to 0.452**	**0**	**0.383**	**0.331 to 0.441**	**0**	**0.382**	**0.337 to 0.436**	**0**
Phospholipids in LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.365**	**0.307 to 0.43**	**0**	**0.361**	**0.309 to 0.421**	**0**	**0.357**	**0.304 to 0.416**	**0**
Phospholipids in medium LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.242**	**0.211 to 0.275**	**0**	**0.236**	**0.206 to 0.269**	**0**	**0.233**	**0.218 to 0.264**	**0**
Phospholipids in small LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.180**	**0.158 to 0.202**	**0**	**0.176**	**0.156 to 0.198**	**0**	**0.178**	**0.161 to 0.197**	**0**

Total cholesterol in large LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**1.094**	**0.886 to 1.322**	**0**	**1.075**	**0.891 to 1.277**	**0**	**1.082**	**0.884 to 1.259**	**0**
Total cholesterol in LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.854**	**0.723 to 1.032**	**0**	**0.851**	**0.714 to 1.003**	**0**	**0.837**	**0.712 to 1**	**0**
Total cholesterol in medium LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.633**	**0.509 to 0.775**	**0**	**0.620**	**0.505 to 0.748**	**0**	**0.625**	**0.497 to 0.73**	**0**
Total cholesterol in small LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.386**	**0.315 to 0.474**	**0**	**0.382**	**0.312 to 0.459**	**0**	**0.387**	**0.309 to 0.455**	**0**
Total lipids in large LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**1.635**	**1.362 to 1.943**	**0**	**1.612**	**1.356 to 1.892**	**0**	**1.615**	**1.375 to 1.847**	**0**
Total lipids in LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**1.394**	**1.172 to 1.646**	**0**	**1.376**	**1.163 to 1.606**	**5**	**1.364**	**1.16 to 1.584**	**0**
Total lipids in medium LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.957**	**0.796 to 1.138**	**0**	**0.935**	**0.78 to 1.104**	**0**	**0.932**	**0.796 to 1.089**	**0**
Total lipids in small LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.618**	**0.523 to 0.728**	**0**	**0.605**	**0.511 to 0.71**	**0**	**0.599**	**0.516 to 0.698**	**0**
Triglycerides in large LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.158**	**0.134 to 0.183**	**0**	**0.154**	**0.131 to 0.179**	**0**	**0.155**	**0.134 to 0.179**	**0**
Triglycerides in LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.168**	**0.141 to 0.194**	**0**	**0.161**	**0.138 to 0.19**	**0**	**0.160**	**0.141 to 0.19**	**0**
Triglycerides in medium LDL	**Lipoprotein subclasses LDL**	**mmol/l**	**0.081**	**0.069 to 0.095**	**0**	**0.079**	**0.068 to 0.092**	**0**	**0.080**	**0.07 to 0.091**	**0**

Triglycerides in small LDL	Lipoprotein subclasses LDL	mmol/l	0.049	0.042 to 0.058	0	0.047	0.041 to 0.056	0	0.047	0.042 to 0.055	0
Cholesterol esters in cms & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.003	0.002 to 0.005	0	0.003	0.002 to 0.005	0	0.003	0.002 to 0.005	0
Cholesterol esters in large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.032	0.02 to 0.048	0	0.029	0.019 to 0.044	0	0.028	0.016 to 0.049	0
Cholesterol esters in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	0.107	0.081 to 0.135	0	0.101	0.078 to 0.13	0	0.101	0.076 to 0.129	0
Cholesterol esters in small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.162	0.127 to 0.198	0	0.155	0.123 to 0.192	0	0.153	0.123 to 0.184	0
Cholesterol esters in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.006	0.004 to 0.011	0	0.006	0.003 to 0.01	0	0.006	0.003 to 0.011	0
Cholesterol esters in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.188	0.155 to 0.226	0	0.186	0.154 to 0.222	0	0.180	0.156 to 0.228	0
Conc. Of cms & extremely large VLDL particles	Lipoprotein subclasses VLDL	mol/l	0.000	5.956e-11 to 1.593e-10	0	0.000	5.315e-11 to 1.457e-10	0	0.000	4.559e-11 to 1.468e-10	0
Concentration of large VLDL particles	Lipoprotein subclasses VLDL	mol/l	0.000	3.105e-09 to 7.507e-09	0	0.000	2.799e-09 to 6.647e-09	0	0.000	2.625e-09 to 7.436e-09	0
Concentration of medium VLDL particles	Lipoprotein subclasses VLDL	mol/l	0.000	1.270e-08 to 2.394e-08	0	0.000	1.160e-08 to 2.190e-08	0	0.000	1.134e-08 to 2.382e-08	0

Concentration of small VLDL particles	**Lipoprotein subclasses VLDL**	**mol/l**	**0.000**	**2.586e-08 to 3.996e-08**	**0**	**0.000**	**2.473e-08 to 3.830e-08**	**0**	**0.000**	**2.518e-08 to 3.860e-08**	**0**
Concentration of very large VLDL particles	**Lipoprotein subclasses VLDL**	**mol/l**	**0.000**	**3.417e-10 to 1.079e-09**	**0**	**0.000**	**3.090e-10 to 9.437e-10**	**0**	**0.000**	**2.689e-10 to 1.105e-09**	**0**
Concentration of very small VLDL particles	**Lipoprotein subclasses VLDL**	**mol/l**	**0.000**	**4.089e-08 to 5.728e-08**	**0**	**0.000**	**3.946e-08 to 5.567e-08**	**0**	**0.000**	**4.139e-08 to 5.536e-08**	**0**
Free cholesterol in cms & extremely large VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.002**	**0.002 to 0.004**	**0**	**0.002**	**0.001 to 0.003**	**0**	**0.002**	**0.001 to 0.004**	**0**
Free cholesterol in large VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.032**	**0.019 to 0.049**	**0**	**0.028**	**0.017 to 0.044**	**0**	**0.028**	**0.017 to 0.049**	**0**
Free cholesterol in medium VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.074**	**0.053 to 0.102**	**0**	**0.067**	**0.049 to 0.093**	**0**	**0.066**	**0.049 to 0.101**	**0**
Free cholesterol in small VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.099**	**0.081 to 0.119**	**0**	**0.094**	**0.077 to 0.115**	**0**	**0.092**	**0.08 to 0.115**	**0**
Free cholesterol in very large VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.006**	**0.004 to 0.01**	**0**	**0.005**	**0.003 to 0.009**	**0**	**0.005**	**0.003 to 0.009**	**0**
Free cholesterol in very small VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.097**	**0.08 to 0.114**	**0**	**0.093**	**0.078 to 0.11**	**0**	**0.091**	**0.08 to 0.111**	**0**
Phospholipids in cms & extremely large VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.003**	**0.002 to 0.005**	**0**	**0.003**	**0.002 to 0.005**	**0**	**0.003**	**0.002 to 0.005**	**0**

Phospholipids in large VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.056**	**0.037 to 0.083**	**0**	**0.050**	**0.033 to 0.074**	**0**	**0.049**	**0.031 to 0.082**	**0**
Phospholipids in medium VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.120**	**0.091 to 0.164**	**0**	**0.112**	**0.083 to 0.151**	**0**	**0.110**	**0.082 to 0.162**	**0**
Phospholipids in small VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.150**	**0.123 to 0.181**	**0**	**0.143**	**0.118 to 0.173**	**0**	**0.142**	**0.126 to 0.172**	**0**
Phospholipids in very large VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.011**	**0.007 to 0.019**	**0**	**0.010**	**0.006 to 0.017**	**0**	**0.011**	**0.006 to 0.018**	**0**
Phospholipids in very small VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.188**	**0.153 to 0.22**	**0**	**0.181**	**0.15 to 0.216**	**0**	**0.178**	**0.155 to 0.211**	**0**
Total cholesterol in cms & extremely large VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.005**	**0.003 to 0.008**	**0**	**0.005**	**0.003 to 0.007**	**0**	**0.005**	**0.003 to 0.007**	**0**
Total cholesterol in large VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.064**	**0.039 to 0.097**	**0**	**0.057**	**0.036 to 0.088**	**0**	**0.056**	**0.031 to 0.097**	**0**
Total cholesterol in medium VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.179**	**0.135 to 0.235**	**0**	**0.169**	**0.127 to 0.222**	**0**	**0.165**	**0.123 to 0.229**	**0**
Total cholesterol in small VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.265**	**0.209 to 0.314**	**0**	**0.249**	**0.201 to 0.304**	**0**	**0.247**	**0.202 to 0.299**	**0**
Total cholesterol in very large VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.012**	**0.007 to 0.02**	**0**	**0.011**	**0.006 to 0.019**	**0**	**0.012**	**0.005 to 0.02**	**0**
Total cholesterol in very small VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.283**	**0.234 to 0.34**	**0**	**0.278**	**0.232 to 0.332**	**0**	**0.273**	**0.233 to 0.336**	**0**

Total lipids in cms & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.023	0.014 to 0.035	0	0.020	0.012 to 0.032	0	0.020	0.01 to 0.033	0
Total lipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.291	0.18 to 0.435	0	0.258	0.162 to 0.386	0	0.256	0.152 to 0.431	0
Total lipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	0.581	0.433 to 0.808	0	0.545	0.395 to 0.74	0	0.522	0.381 to 0.802	0
Total lipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.648	0.522 to 0.796	0	0.619	0.496 to 0.761	0	0.604	0.51 to 0.775	0
Total lipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.065	0.034 to 0.105	0	0.057	0.031 to 0.093	0	0.057	0.027 to 0.107	0
Total lipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.615	0.511 to 0.718	0	0.593	0.498 to 0.703	0	0.590	0.514 to 0.707	0
Triglycerides in cms & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.015	0.009 to 0.024	0	0.013	0.008 to 0.021	0	0.014	0.007 to 0.022	0
Triglycerides in large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.171	0.105 to 0.254	0	0.150	0.095 to 0.226	0	0.149	0.089 to 0.254	0
Triglycerides in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	0.284	0.198 to 0.4	0	0.264	0.185 to 0.371	0	0.253	0.186 to 0.412	0
Triglycerides in small VLDL	Lipoprotein subclasses VLDL	mmol/l	0.236	0.184 to 0.31	0	0.224	0.175 to 0.289	0	0.214	0.177 to 0.319	0
Triglycerides in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	0.042	0.022 to 0.067	0	0.035	0.019 to 0.059	0	0.037	0.016 to 0.07	0

Triglycerides in very small VLDL	**Lipoprotein subclasses VLDL**	**mmol/l**	**0.141**	**0.115 to 0.169**	**0**	**0.133**	**0.111 to 0.162**	**0**	**0.135**	**0.116 to 0.161**	**0**

APPENDIX 4A3: Results of logistical regression (adjusted) comparing maternal metabolites of those women who delivered preterm (<259 days or <37 completed weeks) compared with those who delivered at term (259 days to 294 days, or 37 to 42 completed weeks), excluding those who delivered post-term (>294 days), showing odds ratio per 1 standard deviation (SD) increase in metabolite measured. N=399 for preterm, n=6994 for term, and n=54 excluded for post-term. The biochemical group of the metabolite, units, upper and lower 95% confidence interval and p-values are also presented (bold if significant at p<0.0029).

Metabolites	Group	Units	OR per 1SD increase in metabolite	SD	Lower 95%CI	Upper 95%CI	p-value	N
Alanine	Amino acids	mmol/l	**0.069**	0.048	-0.035	0.173	1.929E-01	7347
Glutamine	Amino acids	mmol/l	**0.003**	0.046	-0.103	0.109	9.529E-01	7370
Glycine	Amino acids	mmol/l	**0.059**	0.032	-0.042	0.161	2.526E-01	7360
Histidine	Amino acids	mmol/l	**0.043**	0.010	-0.061	0.146	4.178E-01	7369
Apolipoprotein A-I	Apolipoproteins	g/l	**0.031**	0.220	-0.073	0.136	5.575E-01	7374
Apolipoprotein B	Apolipoproteins	g/l	**0.128**	0.228	0.028	0.229	1.251E-02	7374
Ratio of apolipoprotein B to apolipoprotein A-I	Apolipoproteins	#N/A	**0.118**	0.121	0.018	0.218	2.074E-02	7374
Phenylalanine	Aromatic amino acids	mmol/l	**0.151**	0.011	0.044	0.257	5.638E-03	7375
Tyrosine	Aromatic amino acids	mmol/l	**0.069**	0.006	-0.037	0.176	2.012E-01	7366
Isoleucine	Branched--chain amino acids	mmol/l	**0.095**	0.010	-0.007	0.197	6.692E-02	7372
Leucine	Branched--chain amino acids	mmol/l	**0.121**	0.010	0.017	0.224	2.199E-02	7372
Valine	Branched--chain amino acids	mmol/l	**0.036**	0.018	-0.070	0.142	5.058E-01	7365
Esterified cholesterol	Cholesterol	mmol/l	**0.069**	0.807	-0.035	0.173	1.911E-01	7348
Free cholesterol	Cholesterol	mmol/l	**0.088**	0.334	-0.015	0.192	9.328E-02	7348

Remnant cholesterol (non-HDL, non-LDL - cholesterol)	Cholesterol	mmol/l	**0.114**	0.467	0.013	0.215	2.707E-02	7374
Serum total cholesterol	Cholesterol	mmol/l	**0.073**	1.157	-0.030	0.176	1.641E-01	7374
Total cholesterol in HDL	Cholesterol	mmol/l	**-0.028**	0.370	-0.133	0.078	6.090E-01	7374
Total cholesterol in HDL2	Cholesterol	mmol/l	**-0.039**	0.335	-0.145	0.067	4.752E-01	7374
Total cholesterol in HDL3	Cholesterol	mmol/l	**0.059**	0.044	-0.044	0.162	2.625E-01	7374
Total cholesterol in LDL	Cholesterol	mmol/l	**0.065**	0.617	-0.038	0.168	2.186E-01	7374
Total cholesterol in VLDL	Cholesterol	mmol/l	**0.137**	0.276	0.039	0.236	6.197E-03	7374
Ratio of 18:2 linoleic acid to total fatty acids	Fatty acids (%)	%	**-0.123**	2.693	-0.239	-0.007	3.716E-02	7325
Ratio of 22:6 docosahexaenoic acid to total fatty acids	Fatty acids (%)	%	**-0.102**	0.230	-0.213	0.008	6.925E-02	7325
Ratio of monounsaturated fatty acids to total fatty acids	Fatty acids (%)	%	**0.104**	2.416	-0.017	0.224	9.223E-02	7325
Ratio of omega-3 fatty acids to total fatty acids	Fatty acids (%)	%	**-0.084**	0.492	-0.195	0.028	1.421E-01	7325
Ratio of omega-6 fatty acids to total fatty acids	Fatty acids (%)	%	**-0.114**	2.207	-0.226	-0.002	4.641E-02	7325
Ratio of polyunsaturated fatty acids to total fatty acids	Fatty acids (%)	%	**-0.122**	2.387	-0.233	-0.010	3.236E-02	7325
Ratio of saturated fatty acids to total fatty acids	Fatty acids (%)	%	**0.047**	1.508	-0.060	0.154	3.917E-01	7325
18:2, linoleic acid	Fatty acids and saturation measures	mmol/l	**0.066**	0.766	-0.039	0.170	2.176E-01	7325
22:6, docosahexaenoic acid	Fatty acids and saturation measures	mmol/l	**0.061**	0.043	-0.046	0.168	2.630E-01	7325
Estimated degree of unsaturation	Fatty acids and saturation measures	#N/A	**-0.054**	0.043	-0.159	0.052	3.193E-01	7325

Monounsaturated fatty acids; 16:1, 18:1	Fatty acids and saturation measures	mmol/l	**0.136**	0.996	0.030	0.242	1.184E-02	7325
Omega-3 fatty acids	Fatty acids and saturation measures	mmol/l	**0.071**	0.104	-0.035	0.178	1.890E-01	7325
Omega-6 fatty acids	Fatty acids and saturation measures	mmol/l	**0.083**	0.840	-0.021	0.187	1.180E-01	7325
Polyunsaturated fatty acids	Fatty acids and saturation measures	mmol/l	**0.084**	0.917	-0.020	0.188	1.143E-01	7325
Saturated fatty acids	Fatty acids and saturation measures	mmol/l	**0.133**	1.044	0.029	0.237	1.228E-02	7325
Total fatty acids	Fatty acids and saturation measures	mmol/l	**0.124**	2.779	0.020	0.229	1.913E-02	7325
Albumin	Fluid balance and inflammation	signal area	**0.108**	0.006	0.007	0.208	3.522E-02	7375
Creatinine	Fluid balance and inflammation	mmol/l	**0.192**	0.006	0.097	0.286	**6.761E-05**	7370
Glycoprotein acetyls, mainly a1-acid glycoprotein	Fluid balance and inflammation	mmol/l	**0.202**	0.198	0.105	0.298	**4.301E-05**	7375
Phosphatidylcholine and other cholines	Glycerides and phospholipids	mmol/l	**0.114**	0.459	0.011	0.217	2.977E-02	7346
Ratio of triglycerides to phosphoglycerides	Glycerides and phospholipids	#N/A	**0.119**	0.160	0.018	0.220	2.091E-02	7348
Serum total triglycerides	Glycerides and phospholipids	mmol/l	**0.154**	0.580	0.061	0.246	**1.141E-03**	7374
Sphingomyelins	Glycerides and phospholipids	mmol/l	**0.057**	0.086	-0.047	0.162	2.841E-01	7346

Total cholines	Glycerides and phospholipids	mmol/l	**0.116**	0.485	0.013	0.219	2.780E-02	7346
Total phosphoglycerides	Glycerides and phospholipids	mmol/l	**0.129**	0.398	0.026	0.233	1.422E-02	7348
Triglycerides in HDL	Glycerides and phospholipids	mmol/l	**0.125**	0.042	0.025	0.224	1.438E-02	7374
Triglycerides in LDL	Glycerides and phospholipids	mmol/l	**0.129**	0.070	0.028	0.231	1.256E-02	7374
Triglycerides in VLDL	Glycerides and phospholipids	mmol/l	**0.149**	0.460	0.059	0.240	**1.182E-03**	7374
Citrate	Glycolysis related metabolites	mmol/l	**0.089**	0.017	-0.022	0.200	1.177E-01	7247
Glucose	Glycolysis related metabolites	mmol/l	**0.009**	0.490	-0.099	0.117	8.722E-01	7350
Glycerol	Glycolysis related metabolites	mmol/l	**0.230**	0.016	0.130	0.330	**6.879E-06**	7361
Lactate	Glycolysis related metabolites	mmol/l	**0.049**	0.492	-0.053	0.151	3.448E-01	7363
Pyruvate	Glycolysis related metabolites	mmol/l	**0.049**	0.022	-0.053	0.151	3.468E-01	7359
3-hydroxybutyrate	Ketone bodies	mmol/l	**0.028**	0.038	-0.076	0.132	6.020E-01	7374
Acetate	Ketone bodies	mmol/l	**-0.035**	0.006	-0.140	0.071	5.193E-01	7368
Mean diameter for HDL particles	Lipoprotein particle size	nm	**-0.156**	0.205	-0.263	-0.050	4.063E-03	7374
Mean diameter for LDL particles	Lipoprotein particle size	nm	**-0.071**	0.063	-0.179	0.037	1.993E-01	7374
Mean diameter for VLDL particles	Lipoprotein particle size	nm	**0.124**	1.009	0.019	0.230	2.069E-02	7374
Cholesterol esters in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.086**	0.144	-0.194	0.021	1.139E-01	7379
Cholesterol esters in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.066**	0.087	-0.038	0.170	2.131E-01	7379

Cholesterol esters in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.103**	0.059	0.000	0.207	5.089E-02	7379
Cholesterol esters in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.104**	0.074	-0.212	0.003	5.669E-02	7379
Concentration of large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.066**	0.000	-0.172	0.041	2.261E-01	7379
Concentration of medium HDL particles	Lipoprotein subclasses HDL	mol/l	**0.109**	0.000	0.006	0.212	3.833E-02	7379
Concentration of small HDL particles	Lipoprotein subclasses HDL	mol/l	**0.180**	0.000	0.077	0.284	6.512E-04	7379
Concentration of very large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.106**	0.000	-0.214	0.001	5.243E-02	7379
Free cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.086**	0.045	-0.193	0.021	1.161E-01	7379
Free cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.090**	0.023	-0.013	0.193	8.650E-02	7379
Free cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.144**	0.017	0.041	0.246	6.135E-03	7379
Free cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.108**	0.030	-0.216	-0.001	4.865E-02	7379
Phospholipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.048**	0.157	-0.154	0.058	3.727E-01	7379
Phospholipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.113**	0.087	0.010	0.216	3.169E-02	7379
Phospholipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.144**	0.094	0.041	0.248	6.136E-03	7379
Phospholipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.112**	0.122	-0.219	-0.004	4.148E-02	7379
Total cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.086**	0.189	-0.193	0.021	1.143E-01	7379
Total cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.071**	0.109	-0.032	0.175	1.777E-01	7379

Total cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.128**	0.067	0.024	0.231	1.555E-02	7379
Total cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.106**	0.104	-0.213	0.002	5.334E-02	7379
Total lipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.068**	0.356	-0.175	0.038	2.094E-01	7379
Total lipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.103**	0.195	0.000	0.206	4.989E-02	7379
Total lipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.175**	0.142	0.072	0.279	**8.974E-04**	7379
Total lipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.107**	0.230	-0.215	0.000	5.064E-02	7379
Triglycerides in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.028**	0.016	-0.134	0.077	5.997E-01	7379
Triglycerides in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.211**	0.013	0.110	0.313	**4.502E-05**	7379
Triglycerides in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.157**	0.016	0.055	0.258	**2.489E-03**	7379
Triglycerides in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.001**	0.010	-0.105	0.103	9.842E-01	7379
Cholesterol esters in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.072**	0.234	-0.031	0.175	1.703E-01	7379
Cholesterol esters in LDL	Lipoprotein subclasses LDL	mmol/l	**0.072**	0.163	-0.031	0.175	1.703E-01	7379
Cholesterol esters in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.068**	0.157	-0.036	0.171	1.991E-01	7379
Cholesterol esters in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.062**	0.095	-0.042	0.165	2.429E-01	7379
Concentration of large LDL particles	Lipoprotein subclasses LDL	mol/l	**0.079**	0.000	-0.024	0.181	1.345E-01	7379
Concentration of LDL particles	Lipoprotein subclasses LDL	mol/l	**0.078**	0.000	-0.025	0.180	1.392E-01	7374

Concentration of medium LDL particles	Lipoprotein subclasses LDL	mol/l	**0.083**	0.000	-0.019	0.186	1.114E-01	7379
Concentration of small LDL particles	Lipoprotein subclasses LDL	mol/l	**0.088**	0.000	-0.015	0.190	9.445E-02	7379
Free cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.049**	0.076	-0.055	0.153	3.541E-01	7379
Free cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**0.048**	0.067	-0.056	0.152	3.650E-01	7379
Free cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.072**	0.037	-0.031	0.176	1.696E-01	7379
Free cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.078**	0.023	-0.025	0.181	1.377E-01	7379
Phospholipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.074**	0.088	-0.029	0.177	1.606E-01	7379
Phospholipids in LDL	Lipoprotein subclasses LDL	mmol/l	**0.061**	0.090	-0.042	0.164	2.480E-01	7379
Phospholipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.101**	0.050	-0.001	0.204	5.310E-02	7379
Phospholipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.107**	0.034	0.004	0.209	4.172E-02	7379
Total cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.067**	0.310	-0.037	0.170	2.066E-01	7379
Total cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**0.065**	0.230	-0.038	0.169	2.156E-01	7379
Total cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.069**	0.193	-0.035	0.172	1.922E-01	7379
Total cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.065**	0.117	-0.038	0.168	2.170E-01	7379
Total lipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.074**	0.428	-0.029	0.177	1.566E-01	7379
Total lipids in LDL	Lipoprotein subclasses LDL	mmol/l	**0.072**	0.349	-0.031	0.175	1.684E-01	7374

Total lipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.081**	0.258	-0.022	0.184	1.241E-01	7379
Total lipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.084**	0.159	-0.019	0.187	1.110E-01	7379
Triglycerides in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.125**	0.039	0.023	0.227	1.654E-02	7379
Triglycerides in LDL	Lipoprotein subclasses LDL	mmol/l	**0.137**	0.042	0.036	0.239	7.695E-03	7379
Triglycerides in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.127**	0.020	0.025	0.229	1.433E-02	7379
Triglycerides in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.158**	0.013	0.059	0.258	**1.796E-03**	7379
Cholesterol esters in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.097**	0.002	0.006	0.189	3.747E-02	7379
Cholesterol esters in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.123**	0.022	0.033	0.213	7.301E-03	7379
Cholesterol esters in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.130**	0.043	0.034	0.225	7.755E-03	7379
Cholesterol esters in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.119**	0.054	0.018	0.220	2.138E-02	7379
Cholesterol esters in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.108**	0.006	0.020	0.196	1.609E-02	7379
Cholesterol esters in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.063**	0.053	-0.040	0.166	2.301E-01	7379
Conc. of chylomicrons & extremely large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.114**	0.000	0.029	0.200	9.011E-03	7379
Concentration of large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.142**	0.000	0.053	0.231	**1.755E-03**	7379
Concentration of medium VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.148**	0.000	0.057	0.238	**1.354E-03**	7379
Concentration of small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.167**	0.000	0.071	0.263	**6.594E-04**	7379

Concentration of very large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.131**	0.000	0.043	0.218	3.451E-03	7379
Concentration of very small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.118**	0.000	0.016	0.220	2.315E-02	7379
Free cholesterol in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.125**	0.002	0.038	0.211	4.700E-03	7379
Free cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.141**	0.023	0.052	0.229	**1.832E-03**	7379
Free cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.151**	0.039	0.061	0.242	**1.055E-03**	7379
Free cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.176**	0.031	0.079	0.274	**3.787E-04**	7379
Free cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.123**	0.005	0.036	0.210	5.607E-03	7379
Free cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.103**	0.026	0.001	0.205	4.850E-02	7379
Phospholipids in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.125**	0.002	0.040	0.211	4.217E-03	7379
Phospholipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.145**	0.037	0.055	0.234	**1.505E-03**	7379
Phospholipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.152**	0.058	0.061	0.243	**1.103E-03**	7379
Phospholipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.181**	0.044	0.084	0.279	**2.594E-04**	7379
Phospholipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.133**	0.009	0.045	0.221	2.978E-03	7379
Phospholipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.090**	0.052	-0.013	0.192	8.602E-02	7379
Total cholesterol in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.111**	0.004	0.022	0.200	1.417E-02	7379
Total cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.132**	0.046	0.043	0.221	3.525E-03	7379

Total cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.142**	0.080	0.050	0.235	**2.602E-03**	7379
Total cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.146**	0.082	0.046	0.246	4.374E-03	7379
Total cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.115**	0.011	0.028	0.202	9.914E-03	7379
Total cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.076**	0.078	-0.026	0.179	1.445E-01	7379
Total lipids in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.115**	0.018	0.029	0.201	8.891E-03	7379
Total lipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.141**	0.199	0.053	0.230	**1.819E-03**	7379
Total lipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.148**	0.298	0.057	0.238	**1.373E-03**	7379
Total lipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.167**	0.215	0.071	0.264	**6.952E-04**	7379
Total lipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.130**	0.055	0.042	0.217	3.675E-03	7379
Total lipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.111**	0.161	0.009	0.213	3.335E-02	7379
Triglycerides in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.113**	0.012	0.028	0.199	9.165E-03	7379
Triglycerides in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.144**	0.117	0.055	0.232	**1.525E-03**	7379
Triglycerides in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.148**	0.163	0.058	0.237	**1.265E-03**	7379
Triglycerides in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.160**	0.097	0.068	0.253	**6.878E-04**	7379
Triglycerides in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.133**	0.035	0.045	0.220	2.924E-03	7379
Triglycerides in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.167**	0.042	0.069	0.265	**8.432E-04**	7379

APPENDIX 4A4: Results of logistical regression (adjusted) comparing maternal metabolites of those women who delivered post-term (>294 days, or >42 completed weeks) compared with those who delivered at term (259 days to 294 days, or 37 to 42 completed weeks), excluding those who delivered pre-term (<259 days, of <37 completed weeks), showing odds ratio per 1 standard deviation (SD) increase in metabolite measured. N=54 for post-term, n=6994 for term, and n=392 excluded for pre-term. The biochemical group of the metabolite, units, upper and lower 95% confidence interval. No p-values were significant at p<0.0029.

Metabolites	Group	Units	OR per 1SD increase in metabolite	SD	Lower 95%CI	Upper 95%CI	p-value	N
Acetate	Ketone bodies	mmol/l	**-0.101**	0.006	-0.386	0.184	4.862E-01	6350
Alanine	Amino acids	mmol/l	**-0.194**	0.048	-0.487	0.099	1.943E-01	6334
Albumin	Fluid balance and inflammation	signal area	**-0.010**	0.006	-0.286	0.265	9.407E-01	6356
Apolipoprotein A-I	Apolipoproteins	g/l	**-0.142**	0.220	-0.432	0.148	3.361E-01	6355
Apolipoprotein B	Apolipoproteins	g/l	**0.012**	0.228	-0.264	0.287	9.339E-01	6355
Ratio of apolipoprotein B to apolipoprotein A-I	Apolipoproteins	#N/A	**0.097**	0.120	-0.167	0.362	4.721E-01	6355
3-hydroxybutyrate	Ketone bodies	mmol/l	**-0.080**	0.038	-0.401	0.241	6.268E-01	6356
Citrate	Glycolysis related metabolites	mmol/l	**-0.049**	0.017	-0.356	0.257	7.520E-01	6255
Creatinine	Fluid balance and inflammation	mmol/l	**0.052**	0.006	-0.192	0.296	6.749E-01	6353
22:6, docosahexaenoic acid	Fatty acids and saturation measures	mmol/l	**0.084**	0.043	-0.190	0.358	5.492E-01	6317
Ratio of 22:6 docosahexaenoic acid to total fatty acids	Fatty acids (%)	%	**0.030**	0.230	-0.246	0.306	8.319E-01	6317
Esterified cholesterol	Cholesterol	mmol/l	**-0.009**	0.805	-0.291	0.272	9.476E-01	6336

Omega-3 fatty acids	Fatty acids and saturation measures	mmol/l	**0.011**	0.104	-0.269	0.291	9.375E-01	6317
Ratio of omega-3 fatty acids to total fatty acids	Fatty acids (%)	%	**-0.063**	0.494	-0.349	0.223	6.681E-01	6317
Omega-6 fatty acids	Fatty acids and saturation measures	mmol/l	**-0.040**	0.839	-0.322	0.243	7.837E-01	6317
Ratio of omega-6 fatty acids to total fatty acids	Fatty acids (%)	%	**-0.194**	2.200	-0.492	0.104	2.011E-01	6317
Free cholesterol	Cholesterol	mmol/l	**-0.020**	0.333	-0.302	0.261	8.866E-01	6336
Glucose	Glycolysis related metabolites	mmol/l	**0.035**	0.487	-0.293	0.363	8.327E-01	6334
Glutamine	Amino acids	mmol/l	**-0.109**	0.046	-0.398	0.181	4.614E-01	6351
Glycerol	Glycolysis related metabolites	mmol/l	**0.219**	0.016	-0.063	0.500	1.274E-01	6341
Glycine	Amino acids	mmol/l	**0.104**	0.032	-0.162	0.370	4.450E-01	6348
Glycoprotein acetyls, mainly a1-acid glycoprotein	Fluid balance and inflammation	mmol/l	**0.029**	0.195	-0.258	0.316	8.410E-01	6356
Total cholesterol in HDL2	Cholesterol	mmol/l	**-0.145**	0.333	-0.431	0.142	3.227E-01	6355
Total cholesterol in HDL3	Cholesterol	mmol/l	**-0.009**	0.044	-0.287	0.269	9.502E-01	6355
Total cholesterol in HDL	Cholesterol	mmol/l	**-0.132**	0.368	-0.419	0.154	3.655E-01	6355
Mean diameter for HDL particles	Lipoprotein particle size	nm	**-0.105**	0.204	-0.387	0.177	4.655E-01	6355
Triglycerides in HDL	Glycerides and phospholipids	mmol/l	**0.025**	0.042	-0.253	0.302	8.621E-01	6355
Histidine	Amino acids	mmol/l	**0.085**	0.010	-0.186	0.355	5.393E-01	6352
Total cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**0.005**	0.229	-0.272	0.282	9.741E-01	6360
Cholesterol esters in LDL	Lipoprotein subclasses LDL	mmol/l	**0.002**	0.163	-0.275	0.279	9.877E-01	6360
Free cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**0.010**	0.067	-0.267	0.287	9.435E-01	6360
Total lipids in LDL	Lipoprotein subclasses LDL	mmol/l	**0.014**	0.349	-0.262	0.291	9.186E-01	6355
Concentration of LDL particles	Lipoprotein subclasses LDL	mol/l	**0.019**	0.000	-0.257	0.295	8.938E-01	6355
Phospholipids in LDL	Lipoprotein subclasses LDL	mmol/l	**0.017**	0.089	-0.259	0.293	9.041E-01	6360
Triglycerides in LDL	Lipoprotein subclasses LDL	mmol/l	**0.098**	0.042	-0.170	0.366	4.722E-01	6360
Isoleucine	Branched--chain amino acids	mmol/l	**-0.023**	0.010	-0.306	0.259	8.707E-01	6354

18:2, linoleic acid	Fatty acids and saturation measures	mmol/l	**-0.058**	0.765	-0.345	0.228	6.904E-01	6317
Lactate	Glycolysis related metabolites	mmol/l	**0.151**	0.493	-0.093	0.395	2.240E-01	6345
Ratio of 18:2 linoleic acid to total fatty acids	Fatty acids (%)	%	**-0.175**	2.689	-0.481	0.132	2.638E-01	6317
Total cholesterol in LDL	Cholesterol	mmol/l	**0.014**	0.617	-0.264	0.292	9.212E-01	6355
Mean diameter for LDL particles	Lipoprotein particle size	nm	**-0.048**	0.063	-0.333	0.238	7.436E-01	6355
Triglycerides in LDL	Glycerides and phospholipids	mmol/l	**0.077**	0.070	-0.194	0.348	5.787E-01	6355
Leucine	Branched--chain amino acids	mmol/l	**-0.071**	0.010	-0.361	0.219	6.311E-01	6354
Total cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.118**	0.188	-0.403	0.167	4.171E-01	6360
Cholesterol esters in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.119**	0.143	-0.404	0.166	4.127E-01	6360
Free cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.115**	0.045	-0.399	0.170	4.303E-01	6360
Total lipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.117**	0.354	-0.401	0.167	4.193E-01	6360
Concentration of large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.117**	0.000	-0.400	0.167	4.206E-01	6360
Phospholipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.119**	0.156	-0.402	0.164	4.087E-01	6360
Triglycerides in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.041**	0.016	-0.321	0.239	7.723E-01	6360
Total cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.014**	0.309	-0.263	0.291	9.211E-01	6360
Cholesterol esters in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.014**	0.234	-0.263	0.290	9.215E-01	6360
Free cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.014**	0.076	-0.263	0.291	9.186E-01	6360
Total lipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.020**	0.428	-0.256	0.296	8.865E-01	6360
Concentration of large LDL particles	Lipoprotein subclasses LDL	mol/l	**0.023**	0.000	-0.253	0.298	8.715E-01	6360
Phospholipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.011**	0.088	-0.266	0.287	9.393E-01	6360
Triglycerides in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.086**	0.038	-0.184	0.355	5.330E-01	6360
Total cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.016**	0.045	-0.255	0.288	9.058E-01	6360
Cholesterol esters in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.017**	0.022	-0.254	0.288	9.015E-01	6360
Free cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.016**	0.023	-0.257	0.288	9.108E-01	6360
Total lipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.023**	0.194	-0.249	0.294	8.708E-01	6360
Concentration of large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.023**	0.000	-0.248	0.295	8.675E-01	6360
Phospholipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.025**	0.036	-0.247	0.296	8.590E-01	6360

Triglycerides in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.024**	0.114	-0.247	0.296	8.612E-01	6360
Total cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**-0.155**	0.108	-0.432	0.122	2.738E-01	6360
Cholesterol esters in medium HDL	Lipoprotein subclasses HDL	mmol/l	**-0.159**	0.086	-0.435	0.118	2.603E-01	6360
Free cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**-0.136**	0.023	-0.417	0.144	3.404E-01	6360
Total lipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**-0.138**	0.194	-0.414	0.139	3.294E-01	6360
Concentration of medium HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.136**	0.000	-0.412	0.140	3.351E-01	6360
Phospholipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**-0.117**	0.086	-0.393	0.160	4.076E-01	6360
Triglycerides in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.002**	0.012	-0.280	0.283	9.915E-01	6360
Total cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.023**	0.193	-0.253	0.300	8.683E-01	6360
Cholesterol esters in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.023**	0.157	-0.253	0.299	8.713E-01	6360
Free cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.025**	0.037	-0.251	0.301	8.585E-01	6360
Total lipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.027**	0.258	-0.248	0.303	8.462E-01	6360
Concentration of medium LDL particles	Lipoprotein subclasses LDL	mol/l	**0.029**	0.000	-0.246	0.304	8.367E-01	6360
Phospholipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.018**	0.050	-0.257	0.294	8.956E-01	6360
Triglycerides in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.083**	0.020	-0.188	0.354	5.479E-01	6360
Monounsaturated fatty acids; 16:1, 18:1	Fatty acids and saturation measures	mmol/l	**0.100**	0.993	-0.176	0.377	4.764E-01	6317
Ratio of monounsaturated fatty acids to total fatty acids	Fatty acids (%)	%	**0.209**	2.410	-0.098	0.516	1.826E-01	6317
Total cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.020**	0.078	-0.252	0.292	8.844E-01	6360
Cholesterol esters in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.011**	0.042	-0.262	0.284	9.367E-01	6360
Free cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.030**	0.038	-0.242	0.301	8.301E-01	6360
Total lipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.028**	0.292	-0.244	0.299	8.419E-01	6360
Concentration of medium VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.028**	0.000	-0.243	0.299	8.394E-01	6360
Phospholipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.029**	0.057	-0.242	0.300	8.328E-01	6360
Triglycerides in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.030**	0.159	-0.241	0.301	8.265E-01	6360
Phosphatidylcholine and other cholines	Glycerides and phospholipids	mmol/l	**-0.017**	0.458	-0.297	0.263	9.059E-01	6334
Phenylalanine	Aromatic amino acids	mmol/l	**-0.015**	0.011	-0.302	0.272	9.179E-01	6356

Polyunsaturated fatty acids	Fatty acids and saturation measures	mmol/l	**-0.035**	0.916	-0.317	0.247	8.086E-01	6317
Ratio of polyunsaturated fatty acids to total fatty acids	Fatty acids (%)	%	**-0.190**	2.381	-0.484	0.104	2.061E-01	6317
Pyruvate	Glycolysis related metabolites	mmol/l	**0.176**	0.022	-0.069	0.420	1.586E-01	6341
Remnant cholesterol (non-HDL, non-LDL -cholesterol)	Cholesterol	mmol/l	**0.021**	0.465	-0.254	0.295	8.830E-01	6355
Serum total cholesterol	Cholesterol	mmol/l	**-0.025**	1.156	-0.306	0.256	8.615E-01	6355
Serum total triglycerides	Glycerides and phospholipids	mmol/l	**0.043**	0.568	-0.228	0.314	7.544E-01	6355
Saturated fatty acids	Fatty acids and saturation measures	mmol/l	**0.033**	1.041	-0.245	0.311	8.170E-01	6317
Ratio of saturated fatty acids to total fatty acids	Fatty acids (%)	%	**-0.003**	1.504	-0.284	0.278	9.837E-01	6317
Total cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.007**	0.067	-0.262	0.275	9.618E-01	6360
Cholesterol esters in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.016**	0.059	-0.255	0.287	9.101E-01	6360
Free cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**-0.029**	0.017	-0.304	0.245	8.337E-01	6360
Total lipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**-0.015**	0.141	-0.285	0.255	9.135E-01	6360
Concentration of small HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.012**	0.000	-0.283	0.258	9.291E-01	6360
Phospholipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**-0.049**	0.093	-0.323	0.225	7.277E-01	6360
Triglycerides in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.126**	0.016	-0.145	0.396	3.622E-01	6360
Total cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.021**	0.117	-0.256	0.298	8.828E-01	6360
Cholesterol esters in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.021**	0.095	-0.255	0.298	8.797E-01	6360
Free cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.018**	0.023	-0.258	0.295	8.977E-01	6360
Total lipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.021**	0.159	-0.255	0.298	8.790E-01	6360
Concentration of small LDL particles	Lipoprotein subclasses LDL	mol/l	**0.023**	0.000	-0.253	0.299	8.719E-01	6360
Phospholipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.006**	0.034	-0.271	0.283	9.674E-01	6360
Triglycerides in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.062**	0.013	-0.210	0.334	6.545E-01	6360
Sphingomyelins	Glycerides and phospholipids	mmol/l	**-0.049**	0.086	-0.331	0.233	7.336E-01	6334
Total cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.047**	0.081	-0.224	0.319	7.331E-01	6360
Cholesterol esters in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.039**	0.054	-0.233	0.311	7.785E-01	6360

Free cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.058**	0.030	-0.213	0.330	6.749E-01	6360
Total lipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.052**	0.212	-0.219	0.323	7.079E-01	6360
Concentration of small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.051**	0.000	-0.220	0.322	7.104E-01	6360
Phospholipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.059**	0.044	-0.213	0.331	6.725E-01	6360
Triglycerides in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.048**	0.095	-0.222	0.319	7.267E-01	6360
Ratio of triglycerides to phosphoglycerides	Glycerides and phospholipids	#N/A	**0.098**	0.158	-0.172	0.369	4.757E-01	6336
Total cholines	Glycerides and phospholipids	mmol/l	**-0.025**	0.483	-0.306	0.255	8.595E-01	6334
Total fatty acids	Fatty acids and saturation measures	mmol/l	**0.036**	2.773	-0.241	0.313	7.989E-01	6317
Total phosphoglycerides	Glycerides and phospholipids	mmol/l	**-0.022**	0.397	-0.303	0.259	8.795E-01	6336
Tyrosine	Aromatic amino acids	mmol/l	**0.118**	0.005	-0.159	0.396	4.039E-01	6349
Estimated degree of unsaturation	Fatty acids and saturation measures	#N/A	**-0.149**	0.043	-0.426	0.127	2.900E-01	6317
Valine	Branched--chain amino acids	mmol/l	**-0.058**	0.018	-0.341	0.225	6.867E-01	6349
Total cholesterol in VLDL	Cholesterol	mmol/l	**0.035**	0.273	-0.237	0.307	8.001E-01	6355
Mean diameter for VLDL particles	Lipoprotein particle size	nm	**0.023**	1.005	-0.259	0.305	8.715E-01	6355
Triglycerides in VLDL	Glycerides and phospholipids	mmol/l	**0.032**	0.449	-0.240	0.303	8.181E-01	6355
Total cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.060**	0.104	-0.342	0.222	6.769E-01	6360
Cholesterol esters in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.053**	0.074	-0.335	0.228	7.106E-01	6360
Free cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.076**	0.030	-0.359	0.208	6.003E-01	6360
Total lipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.076**	0.230	-0.359	0.208	6.022E-01	6360
Concentration of very large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.076**	0.000	-0.360	0.208	6.014E-01	6360
Phospholipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.092**	0.122	-0.377	0.194	5.292E-01	6360
Triglycerides in very large HDL	Lipoprotein subclasses HDL	mmol/l	**0.001**	0.010	-0.273	0.276	9.920E-01	6360
Total cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.005**	0.011	-0.280	0.270	9.700E-01	6360
Cholesterol esters in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.001**	0.006	-0.275	0.272	9.920E-01	6360
Free cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.010**	0.005	-0.286	0.267	9.446E-01	6360
Total lipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.001**	0.054	-0.273	0.276	9.918E-01	6360

Concentration of very large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.002**	0.000	-0.272	0.277	9.860E-01	6360
Phospholipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.003**	0.009	-0.279	0.272	9.805E-01	6360
Triglycerides in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.005**	0.034	-0.270	0.280	9.722E-01	6360
Total cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.060**	0.078	-0.211	0.331	6.642E-01	6360
Cholesterol esters in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.062**	0.053	-0.210	0.333	6.563E-01	6360
Free cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.056**	0.026	-0.216	0.328	6.860E-01	6360
Total lipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.064**	0.161	-0.207	0.334	6.444E-01	6360
Concentration of very small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.066**	0.000	-0.204	0.336	6.311E-01	6360
Phospholipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.040**	0.052	-0.234	0.314	7.739E-01	6360
Triglycerides in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.086**	0.041	-0.183	0.355	5.305E-01	6360
Total cholesterol in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.013**	0.004	-0.289	0.262	9.239E-01	6360
Cholesterol esters in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.011**	0.002	-0.286	0.263	9.356E-01	6360
Free cholesterol in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.016**	0.002	-0.294	0.263	9.108E-01	6360
Total lipids in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.031**	0.018	-0.312	0.249	8.261E-01	6360
Conc. of chylomicrons & extremely large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**-0.033**	0.000	-0.314	0.248	8.181E-01	6360
Phospholipids in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.035**	0.002	-0.317	0.247	8.096E-01	6360
Triglycerides in chylomicrons & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.036**	0.012	-0.319	0.246	8.002E-01	6360

APPENDIX 4A5: Results of logistical regression (unadjusted) comparing maternal metabolites of those women who delivered extremely preterm (<196 days, or <28 completed weeks) compared with those who delivered at term (259 days to 294 days, or 37 to 42 completed weeks), excluding those who delivered very or moderate to late preterm (196 days to <259 days, or 28 to <37 completed weeks) and post-term (>294 days, or >42 completed weeks); showing odds ratio per 1 standard deviation (SD) increase in metabolite measured. N=12 for extremely preterm, n=6994 for term, and n=434 excluded from analysis. The biochemical group of the metabolite, units, upper and lower 95% confidence interval. P-values in bold significant at p<0.0029.

Metabolites	Group	Units	OR per 1SD increase in metabolite	SD	Lower 95%CI	Upper 95%CI	p-value	N
Alanine	Amino acids	mmol/l	**0.079**	0.048	-0.468	0.627	7.771E-01	6977
Glutamine	Amino acids	mmol/l	**0.112**	0.046	-0.428	0.651	6.850E-01	6997
Glycine	Amino acids	mmol/l	**0.259**	0.032	-0.160	0.678	2.251E-01	6991
Histidine	Amino acids	mmol/l	**-0.071**	0.010	-0.644	0.503	8.090E-01	6997
Apolipoprotein A-I	Apolipoproteins	g/l	**0.118**	0.220	-0.422	0.658	6.687E-01	7001
Apolipoprotein B	Apolipoproteins	g/l	**0.473**	0.228	0.031	0.915	3.614E-02	7001
apolipoprotein B:apolipoprotein A-I	Apolipoproteins	#N/A	**0.426**	0.120	0.006	0.846	4.656E-02	7001
Phenylalanine	Aromatic amino acids	mmol/l	**0.536**	0.011	0.118	0.954	1.203E-02	7002
Tyrosine	Aromatic amino acids	mmol/l	**-0.202**	0.006	-0.792	0.388	5.022E-01	6993
Isoleucine	Branched--chain amino acids	mmol/l	**0.434**	0.010	0.012	0.857	4.401E-02	7000
Leucine	Branched--chain amino acids	mmol/l	**0.476**	0.010	0.080	0.873	1.851E-02	7000
Valine	Branched--chain amino acids	mmol/l	**0.130**	0.018	-0.407	0.666	6.355E-01	6994
Esterified cholesterol	Cholesterol	mmol/l	**0.194**	0.806	-0.341	0.728	4.778E-01	6977
Free cholesterol	Cholesterol	mmol/l	**0.325**	0.333	-0.185	0.836	2.112E-01	6977
Remnant cholesterol (non-HDL, non-LDL)	Cholesterol	mmol/l	**0.436**	0.466	-0.021	0.892	6.124E-02	7001
Serum total cholesterol	Cholesterol	mmol/l	**0.218**	1.156	-0.298	0.735	4.078E-01	7001
Total cholesterol in HDL	Cholesterol	mmol/l	**-0.133**	0.368	-0.715	0.448	6.529E-01	7001
Total cholesterol in HDL2	Cholesterol	mmol/l	**-0.185**	0.333	-0.770	0.400	5.348E-01	7001

Total cholesterol in HDL3	Cholesterol	mmol/l	**0.242**	0.044	-0.259	0.744	3.439E-01	7001
Total cholesterol in LDL	Cholesterol	mmol/l	**0.114**	0.617	-0.430	0.658	6.818E-01	7001
Total cholesterol in VLDL	Cholesterol	mmol/l	**0.537**	0.274	0.174	0.900	3.710E-03	7001
18:2 linoleic acid to total FAs	Fatty acids (%)	%	**-0.676**	2.694	-1.278	-0.074	2.775E-02	6957
22:6 docosahexaenoic acid to total FAs	Fatty acids (%)	%	**-0.516**	0.230	-1.199	0.167	1.385E-01	6957
Monounsaturated FAs to total FAs	Fatty acids (%)	%	**0.838**	2.413	0.330	1.346	**1.231E-03**	6957
Omega-3 FAs to total FAs	Fatty acids (%)	%	**-0.632**	0.493	-1.290	0.027	6.000E-02	6957
Omega-6 FAs to total FAs	Fatty acids (%)	%	**-0.671**	2.204	-1.220	-0.122	1.650E-02	6957
Polyunsaturated FAs to total FAs	Fatty acids (%)	%	**-0.716**	2.384	-1.259	-0.174	9.658E-03	6957
Saturated FAs to total FAs	Fatty acids (%)	%	**-0.326**	1.505	-0.940	0.288	2.977E-01	6957
18:2, linoleic acid	Fatty acids and saturation measures	mmol/l	**0.132**	0.766	-0.438	0.701	6.501E-01	6957
22:6, docosahexaenoic acid	Fatty acids and saturation measures	mmol/l	**0.170**	0.043	-0.374	0.713	5.403E-01	6957
Estimated degree of unsaturation	Fatty acids and saturation measures	#N/A	**-0.444**	0.043	-1.012	0.125	1.260E-01	6957
Monounsaturated fatty acids; 16:1, 18:1	Fatty acids and saturation measures	mmol/l	**0.648**	0.994	0.193	1.104	5.299E-03	6957
Omega-3 fatty acids	Fatty acids and saturation measures	mmol/l	**0.081**	0.104	-0.491	0.653	7.818E-01	6957
Omega-6 fatty acids	Fatty acids and saturation measures	mmol/l	**0.208**	0.839	-0.355	0.772	4.684E-01	6957
Polyunsaturated fatty acids	Fatty acids and saturation measures	mmol/l	**0.201**	0.916	-0.364	0.766	4.861E-01	6957
Saturated fatty acids	Fatty acids and saturation measures	mmol/l	**0.408**	1.042	-0.123	0.938	1.322E-01	6957
Total fatty acids	Fatty acids and saturation measures	mmol/l	**0.480**	2.774	-0.044	1.003	7.281E-02	6957
Albumin	Fluid balance and inflammation	signal area	**-0.028**	0.006	-0.595	0.539	9.225E-01	7002

Creatinine	Fluid balance and inflammation	mmol/l	**0.186**	0.006	-0.160	0.532	2.932E-01	6998
Glycoprotein acetyls (a1-acid glycoprotein)	Fluid balance and inflammation	mmol/l	**0.449**	0.196	0.117	0.780	7.972E-03	7002
Phosphatidylcholine and other cholines	Glycerides and phospholipids	mmol/l	**0.512**	0.458	0.027	0.996	3.867E-02	6975
Ratio of triglycerides to phosphoglycerides	Glycerides and phospholipids	#N/A	**0.536**	0.159	0.160	0.912	5.177E-03	6977
Serum total triglycerides	Glycerides and phospholipids	mmol/l	**0.528**	0.571	0.269	0.787	**6.583E-05**	7001
Sphingomyelins	Glycerides and phospholipids	mmol/l	**0.162**	0.086	-0.390	0.713	5.656E-01	6975
Total cholines	Glycerides and phospholipids	mmol/l	**0.484**	0.483	-0.015	0.982	5.742E-02	6975
Total phosphoglycerides	Glycerides and phospholipids	mmol/l	**0.611**	0.398	0.131	1.090	1.256E-02	6977
Triglycerides in HDL	Glycerides and phospholipids	mmol/l	**0.638**	0.042	0.289	0.988	**3.450E-04**	7001
Triglycerides in LDL	Glycerides and phospholipids	mmol/l	**0.612**	0.070	0.236	0.989	**1.446E-03**	7001
Triglycerides in VLDL	Glycerides and phospholipids	mmol/l	**0.480**	0.451	0.238	0.723	**1.013E-04**	7001
Citrate	Glycolysis related metabolites	mmol/l	**-0.054**	0.017	-0.658	0.550	8.611E-01	6882
Glucose	Glycolysis related metabolites	mmol/l	**-0.118**	0.488	-0.716	0.480	6.990E-01	6977
Glycerol	Glycolysis related metabolites	mmol/l	**0.612**	0.016	0.207	1.017	3.087E-03	6988
Lactate	Glycolysis related metabolites	mmol/l	**-0.147**	0.492	-0.779	0.485	6.482E-01	6990
Pyruvate	Glycolysis related metabolites	mmol/l	**-0.127**	0.022	-0.735	0.482	6.835E-01	6986
3-hydroxybutyrate	Ketone bodies	mmol/l	**0.017**	0.038	-0.533	0.566	9.520E-01	7002
Acetate	Ketone bodies	mmol/l	**0.115**	0.006	-0.415	0.644	6.711E-01	6996
Mean diameter for HDL particles	Lipoprotein particle size	nm	**-0.380**	0.204	-0.940	0.180	1.839E-01	7001
Mean diameter for LDL particles	Lipoprotein particle size	nm	**-0.023**	0.063	-0.592	0.546	9.370E-01	7001
Mean diameter for VLDL particles	Lipoprotein particle size	nm	**0.574**	1.006	0.061	1.088	2.846E-02	7001
Cholesterol esters in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.299**	0.143	-0.893	0.295	3.235E-01	7006
Cholesterol esters in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.038**	0.086	-0.525	0.601	8.937E-01	7006
Cholesterol esters in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.108**	0.059	-0.451	0.666	7.047E-01	7006
Cholesterol esters in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.174**	0.074	-0.755	0.407	5.580E-01	7006
Concentration of large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.210**	0.000	-0.796	0.377	4.833E-01	7006

Concentration of medium HDL particles	Lipoprotein subclasses HDL	mol/l	**0.298**	0.000	-0.217	0.813	2.568E-01	7006
Concentration of small HDL particles	Lipoprotein subclasses HDL	mol/l	**0.533**	0.000	0.062	1.005	2.664E-02	7006
Concentration of very large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.232**	0.000	-0.820	0.356	4.386E-01	7006
Free cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.305**	0.045	-0.898	0.289	3.146E-01	7006
Free cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.218**	0.023	-0.310	0.746	4.181E-01	7006
Free cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.528**	0.017	0.049	1.007	3.081E-02	7006
Free cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.183**	0.030	-0.769	0.403	5.412E-01	7006
Phospholipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.174**	0.156	-0.755	0.408	5.577E-01	7006
Phospholipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.347**	0.086	-0.156	0.851	1.765E-01	7006
Phospholipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.456**	0.094	-0.058	0.969	8.201E-02	7006
Phospholipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.332**	0.122	-0.925	0.262	2.732E-01	7006
Total cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.301**	0.188	-0.895	0.293	3.212E-01	7006
Total cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.078**	0.108	-0.480	0.636	7.835E-01	7006
Total cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.235**	0.067	-0.294	0.763	3.842E-01	7006
Total cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.177**	0.103	-0.760	0.406	5.515E-01	7006
Total lipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.224**	0.354	-0.811	0.364	4.553E-01	7006
Total lipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.269**	0.195	-0.253	0.791	3.122E-01	7006
Total lipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.504**	0.141	0.029	0.979	3.753E-02	7006
Total lipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.234**	0.229	-0.822	0.354	4.360E-01	7006
Triglycerides in large HDL	Lipoprotein subclasses HDL	mmol/l	**0.233**	0.016	-0.294	0.760	3.861E-01	7006
Triglycerides in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.788**	0.012	0.410	1.166	**4.445E-05**	7006
Triglycerides in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.689**	0.016	0.371	1.007	**2.165E-05**	7006
Triglycerides in very large HDL	Lipoprotein subclasses HDL	mmol/l	**0.410**	0.010	-0.070	0.891	9.438E-02	7006
Cholesterol esters in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.148**	0.234	-0.389	0.685	5.896E-01	7006
Cholesterol esters in LDL	Lipoprotein subclasses LDL	mmol/l	**0.152**	0.163	-0.383	0.688	5.775E-01	7006
Cholesterol esters in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.104**	0.157	-0.442	0.651	7.081E-01	7006
Cholesterol esters in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.071**	0.095	-0.483	0.625	8.028E-01	7006
Concentration of large LDL particles	Lipoprotein subclasses LDL	mol/l	**0.222**	0.000	-0.298	0.741	4.030E-01	7006

Concentration of LDL particles	Lipoprotein subclasses LDL	mol/l	**0.251**	0.000	-0.257	0.760	3.324E-01	7001
Concentration of medium LDL particles	Lipoprotein subclasses LDL	mol/l	**0.230**	0.000	-0.288	0.749	3.834E-01	7006
Concentration of small LDL particles	Lipoprotein subclasses LDL	mol/l	**0.248**	0.000	-0.266	0.762	3.444E-01	7006
Free cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.043**	0.076	-0.516	0.602	8.807E-01	7006
Free cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**0.049**	0.067	-0.508	0.607	8.620E-01	7006
Free cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.166**	0.037	-0.370	0.702	5.437E-01	7006
Free cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.198**	0.023	-0.329	0.725	4.622E-01	7006
Phospholipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.167**	0.088	-0.367	0.701	5.395E-01	7006
Phospholipids in LDL	Lipoprotein subclasses LDL	mmol/l	**0.134**	0.089	-0.407	0.675	6.272E-01	7006
Phospholipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.311**	0.050	-0.189	0.811	2.228E-01	7006
Phospholipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.334**	0.034	-0.156	0.824	1.816E-01	7006
Total cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.123**	0.310	-0.420	0.666	6.572E-01	7006
Total cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**0.123**	0.230	-0.419	0.665	6.565E-01	7006
Total cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.116**	0.193	-0.428	0.661	6.754E-01	7006
Total cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.096**	0.117	-0.452	0.645	7.304E-01	7006
Total lipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.192**	0.428	-0.335	0.719	4.755E-01	7006
Total lipids in LDL	Lipoprotein subclasses LDL	mmol/l	**0.213**	0.349	-0.306	0.732	4.210E-01	7001
Total lipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.208**	0.258	-0.315	0.732	4.355E-01	7006
Total lipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.221**	0.159	-0.300	0.742	4.060E-01	7006
Triglycerides in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.596**	0.039	0.208	0.984	**2.616E-03**	7006
Triglycerides in LDL	Lipoprotein subclasses LDL	mmol/l	**0.631**	0.042	0.260	1.001	**8.504E-04**	7006
Triglycerides in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.607**	0.020	0.224	0.990	**1.895E-03**	7006
Triglycerides in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.668**	0.013	0.320	1.016	**1.686E-04**	7006
Cholesterol esters in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.354**	0.002	0.096	0.613	7.239E-03	7006
Cholesterol esters in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.414**	0.022	0.177	0.651	**6.157E-04**	7006
Cholesterol esters in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.470**	0.042	0.165	0.775	**2.528E-03**	7006
Cholesterol esters in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.452**	0.054	0.001	0.904	4.961E-02	7006
Cholesterol esters in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.371**	0.006	0.151	0.591	**9.410E-04**	7006

Cholesterol esters in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.177**	0.053	-0.353	0.707	5.130E-01	7006
Conc. of CMs & extremely large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.350**	0.000	0.135	0.565	**1.397E-03**	7006
Concentration of large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.450**	0.000	0.218	0.683	**1.460E-04**	7006
Concentration of medium VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.473**	0.000	0.224	0.721	**1.942E-04**	7006
Concentration of small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.589**	0.000	0.285	0.893	**1.461E-04**	7006
Concentration of very large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.414**	0.000	0.194	0.635	**2.267E-04**	7006
Concentration of very small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.473**	0.000	0.029	0.918	3.671E-02	7006
Free cholesterol in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.382**	0.002	0.170	0.594	**4.163E-04**	7006
Free cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.440**	0.023	0.213	0.667	**1.418E-04**	7006
Free cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.482**	0.038	0.236	0.727	**1.205E-04**	7006
Free cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.638**	0.030	0.314	0.961	**1.107E-04**	7006
Free cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.385**	0.005	0.168	0.602	**5.078E-04**	7006
Free cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.343**	0.026	-0.138	0.824	**1.618E-01**	7006
Phospholipids in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.370**	0.002	0.155	0.584	**7.336E-04**	7006
Phospholipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.462**	0.036	0.226	0.699	**1.256E-04**	7006
Phospholipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.487**	0.057	0.233	0.741	**1.732E-04**	7006
Phospholipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.638**	0.044	0.325	0.952	**6.501E-05**	7006
Phospholipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.419**	0.009	0.194	0.644	**2.652E-04**	7006
Phospholipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.293**	0.052	-0.205	0.791	2.487E-01	7006
Total cholesterol in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.369**	0.004	0.137	0.601	**1.817E-03**	7006
Total cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.429**	0.045	0.198	0.659	**2.702E-04**	7006

Total cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.483**	0.079	0.212	0.754	**4.855E-04**	7006
Total cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.564**	0.082	0.160	0.969	6.259E-03	7006
Total cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.377**	0.011	0.159	0.595	**6.982E-04**	7006
Total cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.236**	0.078	-0.278	0.750	3.679E-01	7006
Total lipids in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.352**	0.018	0.137	0.568	**1.342E-03**	7006
Total lipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.449**	0.195	0.217	0.681	**1.501E-04**	7006
Total lipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.474**	0.293	0.224	0.724	**1.990E-04**	7006
Total lipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.599**	0.213	0.285	0.914	**1.882E-04**	7006
Total lipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.412**	0.054	0.192	0.632	**2.451E-04**	7006
Total lipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.432**	0.161	-0.025	0.890	6.398E-02	7006
Triglycerides in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.345**	0.012	0.133	0.557	**1.426E-03**	7006
Triglycerides in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.453**	0.115	0.221	0.685	**1.313E-04**	7006
Triglycerides in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.466**	0.160	0.223	0.708	**1.656E-04**	7006
Triglycerides in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.532**	0.096	0.271	0.792	**6.267E-05**	7006
Triglycerides in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.421**	0.034	0.200	0.642	**1.847E-04**	7006
Triglycerides in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.652**	0.041	0.339	0.965	**4.439E-05**	7006

APPENDIX 4A6: Results of logistical regression (unadjusted) comparing maternal metabolites of those women who delivered VERY preterm (196 days to <224 days, or 28 to <32 completed weeks) compared with those who delivered at term (259 days to 294 days, or 37 to 42 completed weeks), excluding those who delivered extremely (<196 days, or <28 completed weeks) or moderate to late preterm (224 days to <259 days, or 32 to <37 completed weeks) and post-term (>294 days, or >42 completed weeks); showing odds ratio per 1 standard deviation (SD) increase in metabolite measured. N=51 for very preterm, n=6994 for term, and n=395 excluded from analysis. The biochemical group of the metabolite, units, upper and lower 95% confidence interval. P-values in bold significant at p<0.0029.

Metabolites	Group	Units	OR per 1SD increase in metabolite	SD	Lower 95%CI	Upper 95%CI	p-value	N
Alanine	Amino acids	mmol/l	**0.057**	0.048	-0.218	0.331	6.849E-01	7014
Glutamine	Amino acids	mmol/l	**0.031**	0.046	-0.241	0.303	8.226E-01	7036
Glycine	Amino acids	mmol/l	**0.055**	0.032	-0.212	0.322	6.866E-01	7030
Histidine	Amino acids	mmol/l	**0.164**	0.010	-0.104	0.432	2.301E-01	7035
Apolipoprotein A-I	Apolipoproteins	g/l	**-0.087**	0.219	-0.369	0.196	5.466E-01	7040
Apolipoprotein B	Apolipoproteins	g/l	**0.237**	0.228	-0.011	0.484	6.083E-02	7040
apolipoprotein B:apolipoprotein A-I	Apolipoproteins	#N/A	**0.279**	0.121	0.043	0.515	2.045E-02	7040
Phenylalanine	Aromatic amino acids	mmol/l	**0.214**	0.011	-0.038	0.466	9.569E-02	7041
Tyrosine	Aromatic amino acids	mmol/l	**0.087**	0.006	-0.182	0.357	5.248E-01	7032
Isoleucine	Branched--chain amino acids	mmol/l	**0.133**	0.010	-0.125	0.392	3.108E-01	7038
Leucine	Branched--chain amino acids	mmol/l	**0.219**	0.010	-0.031	0.470	8.645E-02	7038
Valine	Branched--chain amino acids	mmol/l	**0.087**	0.018	-0.182	0.356	5.244E-01	7032
Esterified cholesterol	Cholesterol	mmol/l	**-0.049**	0.805	-0.328	0.230	7.301E-01	7016
Free cholesterol	Cholesterol	mmol/l	**0.031**	0.333	-0.242	0.305	8.213E-01	7016
Remnant cholesterol (non-HDL, non-LDL)	Cholesterol	mmol/l	**0.190**	0.466	-0.064	0.444	1.431E-01	7040
Serum total cholesterol	Cholesterol	mmol/l	**-0.033**	1.156	-0.312	0.245	8.148E-01	7040
Total cholesterol in HDL	Cholesterol	mmol/l	**-0.257**	0.368	-0.544	0.031	8.025E-02	7040

Total cholesterol in HDL2	Cholesterol	mmol/l	**-0.276**	0.334	-0.564	0.011	5.937E-02	7040
Total cholesterol in HDL3	Cholesterol	mmol/l	**-0.048**	0.044	-0.327	0.232	7.392E-01	7040
Total cholesterol in LDL	Cholesterol	mmol/l	**-0.072**	0.617	-0.354	0.209	6.143E-01	7040
Total cholesterol in VLDL	Cholesterol	mmol/l	**0.330**	0.275	0.112	0.549	3.031E-03	7040
18:2 linoleic acid to total FAs	Fatty acids (%)	%	**-0.256**	2.692	-0.540	0.029	7.868E-02	6995
22:6 docosahexaenoic acid to total FAs	Fatty acids (%)	%	**-0.245**	0.230	-0.551	0.060	1.156E-01	6995
Monounsaturated FAs to total FAs	Fatty acids (%)	%	**0.290**	2.414	0.020	0.559	3.538E-02	6995
Omega-3 FAs to total FAs	Fatty acids (%)	%	**-0.321**	0.493	-0.626	-0.016	3.926E-02	6995
Omega-6 FAs to total FAs	Fatty acids (%)	%	**-0.304**	2.203	-0.578	-0.030	2.977E-02	6995
Polyunsaturated FAs to total FAs	Fatty acids (%)	%	**-0.337**	2.384	-0.609	-0.065	1.516E-02	6995
Saturated FAs to total FAs	Fatty acids (%)	%	**0.068**	1.507	-0.211	0.346	6.350E-01	6995
18:2, linoleic acid	Fatty acids and saturation measures	mmol/l	**0.035**	0.766	-0.244	0.313	8.067E-01	6995
22:6, docosahexaenoic acid	Fatty acids and saturation measures	mmol/l	**-0.019**	0.043	-0.302	0.265	8.976E-01	6995
Estimated degree of unsaturation	Fatty acids and saturation measures	#N/A	**-0.271**	0.043	-0.547	0.005	5.406E-02	6995
Monounsaturated fatty acids; 16:1, 18:1	Fatty acids and saturation measures	mmol/l	**0.264**	0.995	0.009	0.519	4.278E-02	6995
Omega-3 fatty acids	Fatty acids and saturation measures	mmol/l	**-0.080**	0.104	-0.369	0.210	5.896E-01	6995
Omega-6 fatty acids	Fatty acids and saturation measures	mmol/l	**0.047**	0.839	-0.232	0.325	7.428E-01	6995
Polyunsaturated fatty acids	Fatty acids and saturation measures	mmol/l	**0.034**	0.916	-0.245	0.313	8.106E-01	6995
Saturated fatty acids	Fatty acids and saturation measures	mmol/l	**0.193**	1.043	-0.074	0.460	1.562E-01	6995
Total fatty acids	Fatty acids and saturation measures	mmol/l	**0.183**	2.776	-0.085	0.452	1.812E-01	6995

Albumin	Fluid balance and inflammation	signal area	**0.108**	0.006	-0.152	0.368	4.154E-01	7041
Creatinine	Fluid balance and inflammation	mmol/l	**0.212**	0.006	0.039	0.386	1.647E-02	7036
Glycoprotein acetyls (a1-acid glycoprotein)	Fluid balance and inflammation	mmol/l	**0.372**	0.197	0.196	0.548	3.318E-05	7041
Phosphatidylcholine and other cholines	Glycerides and phospholipids	mmol/l	**0.157**	0.458	-0.108	0.422	2.450E-01	7014
Ratio of triglycerides to phosphoglycerides	Glycerides and phospholipids	#N/A	**0.374**	0.160	0.170	0.579	3.350E-04	7016
Serum total triglycerides	Glycerides and phospholipids	mmol/l	**0.378**	0.578	0.215	0.540	5.334E-06	7040
Sphingomyelins	Glycerides and phospholipids	mmol/l	**-0.131**	0.086	-0.412	0.149	3.595E-01	7014
Total cholines	Glycerides and phospholipids	mmol/l	**0.131**	0.483	-0.137	0.398	3.396E-01	7014
Total phosphoglycerides	Glycerides and phospholipids	mmol/l	**0.224**	0.398	-0.039	0.486	9.475E-02	7016
Triglycerides in HDL	Glycerides and phospholipids	mmol/l	**0.316**	0.042	0.092	0.540	5.784E-03	7040
Triglycerides in LDL	Glycerides and phospholipids	mmol/l	**0.247**	0.070	0.003	0.490	4.686E-02	7040
Triglycerides in VLDL	Glycerides and phospholipids	mmol/l	**0.368**	0.458	0.218	0.518	1.569E-06	7040
Citrate	Glycolysis related metabolites	mmol/l	**0.135**	0.017	-0.128	0.397	3.141E-01	6921
Glucose	Glycolysis related metabolites	mmol/l	**0.026**	0.488	-0.243	0.295	8.501E-01	7016
Glycerol	Glycolysis related metabolites	mmol/l	**0.428**	0.016	0.204	0.652	1.850E-04	7027
Lactate	Glycolysis related metabolites	mmol/l	**0.096**	0.492	-0.158	0.350	4.591E-01	7029
Pyruvate	Glycolysis related metabolites	mmol/l	**0.220**	0.022	-0.019	0.459	7.063E-02	7025
3-hydroxybutyrate	Ketone bodies	mmol/l	**0.044**	0.038	-0.213	0.300	7.383E-01	7040
Acetate	Ketone bodies	mmol/l	**0.054**	0.006	-0.216	0.324	6.952E-01	7034
Mean diameter for HDL particles	Lipoprotein particle size	nm	**-0.517**	0.204	-0.790	-0.245	1.986E-04	7040
Mean diameter for LDL particles	Lipoprotein particle size	nm	**-0.252**	0.063	-0.531	0.028	7.774E-02	7040
Mean diameter for VLDL particles	Lipoprotein particle size	nm	**0.416**	1.008	0.157	0.674	1.604E-03	7040
Cholesterol esters in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.409**	0.143	-0.702	-0.117	6.110E-03	7045
Cholesterol esters in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.117**	0.086	-0.153	0.387	3.973E-01	7045
Cholesterol esters in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.038**	0.059	-0.237	0.313	7.853E-01	7045
Cholesterol esters in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.500**	0.074	-0.794	-0.207	8.298E-04	7045

Concentration of large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.370**	0.000	-0.660	-0.080	1.246E-02	7045
Concentration of medium HDL particles	Lipoprotein subclasses HDL	mol/l	**0.216**	0.000	-0.044	0.476	1.033E-01	7045
Concentration of small HDL particles	Lipoprotein subclasses HDL	mol/l	**0.320**	0.000	0.065	0.576	1.404E-02	7045
Concentration of very large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.501**	0.000	-0.796	-0.205	9.126E-04	7045
Free cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.404**	0.045	-0.696	-0.111	6.790E-03	7045
Free cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.145**	0.023	-0.120	0.409	2.843E-01	7045
Free cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.304**	0.017	0.047	0.561	2.046E-02	7045
Free cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.480**	0.030	-0.778	-0.181	1.669E-03	7045
Phospholipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.325**	0.156	-0.611	-0.038	2.635E-02	7045
Phospholipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.210**	0.086	-0.050	0.470	1.135E-01	7045
Phospholipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.325**	0.094	0.065	0.585	1.444E-02	7045
Phospholipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.502**	0.122	-0.797	-0.208	8.177E-04	7045
Total cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.408**	0.188	-0.701	-0.116	6.257E-03	7045
Total cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.123**	0.109	-0.146	0.392	3.697E-01	7045
Total cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.112**	0.067	-0.158	0.383	4.160E-01	7045
Total cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.497**	0.104	-0.792	-0.201	9.770E-04	7045
Total lipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.374**	0.354	-0.664	-0.083	1.164E-02	7045
Total lipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.200**	0.195	-0.061	0.461	1.336E-01	7045
Total lipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.306**	0.141	0.050	0.562	1.926E-02	7045
Total lipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.501**	0.230	-0.797	-0.205	9.080E-04	7045
Triglycerides in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.303**	0.016	-0.596	-0.010	4.233E-02	7045
Triglycerides in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.507**	0.012	0.293	0.721	3.328E-06	7045
Triglycerides in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.325**	0.016	0.096	0.553	5.345E-03	7045
Triglycerides in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.183**	0.010	-0.471	0.105	2.126E-01	7045
Cholesterol esters in large LDL	Lipoprotein subclasses LDL	mmol/l	**-0.046**	0.234	-0.325	0.233	7.474E-01	7045
Cholesterol esters in LDL	Lipoprotein subclasses LDL	mmol/l	**-0.040**	0.163	-0.319	0.239	7.767E-01	7045
Cholesterol esters in medium LDL	Lipoprotein subclasses LDL	mmol/l	**-0.067**	0.157	-0.348	0.214	6.380E-01	7045
Cholesterol esters in small LDL	Lipoprotein subclasses LDL	mmol/l	**-0.092**	0.095	-0.374	0.191	5.239E-01	7045

Concentration of large LDL particles	Lipoprotein subclasses LDL	mol/l	**-0.019**	0.000	-0.297	0.258	8.913E-01	7045
Concentration of LDL particles	Lipoprotein subclasses LDL	mol/l	**-0.008**	0.000	-0.285	0.268	9.524E-01	7040
Concentration of medium LDL particles	Lipoprotein subclasses LDL	mol/l	**-0.002**	0.000	-0.277	0.274	9.907E-01	7045
Concentration of small LDL particles	Lipoprotein subclasses LDL	mol/l	**0.015**	0.000	-0.260	0.289	9.170E-01	7045
Free cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**-0.148**	0.076	-0.433	0.138	3.101E-01	7045
Free cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**-0.155**	0.067	-0.441	0.132	2.902E-01	7045
Free cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**-0.042**	0.037	-0.321	0.236	7.671E-01	7045
Free cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**-0.016**	0.023	-0.293	0.261	9.087E-01	7045
Phospholipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**-0.045**	0.088	-0.324	0.234	7.539E-01	7045
Phospholipids in LDL	Lipoprotein subclasses LDL	mmol/l	**-0.094**	0.089	-0.377	0.189	5.130E-01	7045
Phospholipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.073**	0.050	-0.196	0.342	5.955E-01	7045
Phospholipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.091**	0.034	-0.177	0.359	5.047E-01	7045
Total cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**-0.071**	0.310	-0.352	0.211	6.224E-01	7045
Total cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**-0.074**	0.230	-0.355	0.208	6.086E-01	7045
Total cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**-0.063**	0.193	-0.343	0.218	6.613E-01	7045
Total cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**-0.078**	0.117	-0.359	0.204	5.892E-01	7045
Total lipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**-0.037**	0.428	-0.316	0.241	7.927E-01	7045
Total lipids in LDL	Lipoprotein subclasses LDL	mmol/l	**-0.033**	0.349	-0.312	0.246	8.157E-01	7040
Total lipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**-0.013**	0.258	-0.290	0.263	9.256E-01	7045
Total lipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**-0.002**	0.159	-0.278	0.274	9.885E-01	7045
Triglycerides in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.224**	0.039	-0.024	0.472	7.729E-02	7045
Triglycerides in LDL	Lipoprotein subclasses LDL	mmol/l	**0.292**	0.042	0.056	0.528	1.523E-02	7045
Triglycerides in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.221**	0.020	-0.028	0.470	8.126E-02	7045
Triglycerides in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.350**	0.013	0.126	0.574	2.167E-03	7045
Cholesterol esters in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.265**	0.002	0.104	0.427	1.269E-03	7045
Cholesterol esters in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.318**	0.022	0.171	0.464	2.184E-05	7045
Cholesterol esters in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.322**	0.042	0.134	0.511	8.083E-04	7045
Cholesterol esters in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.200**	0.054	-0.054	0.453	1.223E-01	7045

Cholesterol esters in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.287**	0.006	0.149	0.425	4.708E-05	7045
Cholesterol esters in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**-0.036**	0.053	-0.315	0.243	7.994E-01	7045
Conc. of CMs & extremely large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.284**	0.000	0.151	0.417	2.948E-05	7045
Concentration of large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.349**	0.000	0.205	0.494	2.227E-06	7045
Concentration of medium VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.363**	0.000	0.211	0.515	2.837E-06	7045
Concentration of small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.411**	0.000	0.227	0.595	1.198E-05	7045
Concentration of very large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.324**	0.000	0.185	0.463	4.746E-06	7045
Concentration of very small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.184**	0.000	-0.071	0.439	1.570E-01	7045
Free cholesterol in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.301**	0.002	0.167	0.436	1.188E-05	7045
Free cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.339**	0.023	0.196	0.482	3.339E-06	7045
Free cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.364**	0.039	0.212	0.516	2.838E-06	7045
Free cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.421**	0.031	0.225	0.618	2.662E-05	7045
Free cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.302**	0.005	0.164	0.439	1.645E-05	7045
Free cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.081**	0.026	-0.186	0.348	5.518E-01	7045
Phospholipids in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.297**	0.002	0.162	0.431	1.587E-05	7045
Phospholipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.354**	0.036	0.206	0.502	2.679E-06	7045
Phospholipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.369**	0.058	0.213	0.525	3.521E-06	7045
Phospholipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.438**	0.044	0.248	0.629	6.483E-06	7045
Phospholipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.324**	0.009	0.182	0.466	7.884E-06	7045
Phospholipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.040**	0.052	-0.232	0.311	7.746E-01	7045
Total cholesterol in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.284**	0.004	0.139	0.429	1.285E-04	7045

Total cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.329**	0.046	0.185	0.473	7.944E-06	7045
Total cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.349**	0.080	0.183	0.516	3.945E-05	7045
Total cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.310**	0.082	0.074	0.546	1.014E-02	7045
Total cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.293**	0.011	0.156	0.431	2.855E-05	7045
Total cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.003**	0.078	-0.272	0.278	9.831E-01	7045
Total lipids in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.285**	0.018	0.151	0.418	3.036E-05	7045
Total lipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.348**	0.198	0.203	0.493	2.452E-06	7045
Total lipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.363**	0.297	0.210	0.515	3.354E-06	7045
Total lipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.411**	0.214	0.220	0.601	2.355E-05	7045
Total lipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.322**	0.055	0.183	0.460	5.480E-06	7045
Total lipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.149**	0.161	-0.110	0.408	2.591E-01	7045
Triglycerides in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.282**	0.012	0.151	0.414	2.401E-05	7045
Triglycerides in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.353**	0.116	0.209	0.497	1.551E-06	7045
Triglycerides in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.365**	0.162	0.217	0.513	1.332E-06	7045
Triglycerides in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.396**	0.097	0.237	0.555	1.082E-06	7045
Triglycerides in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.330**	0.035	0.192	0.469	3.091E-06	7045
Triglycerides in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.410**	0.042	0.211	0.609	5.200E-05	7045

APPENDIX 4A7: Results of logistical regression (unadjusted) comparing maternal metabolites of those women who delivered MODERATE TO LATE preterm (224 days to <259 days, or 32 to <37 completed weeks) compared with those who delivered at term (259 days to 294 days, or 37 to 42 completed weeks), excluding those who delivered extremely or very preterm (<224 days, or <32 weeks) or post-term (>294 days, or >42 completed weeks); showing odds ratio per 1 standard deviation (SD) increase in metabolite measured. N=329 for moderate to late preterm, n=6994 for term, and n=117 excluded from analysis. The biochemical group of the metabolite, units, upper and lower 95% confidence interval. P-values in bold significant at p<0.0029.

Metabolites	Group	Units	OR per 1SD increase in metabolite	SD	Lower 95%CI	Upper 95%CI	p-value	N
Acetate	Ketone bodies	mmol/l	**-0.045**	0.006	-0.158	0.068	4.389E-01	7313
Alanine	Amino acids	mmol/l	**0.103**	0.048	-0.004	0.210	5.831E-02	7293
Albumin	Fluid balance and inflammation	signal area	**0.126**	0.006	0.023	0.230	1.674E-02	7319
Apolipoprotein A-I	Apolipoproteins	g/l	**0.037**	0.221	-0.072	0.146	5.078E-01	7318
Apolipoprotein B	Apolipoproteins	g/l	**0.096**	0.228	-0.011	0.203	7.831E-02	7318
apolipoprotein B:apolipoprotein A-I	Apolipoproteins	#N/A	**0.085**	0.120	-0.022	0.192	1.216E-01	7318
3-hydroxybutyrate	Ketone bodies	mmol/l	**0.020**	0.038	-0.088	0.127	7.214E-01	7319
Citrate	Glycolysis related metabolites	mmol/l	**0.059**	0.017	-0.050	0.169	2.891E-01	7193
Creatinine	Fluid balance and inflammation	mmol/l	**0.195**	0.006	0.099	0.291	7.040E-05	7315
22:6, docosahexaenoic acid	Fatty acids and saturation measures	mmol/l	**0.085**	0.043	-0.022	0.192	1.209E-01	7272
22:6 docosahexaenoic acid to total FAs	Fatty acids (%)	%	**-0.059**	0.230	-0.173	0.055	3.085E-01	7272
Esterified cholesterol	Cholesterol	mmol/l	**0.064**	0.807	-0.045	0.174	2.484E-01	7292

Omega-3 fatty acids	Fatty acids and saturation measures	mmol/l	**0.096**	0.104	-0.011	0.204	7.851E-02	7272
Omega-3 FAs to total FAs	Fatty acids (%)	%	**-0.036**	0.492	-0.149	0.077	5.333E-01	7272
Omega-6 fatty acids	Fatty acids and saturation measures	mmol/l	**0.066**	0.840	-0.044	0.175	2.392E-01	7272
Omega-6 FAs to total FAs	Fatty acids (%)	%	**-0.128**	2.204	-0.238	-0.018	2.310E-02	7272
Free cholesterol	Cholesterol	mmol/l	**0.073**	0.334	-0.036	0.182	1.911E-01	7292
Glucose	Glycolysis related metabolites	mmol/l	**0.054**	0.490	-0.051	0.160	3.142E-01	7294
Glutamine	Amino acids	mmol/l	**-0.015**	0.046	-0.126	0.096	7.890E-01	7314
Glycerol	Glycolysis related metabolites	mmol/l	**0.237**	0.016	0.136	0.338	4.346E-06	7305
Glycine	Amino acids	mmol/l	**0.060**	0.032	-0.048	0.167	2.780E-01	7304
Glycoprotein acetyls (a1-acid glycoprotein)	Fluid balance and inflammation	mmol/l	**0.162**	0.196	0.062	0.263	1.577E-03	7319
Total cholesterol in HDL2	Cholesterol	mmol/l	**-0.014**	0.335	-0.125	0.097	7.996E-01	7318
Total cholesterol in HDL3	Cholesterol	mmol/l	**0.069**	0.044	-0.039	0.176	2.132E-01	7318
Total cholesterol in HDL	Cholesterol	mmol/l	**-0.005**	0.369	-0.115	0.106	9.363E-01	7318
Mean diameter for HDL particles	Lipoprotein particle size	nm	**-0.134**	0.205	-0.244	-0.023	1.754E-02	7318
Triglycerides in HDL	Glycerides and phospholipids	mmol/l	**0.093**	0.042	-0.013	0.200	8.596E-02	7318
Histidine	Amino acids	mmol/l	**0.041**	0.010	-0.069	0.151	4.658E-01	7314
Total cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**0.058**	0.230	-0.051	0.167	2.952E-01	7323
Cholesterol esters in LDL	Lipoprotein subclasses LDL	mmol/l	**0.062**	0.163	-0.047	0.171	2.626E-01	7323
Free cholesterol in LDL	Lipoprotein subclasses LDL	mmol/l	**0.048**	0.067	-0.061	0.157	3.902E-01	7323
Total lipids in LDL	Lipoprotein subclasses LDL	mmol/l	**0.062**	0.349	-0.047	0.170	2.650E-01	7318

Concentration of LDL particles	Lipoprotein subclasses LDL	mol/l	**0.065**	0.000	-0.043	0.173	2.403E-01	7318
Phospholipids in LDL	Lipoprotein subclasses LDL	mmol/l	**0.056**	0.089	-0.053	0.164	3.175E-01	7323
Triglycerides in LDL	Lipoprotein subclasses LDL	mmol/l	**0.100**	0.042	-0.006	0.206	6.434E-02	7323
Isoleucine	Branched--chain amino acids	mmol/l	**0.112**	0.010	0.008	0.217	3.561E-02	7317
18:2, linoleic acid	Fatty acids and saturation measures	mmol/l	**0.044**	0.766	-0.066	0.154	4.344E-01	7272
Lactate	Glycolysis related metabolites	mmol/l	**0.049**	0.493	-0.057	0.156	3.657E-01	7307
18:2 linoleic acid to total FAs	Fatty acids (%)	%	**-0.133**	2.691	-0.245	-0.021	1.963E-02	7272
Total cholesterol in LDL	Cholesterol	mmol/l	**0.059**	0.617	-0.049	0.168	2.840E-01	7318
Mean diameter for LDL particles	Lipoprotein particle size	nm	**-0.064**	0.062	-0.175	0.048	2.643E-01	7318
Triglycerides in LDL	Glycerides and phospholipids	mmol/l	**0.098**	0.070	-0.009	0.204	7.233E-02	7318
Leucine	Branched--chain amino acids	mmol/l	**0.126**	0.010	0.020	0.232	1.925E-02	7317
Total cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.057**	0.188	-0.168	0.055	3.205E-01	7323
Cholesterol esters in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.057**	0.143	-0.168	0.055	3.200E-01	7323
Free cholesterol in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.056**	0.045	-0.168	0.055	3.226E-01	7323
Total lipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.041**	0.355	-0.152	0.070	4.714E-01	7323
Concentration of large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.039**	0.000	-0.150	0.073	4.948E-01	7323
Phospholipids in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.023**	0.157	-0.134	0.088	6.802E-01	7323

Triglycerides in large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.013**	0.016	-0.124	0.098	8.212E-01	7323
Total cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.061**	0.310	-0.048	0.169	2.749E-01	7323
Cholesterol esters in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.064**	0.234	-0.044	0.173	2.458E-01	7323
Free cholesterol in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.049**	0.076	-0.060	0.158	3.804E-01	7323
Total lipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.066**	0.428	-0.042	0.174	2.332E-01	7323
Concentration of large LDL particles	Lipoprotein subclasses LDL	mol/l	**0.069**	0.000	-0.040	0.177	2.137E-01	7323
Phospholipids in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.066**	0.088	-0.043	0.174	2.339E-01	7323
Triglycerides in large LDL	Lipoprotein subclasses LDL	mmol/l	**0.096**	0.038	-0.011	0.202	7.916E-02	7323
Total cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.096**	0.045	-0.005	0.197	6.166E-02	7323
Cholesterol esters in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.084**	0.022	-0.018	0.187	1.070E-01	7323
Free cholesterol in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.107**	0.023	0.007	0.207	3.583E-02	7323
Total lipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.106**	0.194	0.006	0.206	3.768E-02	7323
Concentration of large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.107**	0.000	0.007	0.207	3.669E-02	7323
Phospholipids in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.109**	0.036	0.009	0.210	3.307E-02	7323
Triglycerides in large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.109**	0.114	0.009	0.209	3.261E-02	7323
Total cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.081**	0.109	-0.028	0.190	1.443E-01	7323

Cholesterol esters in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.076**	0.086	-0.033	0.185	1.723E-01	7323
Free cholesterol in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.099**	0.023	-0.009	0.207	7.309E-02	7323
Total lipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.108**	0.195	0.000	0.216	5.008E-02	7323
Concentration of medium HDL particles	Lipoprotein subclasses HDL	mol/l	**0.112**	0.000	0.004	0.220	4.126E-02	7323
Phospholipids in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.118**	0.087	0.010	0.225	3.190E-02	7323
Triglycerides in medium HDL	Lipoprotein subclasses HDL	mmol/l	**0.162**	0.012	0.057	0.267	2.503E-03	7323
Total cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.063**	0.193	-0.045	0.172	2.532E-01	7323
Cholesterol esters in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.063**	0.157	-0.046	0.171	2.587E-01	7323
Free cholesterol in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.066**	0.037	-0.043	0.174	2.363E-01	7323
Total lipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.071**	0.258	-0.037	0.180	1.967E-01	7323
Concentration of medium LDL particles	Lipoprotein subclasses LDL	mol/l	**0.073**	0.000	-0.035	0.181	1.852E-01	7323
Phospholipids in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.085**	0.050	-0.023	0.193	1.226E-01	7323
Triglycerides in medium LDL	Lipoprotein subclasses LDL	mmol/l	**0.097**	0.020	-0.009	0.204	7.396E-02	7323
Monounsaturated fatty acids; 16:1, 18:1	Fatty acids and saturation measures	mmol/l	**0.115**	0.993	0.007	0.222	3.623E-02	7272
Monounsaturated FAs to total FAs	Fatty acids (%)	%	**0.076**	2.412	-0.034	0.186	1.745E-01	7272
Total cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.102**	0.078	-0.001	0.205	5.260E-02	7323

Cholesterol esters in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.088**	0.042	-0.017	0.194	1.002E-01	7323
Free cholesterol in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.113**	0.038	0.012	0.214	2.829E-02	7323
Total lipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.109**	0.292	0.008	0.211	3.483E-02	7323
Concentration of medium VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.109**	0.000	0.008	0.210	3.448E-02	7323
Phospholipids in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.112**	0.057	0.011	0.214	2.996E-02	7323
Triglycerides in medium VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.110**	0.160	0.009	0.210	3.229E-02	7323
Phosphatidylcholine and other cholines	Glycerides and phospholipids	mmol/l	**0.092**	0.459	-0.017	0.200	9.817E-02	7290
Phenylalanine	Aromatic amino acids	mmol/l	**0.147**	0.011	0.042	0.252	6.237E-03	7319
Polyunsaturated fatty acids	Fatty acids and saturation measures	mmol/l	**0.071**	0.917	-0.038	0.181	2.010E-01	7272
Polyunsaturated FAs to total FAs	Fatty acids (%)	%	**-0.125**	2.383	-0.235	-0.015	2.596E-02	7272
Pyruvate	Glycolysis related metabolites	mmol/l	**0.066**	0.022	-0.041	0.172	2.259E-01	7303
Remnant cholesterol (non-HDL, non-LDL)	Cholesterol	mmol/l	**0.085**	0.465	-0.022	0.192	1.212E-01	7318
Serum total cholesterol	Cholesterol	mmol/l	**0.065**	1.157	-0.044	0.173	2.425E-01	7318
Serum total triglycerides	Glycerides and phospholipids	mmol/l	**0.113**	0.570	0.011	0.215	2.962E-02	7318
Saturated fatty acids	Fatty acids and saturation measures	mmol/l	**0.127**	1.042	0.019	0.235	2.083E-02	7272
Saturated FAs to total FAs	Fatty acids (%)	%	**0.076**	1.505	-0.034	0.186	1.758E-01	7272
Total cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.137**	0.067	0.028	0.245	1.329E-02	7323
Cholesterol esters in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.109**	0.059	0.000	0.219	5.034E-02	7323

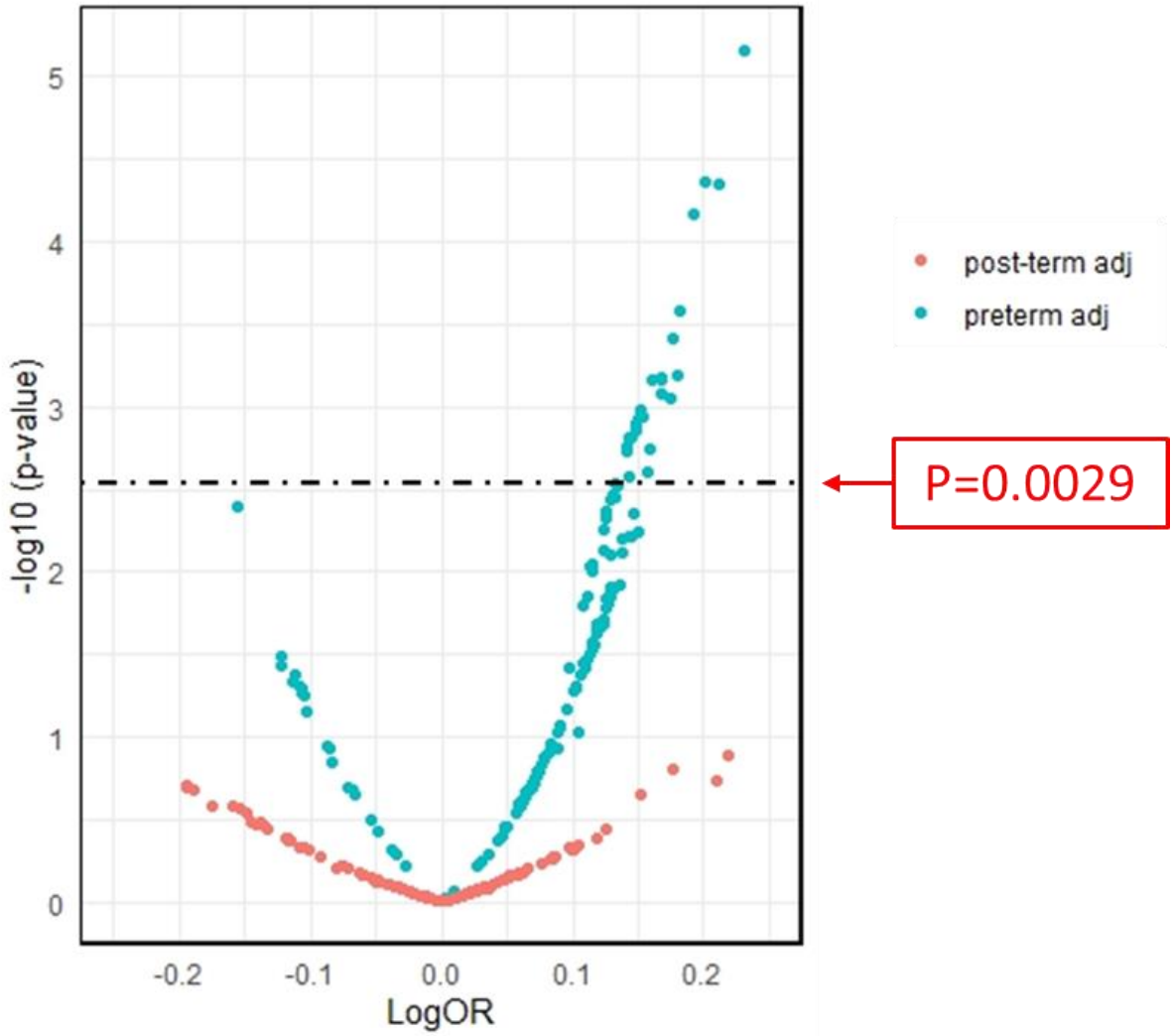
Free cholesterol in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.162**	0.017	0.055	0.269	3.053E-03	7323
Total lipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.187**	0.141	0.080	0.294	5.950E-04	7323
Concentration of small HDL particles	Lipoprotein subclasses HDL	mol/l	**0.191**	0.000	0.084	0.298	4.572E-04	7323
Phospholipids in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.163**	0.094	0.055	0.270	3.164E-03	7323
Triglycerides in small HDL	Lipoprotein subclasses HDL	mmol/l	**0.136**	0.016	0.032	0.241	1.051E-02	7323
Total cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.061**	0.117	-0.047	0.170	2.679E-01	7323
Cholesterol esters in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.059**	0.095	-0.050	0.168	2.877E-01	7323
Free cholesterol in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.070**	0.023	-0.038	0.179	2.040E-01	7323
Total lipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.074**	0.159	-0.034	0.182	1.797E-01	7323
Concentration of small LDL particles	Lipoprotein subclasses LDL	mol/l	**0.077**	0.000	-0.031	0.185	1.644E-01	7323
Phospholipids in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.091**	0.034	-0.017	0.199	9.827E-02	7323
Triglycerides in small LDL	Lipoprotein subclasses LDL	mmol/l	**0.117**	0.013	0.012	0.222	2.963E-02	7323
Sphingomyelins	Glycerides and phospholipids	mmol/l	**0.054**	0.086	-0.056	0.164	3.365E-01	7290
Total cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.105**	0.081	-0.001	0.211	5.299E-02	7323
Cholesterol esters in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.085**	0.054	-0.022	0.193	1.189E-01	7323
Free cholesterol in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.129**	0.030	0.025	0.233	1.490E-02	7323

Total lipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.120**	0.212	0.016	0.224	2.395E-02	7323
Concentration of small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.120**	0.000	0.016	0.224	2.391E-02	7323
Phospholipids in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.132**	0.044	0.028	0.236	1.300E-02	7323
Triglycerides in small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.116**	0.096	0.014	0.218	2.607E-02	7323
Ratio of triglycerides to phosphoglycerides	Glycerides and phospholipids	#N/A	**0.089**	0.159	-0.017	0.196	1.007E-01	7292
Total cholines	Glycerides and phospholipids	mmol/l	**0.095**	0.484	-0.014	0.204	8.745E-02	7290
Total fatty acids	Fatty acids and saturation measures	mmol/l	**0.113**	2.775	0.004	0.221	4.127E-02	7272
Total phosphoglycerides	Glycerides and phospholipids	mmol/l	**0.098**	0.398	-0.011	0.207	7.694E-02	7292
Tyrosine	Aromatic amino acids	mmol/l	**0.068**	0.006	-0.041	0.177	2.201E-01	7310
Estimated degree of unsaturation	Fatty acids and saturation measures	#N/A	**-0.062**	0.043	-0.173	0.049	2.723E-01	7272
Valine	Branched--chain amino acids	mmol/l	**0.052**	0.018	-0.057	0.161	3.501E-01	7310
Total cholesterol in VLDL	Cholesterol	mmol/l	**0.097**	0.273	-0.009	0.203	7.191E-02	7318
Mean diameter for VLDL particles	Lipoprotein particle size	nm	**0.114**	1.006	0.004	0.223	4.162E-02	7318
Triglycerides in VLDL	Glycerides and phospholipids	mmol/l	**0.110**	0.450	0.010	0.211	3.186E-02	7318
Total cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.077**	0.104	-0.189	0.035	1.793E-01	7323
Cholesterol esters in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.075**	0.074	-0.187	0.036	1.863E-01	7323
Free cholesterol in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.079**	0.030	-0.191	0.033	1.684E-01	7323

Total lipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.079**	0.230	-0.191	0.033	1.690E-01	7323
Concentration of very large HDL particles	Lipoprotein subclasses HDL	mol/l	**-0.078**	0.000	-0.190	0.034	1.723E-01	7323
Phospholipids in very large HDL	Lipoprotein subclasses HDL	mmol/l	**-0.084**	0.122	-0.196	0.028	1.435E-01	7323
Triglycerides in very large HDL	Lipoprotein subclasses HDL	mmol/l	**0.009**	0.010	-0.102	0.119	8.796E-01	7323
Total cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.081**	0.011	-0.021	0.182	1.186E-01	7323
Cholesterol esters in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.071**	0.006	-0.031	0.174	1.726E-01	7323
Free cholesterol in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.091**	0.005	-0.009	0.191	7.418E-02	7323
Total lipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.096**	0.054	-0.003	0.196	5.856E-02	7323
Concentration of very large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.097**	0.000	-0.002	0.197	5.598E-02	7323
Phospholipids in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.100**	0.009	0.000	0.200	4.983E-02	7323
Triglycerides in very large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.100**	0.034	0.000	0.199	4.933E-02	7323
Total cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.062**	0.078	-0.046	0.171	2.591E-01	7323
Cholesterol esters in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.051**	0.053	-0.058	0.159	3.624E-01	7323
Free cholesterol in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.085**	0.026	-0.022	0.192	1.193E-01	7323
Total lipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.085**	0.161	-0.023	0.192	1.222E-01	7323
Concentration of very small VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.089**	0.000	-0.018	0.196	1.048E-01	7323

Phospholipids in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.074**	0.052	-0.034	0.182	1.771E-01	7323
Triglycerides in very small VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.117**	0.041	0.012	0.222	2.832E-02	7323
Total cholesterol in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.078**	0.004	-0.025	0.180	1.366E-01	7323
Cholesterol esters in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.060**	0.002	-0.045	0.165	2.610E-01	7323
Free cholesterol in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.096**	0.002	-0.003	0.196	5.674E-02	7323
Total lipids in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.086**	0.018	-0.014	0.186	9.132E-02	7323
Conc. of CMs & extremely large VLDL particles	Lipoprotein subclasses VLDL	mol/l	**0.086**	0.000	-0.014	0.186	9.181E-02	7323
Phospholipids in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.099**	0.002	0.000	0.198	4.965E-02	7323
Triglycerides in CMs & extremely large VLDL	Lipoprotein subclasses VLDL	mmol/l	**0.086**	0.012	-0.014	0.185	9.134E-02	7323

APPENDIX 4A8: Volcano plot comparing logOR values derived from logistic regression outputs of post-term (red dots) and preterm (blue dots) with term for the 157 metabolite conditions measured (x-axis), plotted against -log10 (y-axis). Horizontal dashed line indicates p = 0.0029, above which the OR were significant.



REFERENCES

1. Ratajczak CK, Muglia LJ. Insights into parturition biology from genetically altered mice. *Pediatr Res*. Dec 2008;64(6):581-9. doi:10.1203/PDR.0b013e31818718d2

2. Lopez Bernal A. Overview. Preterm labour: mechanisms and management. *BMC Pregnancy Childbirth*. Jun 1 2007;7 Suppl 1:S2. doi:10.1186/1471-2393-7-s1-s2

3. Sharp GC, Hutchinson JL, Hibbert N, Freeman TC, Saunders PTK, Norman JE. Transcription Analysis of the Myometrium of Labouring and Non-Labouring Women. *PloS one*. 2016;11(5):e0155413-e0155413. doi:10.1371/journal.pone.0155413

4. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't. *Lancet*. Dec 15 2012;380(9859):2095-128. doi:10.1016/S0140-6736(12)61728-0

5. Birchenall KA, Welsh GI, Lopez Bernal A. Metabolite Changes in Maternal and Fetal Plasma Following Spontaneous Labour at Term in Humans Using Untargeted Metabolomics Analysis: A Pilot Study. *Int J Environ Res Public Health*. Apr 30 2019;16(9)doi:10.3390/ijerph16091527

6. Lopez Bernal A. Mechanisms of labour--biochemical aspects. *Bjog*. Apr 2003;110 Suppl 20:39-45.

7. Smith R. Parturition. *N Engl J Med*. Jan 18 2007;356(3):271-83. doi:10.1056/NEJMra061360

8. Mitchell BF, Taggart MJ. Are animal models relevant to key aspects of human parturition? *Am J Physiol Regul Integr Comp Physiol*. Sep 2009;297(3):R525-45. doi:10.1152/ajpregu.00153.2009

9. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. Jan 5 2008;371(9606):75-84. doi:10.1016/s0140-6736(08)60074-4

10. Georgiou HM, Di Quinzio MK, Permezel M, Brennecke SP. Predicting Preterm Labour: Current Status and Future Prospects. *Dis Markers*. 2015;2015:435014. doi:10.1155/2015/435014

11. Kruit H, Heikinheimo O, Sorsa T, Juhila J, Paavonen J, Rahkonen L. Cervical biomarkers as predictors of successful induction of labour by Foley catheter. *J Obstet Gynaecol*. Mar 19 2018:1-6. doi:10.1080/01443615.2018.1434763

12. Vandermolen BI, Hezelgrave NL, Smout EM, Abbott DS, Seed PT, Shennan AH. Quantitative fetal fibronectin and cervical length to predict preterm birth in asymptomatic women with previous cervical surgery. *American journal of obstetrics and gynecology*. 2016;215(4):480.e1-480.e10. doi:10.1016/j.ajog.2016.05.020

13. Nikolova T, Bayev O, Nikolova N, Di Renzo GC. Comparison of a novel test for placental alpha microglobulin-1 with fetal fibronectin and cervical length measurement for the prediction of imminent spontaneous preterm delivery in patients with threatened preterm labor. *J Perinat Med*. Jul 2015;43(4):395-402. doi:10.1515/jpm-2014-0300

14. Lembet A, Eroglu D, Ergin T, et al. New rapid bed-side test to predict preterm delivery: phosphorylated insulin-like growth factor binding protein-1 in cervical secretions. *Acta Obstet Gynecol Scand*. Aug 2002;81(8):706-12. doi:10.1034/j.1600-0412.2002.810804.x

15. Sovio U, McBride N, Wood AM, et al. 4-Hydroxyglutamate is a novel predictor of pre-eclampsia. *International journal of epidemiology*. 2019:dyz098. doi:10.1093/ije/dyz098

16. Sovio U, Goulding N, McBride N, et al. A maternal serum metabolite ratio predicts term fetal growth restriction. *Nature Medicine*. 2020 (in press);

17. Saade GR, Boggess KA, Sullivan SA, et al. Development and validation of a spontaneous preterm delivery predictor in asymptomatic women. *Am J Obstet Gynecol*. May 2016;214(5):633.e1-633.e24. doi:10.1016/j.ajog.2016.02.001

18. Markenson G, Saade G, Laurent L, et al. Performance of a proteomic preterm delivery predictor in a large independent prospective cohort. *American Journal of Obstetrics and Gynaecology MFM*. 2020;doi:https://doi.org/10.1016/j.ajogmf.2020.100140

19. Pearce N, Lawlor DA. Causal inference-so much more than statistics. *International journal of epidemiology*. 2016;45(6):1895-1903. doi:10.1093/ije/dyw328

20. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. *Int J Epidemiol*. Dec 1 2016;45(6):1866-1886. doi:10.1093/ije/dyw314

21. Mayeux R. Biomarkers: potential uses and limitations. *NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics*. 2004;1(2):182-188. doi:10.1602/neurorx.1.2.182

22. Fleming TR, Powers JH. Biomarkers and surrogate endpoints in clinical trials. *Statistics in medicine*. 2012;31(25):2973-2984. doi:10.1002/sim.5403

23. ACOG Committee Opinion No 579: Definition of term pregnancy. *Obstetrics and gynecology*. 2013;122(5):1139-1140. doi:10.1097/01.AOG.0000437385.88715.4a

24. Jukic AM, Baird DD, Weinberg CR, McConnaughey DR, Wilcox AJ. Length of human pregnancy and contributors to its natural variation. *Human reproduction (Oxford, England)*. 2013;28(10):2848-2855. doi:10.1093/humrep/det297

25. Patel RR, Steer P, Doyle P, Little MP, Elliott P. Does gestation vary by ethnic group? A London-based study of over 122,000 pregnancies with spontaneous onset of labour. *Int J Epidemiol*. Feb 2004;33(1):107-13. doi:10.1093/ije/dyg238

26. Dunn PM. Aristotle (384-322 BC): philosopher and scientist of ancient Greece. *Archives of disease in childhood Fetal and neonatal edition*. 2006;91(1):F75-F77. doi:10.1136/adc.2005.074534

27. Koh W, Pan W, Gawad C, et al. Noninvasive in vivo monitoring of tissue-specific global gene expression in humans. *Proc Natl Acad Sci U S A*. May 20 2014;111(20):7361-6. doi:10.1073/pnas.1405528111

28. Ngo TTM, Moufarrej MN, Rasmussen MH, et al. Noninvasive blood tests for fetal development predict gestational age and preterm delivery. *Science*. Jun 8 2018;360(6393):1133-1136. doi:10.1126/science.aar3819

29. National Collaborating Centre for Women's and Children's H. National Institute for Health and Care Excellence: Clinical Guidelines. *Preterm Labour and Birth*. National Institute for Health and Care Excellence (UK) Copyright (c) 2015 National Collaborating Centre for Women's and Children's Health.; 2015.

30. National Collaborating Centre for Women's and Children's H. National Institute for Health and Care Excellence: Clinical Guidelines. *Intrapartum Care: Care of Healthy Women and Their Babies During Childbirth*. National Institute for Health and Care Excellence (UK) Copyright (c) 2014 National Collaborating Centre for Women's and Children's Health.; 2014.

31. National Institute for Health and Care Excellence. Preterm Labour and Birth (NICE Guideline 25). 2015. Accessed 26/11/2017. https://www.nice.org.uk/guidance/ng25

32. Stanfield Z, Lai PF, Lei K, et al. Myometrial Transcriptional Signatures of Human Parturition. *Front Genet*. 2019;10:185. doi:10.3389/fgene.2019.00185

33. Nielsen BW, Bonney EA, Pearce BD, Donahue LR, Sarkar IN. A Cross-Species Analysis of Animal Models for the Investigation of Preterm Birth Mechanisms. *Reprod Sci*. Apr 2016;23(4):482-91. doi:10.1177/1933719115604729

34. Lai PF, Georgiou EX, Tribe RM, Johnson MR. The impact of progesterone and RU-486 on classic pro-labour proteins & contractility in human myometrial tissues during 24-hour exposure to tension & interleukin-1β. *Molecular and cellular endocrinology*. 2020;500:110633-110633. doi:10.1016/j.mce.2019.110633

35. Zakar T, Hertelendy F. Progesterone withdrawal: key to parturition. *Am J Obstet Gynecol*. Apr 2007;196(4):289-96. doi:10.1016/j.ajog.2006.09.005

36. Chang HH, Larson J, Blencowe H, et al. Preventing preterm births: analysis of trends and potential reductions with interventions in 39 countries with very high human development index. *Lancet*. Jan 19 2013;381(9862):223-34. doi:10.1016/s0140-6736(12)61856-x

37. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet*. Dec 17 2016;388(10063):3027-3035. doi:10.1016/s0140-6736(16)31593-8

38. Hässig M, Steinmann B, Wittenbrink MM. [Benefit of clinical and laboratory parameters for the diagnosis of endometritis in dairy cows]. *Schweizer Archiv fur Tierheilkunde*. Sep 2010 2010;152(9):411-416.

39. Manktelow B, Smith L, Prunet C, et al. *MBRRACE-UK Perinatal Mortality Surveillance Report, UK Perinatal Deaths for Births from January to December 2015.* 2017.

40. Sarri G, Davies M, Gholitabar M, Norman JE. Preterm labour: summary of NICE guidance. *Bmj*. Nov 23 2015;351:h6283. doi:10.1136/bmj.h6283

41. MacLennan AH, Thompson SC, Gecz J. Cerebral palsy: causes, pathways, and the role of genetic variants. *American journal of obstetrics and gynecology*. 2015;213(6):779-788. doi:10.1016/j.ajog.2015.05.034

42. Excellence NIoHaC. Cerebral Palsy. 2019.

43. Mealing NM, Roberts CL, Ford JB, Simpson JM, Morris JM. Trends in induction of labour, 1998-2007: a population-based study. *Aust N Z J Obstet Gynaecol*. Dec 2009;49(6):599-605. doi:10.1111/j.1479-828X.2009.01086.x

44. National Institute for Clinical and Health Excellence. Inducing Labour Clinical Guideline CG70. 2008.

45. National Health Service Digital. *NHS Maternity Statistics 2016-17.* 2017.

46. Grobman WA, Rice MM, Reddy UM, et al. Labor Induction versus Expectant Management in Low-Risk Nulliparous Women. *N Engl J Med*. Aug 9 2018;379(6):513-523. doi:10.1056/NEJMoa1800566

47. Middleton P, Shepherd E, Crowther CA. Induction of labour for improving birth outcomes for women at or beyond term. *Cochrane Database Syst Rev*. May 9 2018;5:Cd004945. doi:10.1002/14651858.CD004945.pub4

48. Keirse MJ. Elective induction, selective deduction, and cesarean section. *Birth*. Sep 2010;37(3):252-6. doi:10.1111/j.1523-536X.2010.00413.x

49. Aguilar HN, Mitchell BF. Physiological pathways and molecular mechanisms regulating uterine contractility. *Hum Reprod Update*. Nov-Dec 2010;16(6):725-44. doi:10.1093/humupd/dmq016

50. Mahendroo M. Cervical remodeling in term and preterm birth: insights from an animal model. *Reproduction*. Apr 2012;143(4):429-38. doi:10.1530/rep-11-0466

51. Sokolowski Peter P. Human uterine wall tension trajectories and the onset of parturition. *PLoS One*. 2010;5(6)

52. Caserta MP, Bolan C, Clingan MJ. Through thick and thin: a pictorial review of the endometrium. *Abdominal Radiology*. 2016/12/01 2016;41(12):2312-2329. doi:10.1007/s00261-016-0930-5

53. Tribe RM. Regulation of human myometrial contractility during pregnancy and labour: are calcium homeostatic pathways important? *Exp Physiol*. Mar 2001;86(2):247-54.

54. Word RA, Tang DC, Kamm KE. Activation properties of myosin light chain kinase during contraction/relaxation cycles of tonic and phasic smooth muscles. *The Journal of biological chemistry*. 1994;269(34):21596-21602.

55. Word RA. Myosin phosphorylation and the control of myometrial contraction/relaxation. *Seminars in perinatology*. 1995;19(1):3-14. doi:10.1016/s0146-0005(95)80043-3

56. Yuan W, López Bernal A. Cyclic AMP signalling pathways in the regulation of uterine relaxation. *BMC pregnancy and childbirth*. 2007;7 Suppl 1(Suppl 1):S10-S10. doi:10.1186/1471-2393-7-S1-S10

57. Wray S, Kupittayanant S, Shmygol A, Smith RD, Burdyga T. The physiological basis of uterine contractility: a short review. *Exp Physiol*. Mar 2001;86(2):239-46.

58. Leppert PC. Anatomy and physiology of cervical ripening. *Clin Obstet Gynecol*. Jun 1995;38(2):267-79. doi:10.1097/00003081-199506000-00009

59. Walejko JM, Chelliah A, Keller-Wood M, Gregg A, Edison AS. Global Metabolomics of the Placenta Reveals Distinct Metabolic Profiles between Maternal and Fetal Placental Tissues Following Delivery in Non-Labored Women. *Metabolites*. 2018;8(1):10. doi:10.3390/metabo8010010

60. Jones CJ, Fox H. Ultrastructure of the normal human placenta. *Electron microscopy reviews*. 1991;4(1):129-178. doi:10.1016/0892-0354(91)90019-9

61. Holden D. *MRCOG Part 1: the official companion to the Royal College of Obstetricians and Gynaecologists revision course*. RCOG Press; 2010.

62. Chatuphonprasert W, Jarukamjorn K, Ellinger I. Physiology and Pathophysiology of Steroid Biosynthesis, Transport and Metabolism in the Human Placenta. *Front Pharmacol*. 2018;9:1027. doi:10.3389/fphar.2018.01027

63. D'Souza R. *MRCOG Part One: the official companion to the Royal College of Obstetricians and Gynaecologists revision course*. RCOG Press; 2010:173-184.

64. Travers S, Martinerie L, Boileau P, Lombès M, Pussard E. Alterations of adrenal steroidomic profiles in preterm infants at birth. *Archives of disease in childhood Fetal and neonatal edition*. 2018;103(2):F143-F151. doi:10.1136/archdischild-2016-312457

65. Oh S-Y, Romero R, Shim S-S, Park JS, Jun JK, Yoon BH. Fetal plasma cortisol and dehydroepiandrosterone sulfate concentrations in pregnancy and term parturition. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*. 2006;19(9):529-536. doi:10.1080/14767050600853179

66. Murphy BE. Human fetal serum cortisol levels related to gestational age: evidence of a midgestational fall and a steep late gestational rise, independent of sex or mode of delivery. *American journal of obstetrics and gynecology*. 1982;144(3):276-282. doi:10.1016/0002-9378(82)90579-8

67. Alexandre-Gouabau M-C, Courant F, Moyon T, et al. Maternal and cord blood LC-HRMS metabolomics reveal alterations in energy and polyamine metabolism, and oxidative stress in very-low birth weight infants. *Journal of proteome research*. 2013;12(6):2764-2778. doi:10.1021/pr400122v

68. Rolle-Kampczyk UE, Krumsiek J, Otto W, et al. Metabolomics reveals effects of maternal smoking on endogenous metabolites from lipid metabolism in cord blood of newborns. *Metabolomics : Official journal of the Metabolomic Society*. 2016;12:76-76. doi:10.1007/s11306-016-0983-z

69. Farquharson RG, Klopper AI. Progesterone concentrations in maternal and fetal blood. *British journal of obstetrics and gynaecology*. 1984;91(2):133-137. doi:10.1111/j.1471-0528.1984.tb05896.x

70. Shaxted EJ, Heyes VM, Walker MP, Maynard PV. Umbilical-cord plasma progesterone in term infants delivered by caesarean section. *British journal of obstetrics and gynaecology*. 1982;89(1):73-76. doi:10.1111/j.1471-0528.1982.tb04640.x

71. Mesiano S. *Yen and Jaffe's Reproductive Endocrinology (Eighth Edition): Chapter 11 - Endocrinology of Human Pregnancy and Fetal-Placental Neuroendocrine Development*. 8th ed. Elsevier; 2019.

72. Costa MA. The endocrine function of human placenta: an overview. *Reproductive biomedicine online*. 2016;32(1):14-43. doi:10.1016/j.rbmo.2015.10.005

73. Sugimoto Y, Yamasaki A, Segi E, et al. Failure of parturition in mice lacking the prostaglandin F receptor. *Science (New York, NY)*. 1997;277(5326):681-683. doi:10.1126/science.277.5326.681

74. Liggins GC. Premature parturition after infusion of corticotrophin or cortisol into foetal lambs. *J Endocrinol*. Oct 1968;42(2):323-9. doi:10.1677/joe.0.0420323

75. Liggins GC. Premature delivery of foetal lambs infused with glucocorticoids. *J Endocrinol*. Dec 1969;45(4):515-23. doi:10.1677/joe.0.0450515

76. Liggins GC, Kennedy PC, Holm LW. Failure of initiation of parturition after electrocoagulation of the pituitary of the fetal lamb. *American journal of obstetrics and gynecology*. 1967;98(8):1080-1086. doi:10.1016/0002-9378(67)90031-2

77. Anderson GB, Bradford GE, Cupps PT. Length of gestation in ewes carrying lambs of two different breeds. *Theriogenology*. 1981;16(1):119-129. doi:10.1016/0093-691x(81)90120-5

78. Honnebier WJ, Swaab DF. The influence of anencephaly upon intrauterine growth of fetus and placenta and upon gestation length. *J Obstet Gynaecol Br Commonw*. Jul 1973;80(7):577-88.

79. Mansour S, Devedeux D, Germain G, Marque C, Duchêne J. Uterine EMG spectral analysis and relationship to mechanical activity in pregnant monkeys. *Med Biol Eng Comput*. Mar 1996;34(2):115-21. doi:10.1007/bf02520015

80. Wildman DE, Uddin M, Romero R, et al. Spontaneous abortion and preterm labor and delivery in nonhuman primates: evidence from a captive colony of chimpanzees (Pan troglodytes). *PLoS One*. 2011;6(9):e24509. doi:10.1371/journal.pone.0024509

81. Sadowsky DW, Haluska GJ, Gravett MG, Witkin SS, Novy MJ. Indomethacin blocks interleukin 1beta-induced myometrial contractions in pregnant rhesus monkeys. *Am J Obstet Gynecol*. Jul 2000;183(1):173-80. doi:10.1067/mob.2000.105968

82. Sadowsky DW, Novy MJ, Witkin SS, Gravett MG. Dexamethasone or interleukin-10 blocks interleukin-1beta-induced uterine contractions in pregnant rhesus monkeys. *Am J Obstet Gynecol*. Jan 2003;188(1):252-63. doi:10.1067/mob.2003.70

83. Phillips JB, Abbot P, Rokas A. Is preterm birth a human-specific syndrome? *Evol Med Public Health*. Jun 14 2015;2015(1):136-48. doi:10.1093/emph/eov010

84. Gravett MG, Witkin SS, Haluska GJ, Edwards JL, Cook MJ, Novy MJ. An experimental model for intraamniotic infection and preterm labor in rhesus monkeys. *Am J Obstet Gynecol*. Dec 1994;171(6):1660-7. doi:10.1016/0002-9378(94)90418-9

85. Dunsworth HM, Warrener AG, Deacon T, Ellison PT, Pontzer H. Metabolic hypothesis for human altriciality. *Proc Natl Acad Sci U S A*. Sep 18 2012;109(38):15212-6. doi:10.1073/pnas.1205282109

86. Hapangama D, Neilson JP. Mifepristone for induction of labour. *The Cochrane database of systematic reviews*. 2009;(3):CD002865-CD002865. doi:10.1002/14651858.CD002865.pub2

87. Csapo AI, Knobil E, van der Molen HJ, Wiest WG. Peripheral plasma progesterone levels during human pregnancy and labor. *American journal of obstetrics and gynecology*. 1971;110(5):630-632. doi:10.1016/0002-9378(71)90242-0

88. Editorial: Hormonal functions of the placenta. *British medical journal*. 1976;1(6016):975-976. doi:10.1136/bmj.1.6016.975

89. Diczfalusy E. Endocrine functions of the human fetoplacental unit. 1964. *Am J Obstet Gynecol*. Dec 2005;193(6):2024; discussion 2025. doi:10.1016/j.ajog.2005.02.117

90. Benagiano G, Merialdi M. Egon R. Diczfalusy, the discovery of the fetoplacental unit and much more. *Contraception*. 2011;84(6):544-548. doi:10.1016/j.contraception.2011.03.004

91. Albrecht ED, Pepe GJ. Placental steroid hormone biosynthesis in primate pregnancy. *Endocrine reviews*. 1990;11(1):124-150. doi:10.1210/edrv-11-1-124

92. Lieberman S. The generally accepted version of steroidogenesis is not free of uncertainties: other tenable and possibly superior renditions may be invented. *The Journal of steroid biochemistry and molecular biology*. 2008;109(1-2):197-199. doi:10.1016/j.jsbmb.2008.02.001

93. Ishimoto H, Jaffe RB. Development and function of the human fetal adrenal cortex: a key component in the feto-placental unit. *Endocrine reviews*. 2011;32(3):317-355. doi:10.1210/er.2010-0001

94. Spencer SJ, Mesiano S, Lee JY, Jaffe RB. Proliferation and apoptosis in the human adrenal cortex during the fetal and perinatal periods: implications for growth and remodeling. *The Journal of clinical endocrinology and metabolism*. 1999;84(3):1110-1115. doi:10.1210/jcem.84.3.5513

95. Everett RB, Porter JC, MacDonald PC, Gant NF. Relationship of maternal placental blood flow to the placental clearance of maternal plasma dehydroisoandrosterone sulfate through placental estradiol formation. *Am J Obstet Gynecol*. Feb 15 1980;136(4):435-9. doi:10.1016/0002-9378(80)90667-5

96. Senner JW, Stanczyk FZ, Fritz MA, Novy MJ. Relationship of uteroplacental blood flow to placental clearance of maternal plasma C-19 steroids: evaluation of mathematical models. *Am J Obstet Gynecol*. Nov 1 1985;153(5):573-5. doi:10.1016/0002-9378(85)90481-8

97. Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G, Smith R. Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. *J Clin Endocrinol Metab*. Jun 2002;87(6):2924-30. doi:10.1210/jcem.87.6.8609

98. Condon JC, Hardy DB, Kovaric K, Mendelson CR. Up-regulation of the progesterone receptor (PR)-C isoform in laboring myometrium by activation of nuclear factor-kappaB may contribute to the onset of labor through inhibition of PR function. *Mol Endocrinol*. Apr 2006;20(4):764-75. doi:10.1210/me.2005-0242

99. Kastner P, Krust A, Turcotte B, et al. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *Embo j*. May 1990;9(5):1603-14.

100. Pieber D, Allport VC, Hills F, Johnson M, Bennett PR. Interactions between progesterone receptor isoforms in myometrial cells in human labour. *Mol Hum Reprod*. Sep 2001;7(9):875-9. doi:10.1093/molehr/7.9.875

101. Anamthathmakula P, Kyathanahalli C, Ingles J, Hassan SS, Condon JC, Jeyasuria P. Estrogen receptor alpha isoform ERdelta7 in myometrium modulates uterine quiescence during pregnancy. *EBioMedicine*. Jan 2019;39:520-530. doi:10.1016/j.ebiom.2018.11.038

102. Smith R, Butler T, Chan E-C. Do estrogen receptor variants explain the enigma of human birth? *EBioMedicine*. 2018;39:25-26. doi:10.1016/j.ebiom.2018.12.012

103. Smith R, Smith JI, Shen X, et al. Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor. *J Clin Endocrinol Metab*. Jun 2009;94(6):2066-74. doi:10.1210/jc.2008-2257

104. Rezapour M, Backstrom T, Lindblom B, Ulmsten U. Sex steroid receptors and human parturition. *Obstet Gynecol*. Jun 1997;89(6):918-24. doi:10.1016/s0029-7844(97)00116-6

105. Merlino A, Welsh T, Erdonmez T, et al. Nuclear progesterone receptor expression in the human fetal membranes and decidua at term before and after labor. *Reprod Sci*. Apr 2009;16(4):357-63. doi:10.1177/1933719108328616

106. Condon JC, Jeyasuria P, Faust JM, Mendelson CR. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. *Proc Natl Acad Sci U S A*. Apr 6 2004;101(14):4978-83. doi:10.1073/pnas.0401124101

107. López Bernal A. The regulation of uterine relaxation. *Seminars in cell & developmental biology*. 2007;18(3):340-347. doi:10.1016/j.semcdb.2007.05.002

108. Singh N, Herbert B, Sooranna GR, et al. Is myometrial inflammation a cause or a consequence of term human labour? *J Endocrinol*. Oct 2017;235(1):69-83. doi:10.1530/joe-17-0318

109. Keski-Nisula LT, Aalto ML, Kirkinen PP, Kosma VM, Heinonen ST. Myometrial inflammation in human delivery and its association with labor and infection. *Am J Clin Pathol*. Aug 2003;120(2):217-24. doi:10.1309/kc6k-dtx9-8lfy-b3j7

110. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harbor perspectives in biology*. 2009;1(6):a001651-a001651. doi:10.1101/cshperspect.a001651

111. Lopez Bernal A, Newman GE, Phizackerley PJ, Turnbull AC. Surfactant stimulates prostaglandin E production in human amnion. *Br J Obstet Gynaecol*. Oct 1988;95(10):1013-7. doi:10.1111/j.1471-0528.1988.tb06506.x

112. Burri PH. Fetal and postnatal development of the lung. *Annual review of physiology*. 1984;46:617-628. doi:10.1146/annurev.ph.46.030184.003153

113. Norwitz ER, Starkey PM, Lopez Bernal A, Turnbull AC. Identification by flow cytometry of the prostaglandin-producing cell populations of term human decidua. *J Endocrinol*. Nov 1991;131(2):327-34. doi:10.1677/joe.0.1310327

114. Heida KY, Velthuis BK, Oudijk MA, et al. Cardiovascular disease risk in women with a history of spontaneous preterm delivery: A systematic review and meta-analysis. *Eur J Prev Cardiol*. Feb 2016;23(3):253-63. doi:10.1177/2047487314566758

115. Moayeri M, Heida KY, Franx A, Spiering W, de Laat MW, Oudijk MA. Maternal lipid profile and the relation with spontaneous preterm delivery: a systematic review. *Arch Gynecol Obstet*. Feb 2017;295(2):313-323. doi:10.1007/s00404-016-4216-5

116. Catov JM, Snyder GG, Bullen BL, Barinas-Mitchell EJM, Holzman C. Women with Preterm Birth Have Evidence of Subclinical Atherosclerosis a Decade After Delivery. *J Womens Health (Larchmt)*. May 2019;28(5):621-627. doi:10.1089/jwh.2018.7148

117. Kelly R, Holzman C, Senagore P, et al. Placental vascular pathology findings and pathways to preterm delivery. *Am J Epidemiol*. Jul 15 2009;170(2):148-58. doi:10.1093/aje/kwp131

118. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science*. Aug 15 2014;345(6198):760-5. doi:10.1126/science.1251816

119. Gonzalez JM, Xu H, Chai J, Ofori E, Elovitz MA. Preterm and term cervical ripening in CD1 Mice (Mus musculus): similar or divergent molecular mechanisms? *Biol Reprod*. Dec 2009;81(6):1226-32. doi:10.1095/biolreprod.108.075309

120. Anum EA, Hill LD, Pandya A, Strauss JF, 3rd. Connective tissue and related disorders and preterm birth: clues to genes contributing to prematurity. *Placenta*. 2009;30(3):207-215. doi:10.1016/j.placenta.2008.12.007

121. Haas DM, Hathaway TJ, Ramsey PS. Progestogen for preventing miscarriage in women with recurrent miscarriage of unclear etiology. *The Cochrane database of systematic reviews*. 2018;10(10):CD003511-CD003511. doi:10.1002/14651858.CD003511.pub4

122. Coomarasamy A, Williams H, Truchanowicz E, et al. A Randomized Trial of Progesterone in Women with Recurrent Miscarriages. *The New England journal of medicine*. 2015;373(22):2141-2148. doi:10.1056/NEJMoa1504927

123. Coomarasamy A, Devall AJ, Cheed V, et al. A Randomized Trial of Progesterone in Women with Bleeding in Early Pregnancy. *The New England journal of medicine*. 2019;380(19):1815-1824. doi:10.1056/NEJMoa1813730

124. Coomarasamy A, Devall AJ, Brosens JJ, et al. Micronized vaginal progesterone to prevent miscarriage: a critical evaluation of randomized evidence. *American journal of obstetrics and gynecology*. 2020:S0002-9378(19)32762-0. doi:10.1016/j.ajog.2019.12.006

125. Norman JE, Marlow N, Messow C-M, et al. Vaginal progesterone prophylaxis for preterm birth (the OPPTIMUM study): a multicentre, randomised, double-blind trial. *Lancet (London, England)*. 2016;387(10033):2106-2116. doi:10.1016/S0140-6736(16)00350-0

126. Grignaffini A, Soncini E, Anfuso S, Ronzoni E. Dinoprostone: slow release vaginal insert (Propess) and intracervical gel (Prepidil) for the induction of labour with unriped cervix. Dinoprostone: inserto vaginale a lento rilascio (Propess) e gel intracervicale (Prepidil) nell'induzione del travaglio di parto con Bishop score sfavorevole. *Minerva ginecologica*. 2004;56(5):413-418.

127. Georgiou EX, Lei K, Lai PF, et al. The study of progesterone action in human myometrial explants. *Molecular human reproduction*. 2016;22(8):877-889. doi:10.1093/molehr/gaw037

128. Horgan RP, Broadhurst DI, Walsh SK, et al. Metabolic profiling uncovers a phenotypic signature of small for gestational age in early pregnancy. *J Proteome Res*. Aug 5 2011;10(8):3660-73. doi:10.1021/pr2002897

129. Willyard C. New human gene tally reignites debate. *Nature*. 2018;558(7710):354-355. doi:10.1038/d41586-018-05462-w

130. Pertea M, Shumate A, Pertea G, et al. CHESS: a new human gene catalog curated from thousands of large-scale RNA sequencing experiments reveals extensive transcriptional noise. *Genome biology*. 2018;19(1):208-208. doi:10.1186/s13059-018-1590-2

131. Wishart DS. Emerging applications of metabolomics in drug discovery and precision medicine. *Nat Rev Drug Discov*. Jul 2016;15(7):473-84. doi:10.1038/nrd.2016.32

132. Lindon JC, Holmes E, Nicholson JK. Metabonomics in pharmaceutical R&D. *Febs j*. Mar 2007;274(5):1140-51. doi:10.1111/j.1742-4658.2007.05673.x

133. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica*. Nov 1999;29(11):1181-9. doi:10.1080/004982599238047

134. Atzori L, Barberini L, Santoru ML, Antonucci R, Fanos V. Metabolomics explained to perinatologists and pediatricians. *J Matern Fetal Neonatal Med*. Oct 2012;25(Suppl 5):10-2. doi:10.3109/14767058.2012.714636

135. Emwas A-H, Roy R, McKay RT, et al. NMR Spectroscopy for Metabolomics Research. *Metabolites*. 2019;9(7):123. doi:10.3390/metabo9070123

136. Würtz P, Kangas AJ, Soininen P, Lawlor DA, Davey Smith G, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technologies. *American Journal of Epidemiology*. 2017;186(9):1084-1096. doi:10.1093/aje/kwx016

137. Caboni P, Meloni A, Lussu M, et al. Urinary metabolomics of pregnant women at term: a combined GC/MS and NMR approach. *J Matern Fetal Neonatal Med*. Oct 2014;27 Suppl 2:4-12. doi:10.3109/14767058.2014.956403

138. Virgiliou C, Gika HG, Witting M, et al. Amniotic Fluid and Maternal Serum Metabolic Signatures in the Second Trimester Associated with Preterm Delivery. *J Proteome Res*. Feb 3 2017;16(2):898-910. doi:10.1021/acs.jproteome.6b00845

139. Wu Z, Jin L, Zheng W, et al. NMR-based serum metabolomics study reveals a innovative diagnostic model for missed abortion. *Biochem Biophys Res Commun*. Feb 5 2018;496(2):679-685. doi:10.1016/j.bbrc.2018.01.096

140. Fattuoni C, Pietrasanta C, Pugni L, et al. Urinary metabolomic analysis to identify preterm neonates exposed to histological chorioamnionitis: A pilot study. *PLoS One*. 2017;12(12):e0189120. doi:10.1371/journal.pone.0189120

141. Fotiou M, Fotakis C, Tsakoumaki F, et al. (1)H NMR-based metabolomics reveals the effect of maternal habitual dietary patterns on human amniotic fluid profile. *Sci Rep*. Mar 6 2018;8(1):4076. doi:10.1038/s41598-018-22230-y

142. Aslam B, Basit M, Nisar MA, Khurshid M, Rasool MH. Proteomics: Technologies and Their Applications. *Journal of Chromatographic Science*. 2017;55(2):182-196. doi:10.1093/chromsci/bmw167

143. Graves PR, Haystead TAJ. Molecular biologist's guide to proteomics. *Microbiology and molecular biology reviews : MMBR*. 2002;66(1):39-63. doi:10.1128/mmbr.66.1.39-63.2002

144. Ong SE, Mann M. Mass spectrometry-based proteomics turns quantitative. *Nat Chem Biol*. Oct 2005;1(5):252-62. doi:10.1038/nchembio736

145. Hudson CA, Lopez Bernal A. Phosphorylation of proteins during human myometrial contractions: A phosphoproteomic approach. *Biochem Biophys Res Commun*. Jan 22 2017;482(4):1393-1399. doi:10.1016/j.bbrc.2016.12.047

146. Burhouse A, Lea C, Ray S, et al. Preventing cerebral palsy in preterm labour: a multiorganisational quality improvement approach to the adoption and spread of magnesium sulphate for neuroprotection. *BMJ open quality*. 2017;6(2):e000189-e000189. doi:10.1136/bmjoq-2017-000189

147. Menon R, Bonney EA, Condon J, Mesiano S, Taylor RN. Novel concepts on pregnancy clocks and alarms: redundancy and synergy in human parturition. *Hum Reprod Update*. Sep 2016;22(5):535-60. doi:10.1093/humupd/dmw022

148. Roberts LD, Souza AL, Gerszten RE, Clish CB. Targeted metabolomics. *Current protocols in molecular biology*. 2012;Chapter 30:Unit30.2-30.2.24. doi:10.1002/0471142727.mb3002s98

149. Wishart DS. Emerging applications of metabolomics in drug discovery and precision medicine. *Nature reviews Drug discovery*. 2016;15(7):473-484. doi:10.1038/nrd.2016.32

150. Yuan W, Heesom K, Phillips R, Chen L, Trinder J, Lopez Bernal A. Low abundance plasma proteins in labour. *Reproduction*. Oct 2012;144(4):505-18. doi:10.1530/rep-12-0114

151. Petraglia F, Imperatore A, Challis JR. Neuroendocrine mechanisms in pregnancy and parturition. *Endocr Rev*. Dec 2010;31(6):783-816. doi:10.1210/er.2009-0019

152. Immenschuh S, Vijayan V, Janciauskiene S, Gueler F. Heme as a Target for Therapeutic Interventions. *Front Pharmacol*. 2017;8:146. doi:10.3389/fphar.2017.00146

153. Rapino C, Battista N, Bari M, Maccarrone M. Endocannabinoids as biomarkers of human reproduction. *Hum Reprod Update*. Jul-Aug 2014;20(4):501-16. doi:10.1093/humupd/dmu004

154. Dehaven CD, Evans AM, Dai H, Lawton KA. Organization of GC/MS and LC/MS metabolomics data into chemical libraries. *J Cheminform*. Oct 18 2010;2(1):9. doi:10.1186/1758-2946-2-9

155. Marco-Ramell A, Palau-Rodriguez M, Alay A, et al. Evaluation and comparison of bioinformatic tools for the enrichment analysis of metabolomics data. *BMC bioinformatics*. 2018;19(1):1-1. doi:10.1186/s12859-017-2006-0

156. Puchades-Carrasco L, Palomino-Schätzlein M, Pérez-Rambla C, Pineda-Lucena A. Bioinformatics tools for the analysis of NMR metabolomics studies focused on the identification of clinically relevant biomarkers. *Brief Bioinform*. May 2016;17(3):541-52. doi:10.1093/bib/bbv077

157. Cambiaghi A, Ferrario M, Masseroli M. Analysis of metabolomic data: tools, current strategies and future challenges for omics data integration. *Brief Bioinform*. May 1 2017;18(3):498-510. doi:10.1093/bib/bbw031

158. Chong J, Soufan O, Li C, et al. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic acids research*. 2018;46(W1):W486-W494. doi:10.1093/nar/gky310

159. Lehmann R, Zhao X, Weigert C, et al. Medium chain acylcarnitines dominate the metabolite pattern in humans under moderate intensity exercise and support lipid oxidation. *PLoS One*. Jul 12 2010;5(7):e11519. doi:10.1371/journal.pone.0011519

160. Peake JM, Tan SJ, Markworth JF, Broadbent JA, Skinner TL, Cameron-Smith D. Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise. *Am J Physiol Endocrinol Metab*. Oct 1 2014;307(7):E539-52. doi:10.1152/ajpendo.00276.2014

161. Yin P, Lehmann R, Xu G. Effects of pre-analytical processes on blood samples used in metabolomics studies. *Analytical and Bioanalytical Chemistry*. 03/0410/13/received02/13/revised02/13/accepted 2015;407(17):4879-4892. doi:10.1007/s00216-015-8565-x

162. Manolopoulos KN, O'Reilly MW, Bujalska IJ, Tomlinson JW, Arlt W. Acute Hypercortisolemia Exerts Depot-Specific Effects on Abdominal and Femoral Adipose Tissue Function. *J Clin Endocrinol Metab*. Apr 1 2017;102(4):1091-1101. doi:10.1210/jc.2016-3600

163. Lindsay KL, Hellmuth C, Uhl O, et al. Longitudinal Metabolomic Profiling of Amino Acids and Lipids across Healthy Pregnancy. *PLoS One*. 2015;10(12):e0145794. doi:10.1371/journal.pone.0145794

164. Hill M, Paskova A, Kanceva R, et al. Steroid profiling in pregnancy: a focus on the human fetus. *J Steroid Biochem Mol Biol*. Jan 2014;139:201-22. doi:10.1016/j.jsbmb.2013.03.008

165. Dahlen HG, Downe S, Wright ML, Kennedy HP, Taylor JY. Childbirth and consequent atopic disease: emerging evidence on epigenetic effects based on the hygiene and EPIIC hypotheses. *BMC Pregnancy Childbirth*. Jan 13 2016;16:4. doi:10.1186/s12884-015-0768-9

166. Dahlen HG, Kennedy HP, Anderson CM, et al. The EPIIC hypothesis: intrapartum effects on the neonatal epigenome and consequent health outcomes. *Med Hypotheses*. May 2013;80(5):656-62. doi:10.1016/j.mehy.2013.01.017

167. Tanoey J, Gulati A, Patterson C, Becher H. Risk of Type 1 Diabetes in the Offspring Born through Elective or Non-elective Caesarean Section in Comparison to Vaginal Delivery: a Meta-Analysis of Observational Studies. *Curr Diab Rep*. Nov 11 2019;19(11):124. doi:10.1007/s11892-019-1253-z

168. Keag OE, Norman JE, Stock SJ. Long-term risks and benefits associated with cesarean delivery for mother, baby, and subsequent pregnancies: Systematic review and meta-analysis. *PLoS Med*. Jan 2018;15(1):e1002494. doi:10.1371/journal.pmed.1002494

169. Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disease: meta-analyses. *Clin Exp Allergy*. Apr 2008;38(4):634-42. doi:10.1111/j.1365-2222.2008.02939.x

170. Darmasseelane K, Hyde MJ, Santhakumaran S, Gale C, Modi N. Mode of delivery and offspring body mass index, overweight and obesity in adult life: a systematic review and meta-analysis. *PLoS One*. 2014;9(2):e87896. doi:10.1371/journal.pone.0087896

171. Ahlqvist VH, Persson M, Magnusson C, Berglind D. Elective and nonelective cesarean section and obesity among young adult male offspring: A Swedish population-based cohort study. *PLoS Med*. Dec 2019;16(12):e1002996. doi:10.1371/journal.pmed.1002996

172. Cianchi F, Papucci L, Schiavone N, et al. Cannabinoid receptor activation induces apoptosis through tumor necrosis factor alpha-mediated ceramide de novo synthesis in colon cancer cells. *Clin Cancer Res*. Dec 1 2008;14(23):7691-700. doi:10.1158/1078-0432.ccr-08-0799

173. Marczylo TH, Lam PM, Amoako AA, Konje JC. Anandamide levels in human female reproductive tissues: solid-phase extraction and measurement by ultraperformance liquid chromatography tandem mass spectrometry. *Anal Biochem*. May 15 2010;400(2):155-62. doi:10.1016/j.ab.2009.12.025

174. Nallendran V, Lam PM, Marczylo TH, et al. The plasma levels of the endocannabinoid, anandamide, increase with the induction of labour. *Bjog*. Jun 2010;117(7):863-9. doi:10.1111/j.1471-0528.2010.02555.x

175. Dennedy MC, Friel AM, Houlihan DD, Broderick VM, Smith T, Morrison JJ. Cannabinoids and the human uterus during pregnancy. *Am J Obstet Gynecol*. Jan 2004;190(1):2-9; discussion 3A. doi:10.1016/j.ajog.2003.07.013

176. Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agro A. Relation between decreased anandamide hydrolase concentrations in human lymphocytes and miscarriage. *Lancet*. Apr 15 2000;355(9212):1326-9. doi:10.1016/s0140-6736(00)02115-2

177. Melland-Smith M, Ermini L, Chauvin S, et al. Disruption of sphingolipid metabolism augments ceramide-induced autophagy in preeclampsia. *Autophagy*. 2015:653-69. vol. 4.

178. Habayeb OM, Taylor AH, Evans MD, et al. Plasma levels of the endocannabinoid anandamide in women--a potential role in pregnancy maintenance and labor? *J Clin Endocrinol Metab*. Nov 2004;89(11):5482-7. doi:10.1210/jc.2004-0681

179. Signorelli P, Avagliano L, Reforgiato MR, et al. De novo ceramide synthesis is involved in acute inflammation during labor. *Biol Chem*. Jan 1 2016;397(2):147-55. doi:10.1515/hsz-2015-0213

180. Hill M, Parizek A, Cibula D, et al. Steroid metabolome in fetal and maternal body fluids in human late pregnancy. *J Steroid Biochem Mol Biol*. Oct 2010;122(4):114-32. doi:10.1016/j.jsbmb.2010.05.007

181. Pavlicev M, Norwitz ER. Human Parturition: Nothing More Than a Delayed Menstruation. *Reprod Sci*. Feb 2018;25(2):166-173. doi:10.1177/1933719117725830

182. Hill M, Parizek A, Kancheva R, et al. Steroid metabolome in plasma from the umbilical artery, umbilical vein, maternal cubital vein and in amniotic fluid in normal and preterm labor. *J Steroid Biochem Mol Biol*. Aug 2010;121(3-5):594-610. doi:10.1016/j.jsbmb.2009.10.012

183. Phillips RJ, Heesom KJ, Trinder J, Bernal AL. Human maternal plasma proteomic changes with parturition. *EuPA Open Proteomics*. 2014/12/01/ 2014;5:10-20. doi:https://doi.org/10.1016/j.euprot.2014.09.001

184. Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agro A. Progesterone up-regulates anandamide hydrolase in human lymphocytes: role of cytokines and implications for fertility. *J Immunol*. Jun 15 2001;166(12):7183-9.

185. Wellington N, Shanmuganathan M, de Souza RJ, et al. Metabolic Trajectories Following Contrasting Prudent and Western Diets from Food Provisions: Identifying Robust Biomarkers of Short-Term Changes in Habitual Diet. *Nutrients*. 2019;11(10):2407. doi:10.3390/nu11102407

186. Naismith DJ, Emery PW. Excretion of 3-methylhistidine by pregnant women: evidence for a biphasic system of protein metabolism in human pregnancy. *Eur J Clin Nutr*. Jun 1988;42(6):483-9.

187. Powell KL, Carrozzi A, Stephens AS, et al. Utility of metabolic profiling of serum in the diagnosis of pregnancy complications. *Placenta*. Jun 2018;66:65-73. doi:10.1016/j.placenta.2018.04.005

188. Mazzola C, Medalie J, Scherma M, et al. Fatty acid amide hydrolase (FAAH) inhibition enhances memory acquisition through activation of PPAR-alpha nuclear receptors. *Learning & memory (Cold Spring Harbor, NY)*. 2009;16(5):332-337. doi:10.1101/lm.1145209

189. Nguyen T, Mitrea C, Draghici S. Network-Based Approaches for Pathway Level Analysis. *Curr Protoc Bioinformatics*. Mar 2018;61(1):8.25.1-8.25.24. doi:10.1002/cpbi.42

190. Saghatelian A, McKinney MK, Bandell M, Patapoutian A, Cravatt BF. A FAAH-regulated class of N-acyl taurines that activates TRP ion channels. *Biochemistry*. 2006;45(30):9007-9015. doi:10.1021/bi0608008

191. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 1997;389(6653):816-824. doi:10.1038/39807

192. Waluk DP, Vielfort K, Derakhshan S, Aro H, Hunt MC. N-Acyl taurines trigger insulin secretion by increasing calcium flux in pancreatic β-cells. *Biochemical and biophysical research communications*. 2013;430(1):54-59. doi:10.1016/j.bbrc.2012.11.026

193. Aichler M, Borgmann D, Krumsiek J, et al. N-acyl Taurines and Acylcarnitines Cause an Imbalance in Insulin Synthesis and Secretion Provoking β Cell Dysfunction in Type 2 Diabetes. *Cell metabolism*. 2017;25(6):1334-1347.e4. doi:10.1016/j.cmet.2017.04.012

194. Smart D, Gunthorpe MJ, Jerman JC, et al. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *British journal of pharmacology*. 2000;129(2):227-230. doi:10.1038/sj.bjp.0703050

195. Zygmunt PM, Petersson J, Andersson DA, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature*. 1999;400(6743):452-457. doi:10.1038/22761

196. Brighton PJ, McDonald J, Taylor AH, et al. Characterization of anandamide-stimulated cannabinoid receptor signaling in human ULTR myometrial smooth muscle cells. *Molecular endocrinology (Baltimore, Md)*. 2009;23(9):1415-1427. doi:10.1210/me.2009-0097

197. Deutsch DG, Glaser ST, Howell JM, et al. The cellular uptake of anandamide is coupled to its breakdown by fatty-acid amide hydrolase. *The Journal of biological chemistry*. 2001;276(10):6967-6973. doi:10.1074/jbc.M003161200

198. O'Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *British journal of pharmacology*. 2007;152(5):576-582. doi:10.1038/sj.bjp.0707423

199. LoVerme J, La Rana G, Russo R, Calignano A, Piomelli D. The search for the palmitoylethanolamide receptor. *Life sciences*. 2005;77(14):1685-1698. doi:10.1016/j.lfs.2005.05.012

200. Barua R, Kirkland JA, Petrucco OM. Xanthogranulomatous endometritis: case report. *Pathology*. Apr 1978 1978;10(2):161-164.

201. O'Sullivan SE. An update on PPAR activation by cannabinoids. *British journal of pharmacology*. 2016;173(12):1899-1910. doi:10.1111/bph.13497

202. Pontis S, Ribeiro A, Sasso O, Piomelli D. Macrophage-derived lipid agonists of PPAR-α as intrinsic controllers of inflammation. *Critical reviews in biochemistry and molecular biology*. 2016;51(1):7-14. doi:10.3109/10409238.2015.1092944

203. Surapureddi S, Yu S, Bu H, et al. Identification of a transcriptionally active peroxisome proliferator-activated receptor alpha -interacting cofactor complex in rat liver and characterization of PRIC285 as a coactivator. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;99(18):11836-11841. doi:10.1073/pnas.182426699

204. Fanani ML, Maggio B. The many faces (and phases) of ceramide and sphingomyelin I - single lipids. *Biophysical reviews*. 2017;9(5):589-600. doi:10.1007/s12551-017-0297-z

205. Spiegel S, Cuvillier O, Edsall LC, et al. Sphingosine-1-phosphate in cell growth and cell death. *Annals of the New York Academy of Sciences*. 1998;845:11-18. doi:10.1111/j.1749-6632.1998.tb09658.x

206. Parveen F, Bender D, Law S-H, Mishra VK, Chen C-C, Ke L-Y. Role of Ceramidases in Sphingolipid Metabolism and Human Diseases. *Cells*. 2019;8(12):1573. doi:10.3390/cells8121573

207. Ermini L, Ausman J, Melland-Smith M, et al. A Single Sphingomyelin Species Promotes Exosomal Release of Endoglin into the Maternal Circulation in Preeclampsia. *Scientific reports*. 2017;7(1):12172-12172. doi:10.1038/s41598-017-12491-4

208. Laaksonen R, Ekroos K, Sysi-Aho M, et al. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. *European heart journal*. 2016;37(25):1967-1976. doi:10.1093/eurheartj/ehw148

209. Filippov V, Song MA, Zhang K, et al. Increased ceramide in brains with Alzheimer's and other neurodegenerative diseases. *Journal of Alzheimer's disease : JAD*. 2012;29(3):537-547. doi:10.3233/JAD-2011-111202

210. Lai PF, Tribe RM, Johnson MR. Differential impact of acute and prolonged cAMP agonist exposure on protein kinase A activation and human myometrium contractile activity. *The Journal of physiology*. 2016;594(21):6369-6393. doi:10.1113/JP272320

211. Itagaki K, Hauser CJ. Sphingosine 1-phosphate, a diffusible calcium influx factor mediating store-operated calcium entry. *The Journal of biological chemistry*. 2003;278(30):27540-27547. doi:10.1074/jbc.M301763200

212. Brancazio LR, Murtha AP, Heine RP. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. *The New England journal of medicine*. 2003;349(11):1087-1088. doi:10.1056/NEJM200309113491115

213. Aisien AO, Towobola OA, Otubu JA, Imade GE. Umbilical cord venous progesterone at term delivery in relation to mode of delivery. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*. 1994;47(1):27-31. doi:10.1016/0020-7292(94)90457-x

214. Johnston ZC, Bellingham M, Filis P, et al. The human fetal adrenal produces cortisol but no detectable aldosterone throughout the second trimester. *BMC medicine*. 2018;16(1):23-23. doi:10.1186/s12916-018-1009-7

215. Okita R, Masters B. *Textbook of Biochemistry with Clinical Correlations: Chapter 11 The cytochromes P450 and nitric oxide synthases.* Fifth ed. Wiley; 2002.

216. Kumar P, Magon N. Hormones in pregnancy. *Nigerian medical journal : journal of the Nigeria Medical Association*. 2012;53(4):179-183. doi:10.4103/0300-1652.107549

217. Romero R, Stanczyk FZ. Progesterone is not the same as 17α-hydroxyprogesterone caproate: implications for obstetrical practice. *American journal of obstetrics and gynecology*. 2013;208(6):421-426. doi:10.1016/j.ajog.2013.04.027

218. Sexton DJ, O'Reilly MW, Friel AM, Morrison JJ. Functional effects of 17alpha-hydroxyprogesterone caproate (17P) on human myometrial contractility in vitro. *Reproductive biology and endocrinology : RB&E*. 2004;2:80-80. doi:10.1186/1477-7827-2-80

219. Ruddock NK, Shi S-Q, Jain S, et al. Progesterone, but not 17-alpha-hydroxyprogesterone caproate, inhibits human myometrial contractions. *American journal of obstetrics and gynecology*. 2008;199(4):391.e1-391.e3917. doi:10.1016/j.ajog.2008.06.085

220. Patel S, Li A, Goodwin TM, et al. Effect of 17-alpha hydroxyprogesterone caproate on the production of tumor necrosis factor-alpha and the expression of cyclooxygenase-2 in lipopolysaccharide-treated gravid human myometrial explants. *Journal of perinatology : official journal of the California Perinatal Association*. 2010;30(9):584-589. doi:10.1038/jp.2010.23

221. Christian MS, Brent RL, Calda P. Embryo-fetal toxicity signals for 17alpha-hydroxyprogesterone caproate in high-risk pregnancies: a review of the non-clinical literature for embryo-fetal toxicity with progestins. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*. 2007;20(2):89-112. doi:10.1080/14767050601178758

222. Konopka CK, Morais EN, Naidon D, et al. Maternal serum progesterone, estradiol and estriol levels in successful dinoprostone-induced labor. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas*. 2013;46(1):91-97. doi:10.1590/1414-431x20122453

223. Hsu BB, Gibson TE, Yeliseyev V, et al. Dynamic Modulation of the Gut Microbiota and Metabolome by Bacteriophages in a Mouse Model. *Cell host & microbe*. 2019;25(6):803-814.e5. doi:10.1016/j.chom.2019.05.001

224. Blackwell LF, Cooke DG, Brown S. The Use of Estrone-3-Glucuronide and Pregnanediol-3-Glucuronide Excretion Rates to Navigate the Continuum of Ovarian Activity. *Frontiers in public health*. 2018;6:153-153. doi:10.3389/fpubh.2018.00153

225. Sauer MV, Vermesh M, Anderson RE, Vijod AG, Stanczyk FZ, Lobo RA. Rapid measurement of urinary pregnanediol glucuronide to diagnose ectopic pregnancy. *American journal of obstetrics and gynecology*. 1988;159(6):1531-1535. doi:10.1016/0002-9378(88)90589-3

226. Velenosi TJ, Thomson BKA, Tonial NC, et al. Untargeted metabolomics reveals N, N, N-trimethyl-L-alanyl-L-proline betaine (TMAP) as a novel biomarker of kidney function. *Scientific reports*. 2019;9(1):6831-6831. doi:10.1038/s41598-019-42992-3227. England PH. PFOS and PFOAGeneral Information. 2009.

228. Olsen GW, Burris JM, Ehresman DJ, et al. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environmental health perspectives*. 2007;115(9):1298-1305. doi:10.1289/ehp.10009

229. Stein CR, Savitz DA, Dougan M. Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. *American journal of epidemiology*. 2009;170(7):837-846. doi:10.1093/aje/kwp212

230. Wang H, Du H, Yang J, et al. PFOS, PFOA, estrogen homeostasis, and birth size in Chinese infants. *Chemosphere*. 2019;221:349-355. doi:10.1016/j.chemosphere.2019.01.061

231. Chaparro-Ortega A, Betancourt M, Rosas P, et al. Endocrine disruptor effect of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) on porcine ovarian cell steroidogenesis. *Toxicology in vitro : an international journal published in association with BIBRA*. 2018;46:86-93. doi:10.1016/j.tiv.2017.09.030

232. Du G, Hu J, Huang H, et al. Perfluorooctane sulfonate (PFOS) affects hormone receptor activity, steroidogenesis, and expression of endocrine-related genes in vitro and in vivo. *Environmental toxicology and chemistry*. 2013;32(2):353-360. doi:10.1002/etc.2034

233. Marks KJ, Cutler AJ, Jeddy Z, Northstone K, Kato K, Hartman TJ. Maternal serum concentrations of perfluoroalkyl substances and birth size in British boys. *International journal of hygiene and environmental health*. 2019;222(5):889-895. doi:10.1016/j.ijheh.2019.03.008

234. Maisonet M, Terrell ML, McGeehin MA, et al. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. *Environmental health perspectives*. 2012;120(10):1432-1437. doi:10.1289/ehp.1003096

235. Shrestha N, Sleep SL, Helman TJ, et al. Maternal diet high in linoleic acid alters offspring fatty acids and cardiovascular function in a rat model. *Br J Nutr*. Apr 16 2021:1-37. doi:10.1017/s0007114521001276

236. Taylor K, Ferreira DLS, West J, Yang T, Caputo M, Lawlor DA. Differences in Pregnancy Metabolic Profiles and Their Determinants between White European and South Asian Women: Findings from the Born in Bradford Cohort. *Metabolites*. 2019;9(9):190. doi:10.3390/metabo9090190

237. Alvheim AR, Malde MK, Osei-Hyiaman D, et al. Dietary linoleic acid elevates endogenous 2-AG and anandamide and induces obesity. *Obesity (Silver Spring)*. Oct 2012;20(10):1984-94. doi:10.1038/oby.2012.38

238. Capdevila JH, Falck JR, Estabrook RW. Cytochrome P450 and the arachidonate cascade. *Faseb j*. Jan 6 1992;6(2):731-6. doi:10.1096/fasebj.6.2.1537463

239. Lizewska B, Teul J, Kuc P, et al. Maternal Plasma Metabolomic Profiles in Spontaneous Preterm Birth: Preliminary Results. *Mediators of inflammation*. 2018;2018:9362820-9362820. doi:10.1155/2018/9362820

240. Considine EC, Khashan AS, Kenny LC. Screening for Preterm Birth: Potential for a Metabolomics Biomarker Panel. *Metabolites*. 2019;9(5):90. doi:10.3390/metabo9050090

241. Ryckman KK, Berberich SL, Dagle JM. Predicting gestational age using neonatal metabolic markers. *American journal of obstetrics and gynecology*. 2016;214(4):515.e1-515.e13. doi:10.1016/j.ajog.2015.11.028

242. Wright J, Small N, Raynor P, et al. Cohort Profile: the Born in Bradford multi-ethnic family cohort study. *Int J Epidemiol*. Aug 2013;42(4):978-91. doi:10.1093/ije/dys112

243. Mills HL, Patel N, White SL, et al. The effect of a lifestyle intervention in obese pregnant women on gestational metabolic profiles: findings from the UK Pregnancies Better Eating and Activity Trial (UPBEAT) randomised controlled trial. *BMC medicine*. 2019;17(1):15-15. doi:10.1186/s12916-018-1248-7

244. Soininen P, Kangas AJ, Würtz P, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *The Analyst*. 2009;134(9):1781-1785. doi:10.1039/b910205a

245. Groenwold RHH, Sterne JAC, Lawlor DA, Moons KGM, Hoes AW, Tilling K. Sensitivity analysis for the effects of multiple unmeasured confounders. *Annals of epidemiology*. 2016;26(9):605-611. doi:10.1016/j.annepidem.2016.07.009

246. Lawlor D, Richmond R, Warrington N, et al. Using Mendelian randomization to determine causal effects of maternal pregnancy (intrauterine) exposures on offspring outcomes: Sources of bias and methods for assessing them. *Wellcome open research*. 2017;2:11-11. doi:10.12688/wellcomeopenres.10567.1

247. Delnord M, Blondel B, Prunet C, Zeitlin J. Are risk factors for preterm and early-term live singleton birth the same? A population-based study in France. *BMJ open*. 2018;8(1):e018745-e018745. doi:10.1136/bmjopen-2017-018745

248. Shah PS, Knowledge Synthesis Group on Determinants of LBWPTb. Parity and low birth weight and preterm birth: a systematic review and meta-analyses. *Acta obstetricia et gynecologica Scandinavica*. 2010;89(7):862-875. doi:10.3109/00016349.2010.486827

249. Sheen J-J, Wright JD, Goffman D, et al. Maternal age and risk for adverse outcomes. *American journal of obstetrics and gynecology*. 2018;219(4):390.e1-390.e15. doi:10.1016/j.ajog.2018.08.034

250. Santos Ferreira DL, Williams DM, Kangas AJ, et al. Association of pre-pregnancy body mass index with offspring metabolic profile: Analyses of 3 European prospective birth cohorts. *PLoS Med*. Aug 2017;14(8):e1002376. doi:10.1371/journal.pmed.1002376

251. Slack E, Best KE, Rankin J, Heslehurst N. Maternal obesity classes, preterm and post-term birth: a retrospective analysis of 479,864 births in England. *BMC pregnancy and childbirth*. 2019;19(1):434-434. doi:10.1186/s12884-019-2585-z

252. Garcia R, Ali N, Guppy A, Griffiths M, Randhawa G. Differences in the pregnancy gestation period and mean birth weights in infants born to Indian, Pakistani, Bangladeshi and white British mothers in Luton, UK: a retrospective analysis of routinely collected data. *BMJ open*. 2017;7(8):e017139-e017139. doi:10.1136/bmjopen-2017-017139

253. Whitehead NS. The relationship of socioeconomic status to preterm contractions and preterm delivery. *Maternal and child health journal*. 2012;16(8):1645-1656. doi:10.1007/s10995-012-0948-4

254. Tian Y, Holzman C, Slaughter-Acey J, Margerison-Zilko C, Luo Z, Todem D. Maternal Socioeconomic Mobility and Preterm Delivery: A Latent Class Analysis. *Maternal and child health journal*. 2018;22(11):1647-1658. doi:10.1007/s10995-018-2562-6

255. Fairley L, Cabieses B, Small N, et al. Using latent class analysis to develop a model of the relationship between socioeconomic position and ethnicity: cross-sectional analyses from a multi-ethnic birth cohort study. *BMC public health*. 2014;14:835-835. doi:10.1186/1471-2458-14-835

256. Mamluk L, Jones T, Ijaz S, et al. Evidence of detrimental effects of prenatal alcohol exposure on offspring birthweight and neurodevelopment from a systematic review of quasi-experimental studies. *International Journal of Epidemiology*. 2020;doi:10.1093/ije/dyz272

257. Würtz P, Cook S, Wang Q, et al. Metabolic profiling of alcohol consumption in 9778 young adults. *International journal of epidemiology*. 2016;45(5):1493-1506. doi:10.1093/ije/dyw175

258. Ion R, Bernal AL. Smoking and Preterm Birth. *Reproductive sciences (Thousand Oaks, Calif)*. 2015;22(8):918-926. doi:10.1177/1933719114556486

259. Ion R, Hudson C, Johnson J, Yuan W, Heesom K, López Bernal A. Smoking alters hydroxyprostaglandin dehydrogenase expression in fetal membranes. *Reproductive toxicology (Elmsford, NY)*. 2018;82:18-24. doi:10.1016/j.reprotox.2018.09.004

260. Ion RC, Wills AK, Bernal AL. Environmental Tobacco Smoke Exposure in Pregnancy is Associated With Earlier Delivery and Reduced Birth Weight. *Reproductive sciences (Thousand Oaks, Calif)*. 2015;22(12):1603-1611. doi:10.1177/1933719115612135

261. Arneth B, Arneth R, Shams M. Metabolomics of Type 1 and Type 2 Diabetes. *International journal of molecular sciences*. 2019;20(10):2467. doi:10.3390/ijms20102467

262. White SL, Pasupathy D, Sattar N, et al. Metabolic profiling of gestational diabetes in obese women during pregnancy. *Diabetologia*. 2017;60(10):1903-1912. doi:10.1007/s00125-017-4380-6

263. Köck K, Köck F, Klein K, Bancher-Todesca D, Helmer H. Diabetes mellitus and the risk of preterm birth with regard to the risk of spontaneous preterm birth. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*. 2010;23(9):1004-1008. doi:10.3109/14767050903551392

264. Billionnet C, Mitanchez D, Weill A, et al. Gestational diabetes and adverse perinatal outcomes from 716,152 births in France in 2012. *Diabetologia*. 2017;60(4):636-644. doi:10.1007/s00125-017-4206-6

265. Tzoulaki I, Iliou A, Mikros E, Elliott P. An Overview of Metabolic Phenotyping in Blood Pressure Research. *Current hypertension reports*. 2018;20(9):78-78. doi:10.1007/s11906-018-0877-8

266. Austdal M, Tangerås LH, Skråstad RB, et al. First Trimester Urine and Serum Metabolomics for Prediction of Preeclampsia and Gestational Hypertension: A Prospective Screening Study. *International journal of molecular sciences*. 2015;16(9):21520-21538. doi:10.3390/ijms160921520

267. Davies EL, Bell JS, Bhattacharya S. Preeclampsia and preterm delivery: A population-based case-control study. *Hypertension in pregnancy*. 2016;35(4):510-519. doi:10.1080/10641955.2016.1190846

268. Premkumar A, Baer RJ, Jelliffe-Pawlowski LL, Norton ME. Hypertensive Disorders of Pregnancy and Preterm Birth Rates among Black Women. *American journal of perinatology*. 2019;36(2):148-154. doi:10.1055/s-0038-1660461

269. Marchi J, Berg M, Dencker A, Olander EK, Begley C. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2015;16(8):621-638. doi:10.1111/obr.12288

270. Dann AT, Kenyon AP, Wierzbicki AS, Seed PT, Shennan AH, Tribe RM. Plasma lipid profiles of women with intrahepatic cholestasis of pregnancy. *Obstetrics and gynecology*. 2006;107(1):106-114. doi:10.1097/01.AOG.0000189096.94874.9c

271. Chappell LC, Chambers J, Dixon PH, et al. Ursodeoxycholic acid versus placebo in the treatment of women with intrahepatic cholestasis of pregnancy (ICP) to improve perinatal outcomes: protocol for a randomised controlled trial (PITCHES). *Trials*. 2018;19(1):657-657. doi:10.1186/s13063-018-3018-4

272. Gynaecologists RCoOa. Green top guideline 43: Obstetric cholestasis. 2011.

273. Hughes RA, Heron J, Sterne JAC, Tilling K. Accounting for missing data in statistical analyses: multiple imputation is not always the answer. *International journal of epidemiology*. 2019;48(4):1294-1304. doi:10.1093/ije/dyz032

274. Feingold K, Grunfeld C. *Introduction to lipids and lipoproteins.* 2000.

275. Bayly GR. CHAPTER 37 - Lipids and disorders of lipoprotein metabolism. In: Marshall WJ, Lapsley M, Day AP, Ayling RM, eds. *Clinical Biochemistry: Metabolic and Clinical Aspects (Third Edition)*. Churchill Livingstone; 2014:702-736.

276. Daniels TF, Killinger KM, Michal JJ, Wright RW, Jr., Jiang Z. Lipoproteins, cholesterol homeostasis and cardiac health. *International journal of biological sciences*. 2009;5(5):474-488. doi:10.7150/ijbs.5.474

277. Catov JM, Mackey RH, Scifres CM, Bertolet M, Simhan HN. Lipoprotein Heterogeneity Early in Pregnancy and Preterm Birth. *American journal of perinatology*. 2017;34(13):1326-1332. doi:10.1055/s-0037-1603471

278. Sulaiman WNW, Caslake MJ, Delles C, et al. Does high-density lipoprotein protect vascular function in healthy pregnancy? *Clinical science (London, England : 1979)*. 2016;130(7):491-497. doi:10.1042/CS20150475

279. Thorp JM, Jr., Rice MM, Harper M, et al. Advanced lipoprotein measures and recurrent preterm birth. *American journal of obstetrics and gynecology*. 2013;209(4):342.e1-342.e3427. doi:10.1016/j.ajog.2013.06.005

280. Sonagra AD, Biradar SM, K D, Murthy D S J. Normal pregnancy- a state of insulin resistance. *Journal of clinical and diagnostic research : JCDR*. 2014;8(11):CC01-CC3. doi:10.7860/JCDR/2014/10068.5081

281. Poston L, Caleyachetty R, Cnattingius S, et al. Preconceptional and maternal obesity: epidemiology and health consequences. *The lancet Diabetes & endocrinology*. 2016;4(12):1025-1036. doi:10.1016/S2213-8587(16)30217-0

282. Smith CJ, Baer RJ, Oltman SP, et al. Maternal dyslipidemia and risk for preterm birth. *PloS one*. 2018;13(12):e0209579-e0209579. doi:10.1371/journal.pone.0209579

283. Spracklen CN, Saftlas AF, Triche EW, et al. Genetic Predisposition to Dyslipidemia and Risk of Preeclampsia. *American Journal of Hypertension*. 2014;28(7):915-923. doi:10.1093/ajh/hpu242

284. Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, Sattar N. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. *The Journal of clinical endocrinology and metabolism*. 2002;87(9):4231-4237. doi:10.1210/jc.2002-020311

285. Saarelainen H, Laitinen T, Raitakari OT, et al. Pregnancy-related hyperlipidemia and endothelial function in healthy women. *Circulation journal : official journal of the Japanese Circulation Society*. 2006;70(6):768-772. doi:10.1253/circj.70.768

286. Anell-Olofsson M, Ahmadi S, Lönnqvist PA, Eksborg S, von Horn H, Bartocci M. Plasma concentrations of alpha-1-acid glycoprotein in preterm and term newborns: influence of mode of delivery and implications for plasma protein binding of local anaesthetics. *British journal of anaesthesia*. 2018;121(2):427-431. doi:10.1016/j.bja.2018.01.034

287. Hudson CA, Heesom KJ, López Bernal A. Phasic contractions of isolated human myometrium are associated with Rho-kinase (ROCK)-dependent phosphorylation of myosin phosphatase-targeting subunit (MYPT1). *Molecular human reproduction*. 2012;18(5):265-279. doi:10.1093/molehr/gar078

288. Sanborn BM. Hormonal signaling and signal pathway crosstalk in the control of myometrial calcium dynamics. *Seminars in cell & developmental biology*. 2007;18(3):305-314. doi:10.1016/j.semcdb.2007.05.007

289. Chaemsaithong P, Madan I, Romero R, et al. Characterization of the myometrial transcriptome in women with an arrest of dilatation during labor. *Journal of perinatal medicine*. 2013;41(6):665-681. doi:10.1515/jpm-2013-0086

290. Mittal P, Romero R, Tarca AL, et al. A molecular signature of an arrest of descent in human parturition. *American journal of obstetrics and gynecology*. 2011;204(2):177.e15-177.e1.77E33. doi:10.1016/j.ajog.2010.09.025

291. Conesa A, Mortazavi A. The common ground of genomics and systems biology. *BMC systems biology*. 2014;8 Suppl 2(Suppl 2):S1-S1. doi:10.1186/1752-0509-8-S2-S1

292. von Stechow L, Francavilla C, Olsen JV. Recent findings and technological advances in phosphoproteomics for cells and tissues. *Expert review of proteomics*. 2015;12(5):469-487. doi:10.1586/14789450.2015.1078730

293. Abdel-Hafiz HA, Horwitz KB. Post-translational modifications of the progesterone receptors. *The Journal of steroid biochemistry and molecular biology*. 2014;140:80-89. doi:10.1016/j.jsbmb.2013.12.008

294. Abdul-Ghani S, Heesom KJ, Angelini GD, Suleiman MS. Cardiac phosphoproteomics during remote ischemic preconditioning: a role for the sarcomeric Z-disk proteins. *BioMed research international*. 2014;2014:767812-767812. doi:10.1155/2014/767812

295. Eng JK, McCormack AL, Yates JR. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *Journal of the American Society for Mass Spectrometry*. 1994;5(11):976-989. doi:10.1016/1044-0305(94)80016-2

296. Eng JK, Fischer B, Grossmann J, Maccoss MJ. A fast SEQUEST cross correlation algorithm. *Journal of proteome research*. 2008;7(10):4598-4602. doi:10.1021/pr800420s

297. Fabregat A, Sidiropoulos K, Viteri G, et al. Reactome pathway analysis: a high-performance in-memory approach. *BMC Bioinformatics*. 2017/03/02 2017;18(1):142. doi:10.1186/s12859-017-1559-2

298. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Frontiers in immunology*. 2018;9:754-754. doi:10.3389/fimmu.2018.00754

299. Smith SA, Travers RJ, Morrissey JH. How it all starts: Initiation of the clotting cascade. *Critical reviews in biochemistry and molecular biology*. 2015;50(4):326-336. doi:10.3109/10409238.2015.1050550

300. Yuan W, Duffner AM, Chen L, Hunt LP, Sellers SM, Bernal AL. Analysis of preterm deliveries below 35 weeks' gestation in a tertiary referral hospital in the UK. A case-control survey. *BMC research notes*. 2010;3:119-119. doi:10.1186/1756-0500-3-119

301. Yang J, Hartmann KE, Savitz DA, et al. Vaginal bleeding during pregnancy and preterm birth. *American journal of epidemiology*. 2004;160(2):118-125. doi:10.1093/aje/kwh180

302. Elovitz MA, Saunders T, Ascher-Landsberg J, Phillippe M. Effects of thrombin on myometrial contractions in vitro and in vivo. *American journal of obstetrics and gynecology*. 2000;183(4):799-804. doi:10.1067/mob.2000.108897

303. Elovitz MA, Ascher-Landsberg J, Saunders T, Phillippe M. The mechanisms underlying the stimulatory effects of thrombin on myometrial smooth muscle. *American journal of obstetrics and gynecology*. 2000;183(3):674-681. doi:10.1067/mob.2000.106751

304. Mogami H, Keller PW, Shi H, Word RA. Effect of thrombin on human amnion mesenchymal cells, mouse fetal membranes, and preterm birth. *The Journal of biological chemistry*. 2014;289(19):13295-13307. doi:10.1074/jbc.M114.550541

305. Boscardin E, Alijevic O, Hummler E, Frateschi S, Kellenberger S. The function and regulation of acid-sensing ion channels (ASICs) and the epithelial Na(+) channel (ENaC): IUPHAR Review 19. *British journal of pharmacology*. 2016;173(18):2671-2701. doi:10.1111/bph.13533

306. Boiko N, Kucher V, Wang B, Stockand JD. Restrictive expression of acid-sensing ion channel 5 (asic5) in unipolar brush cells of the vestibulocerebellum. *PloS one*. 2014;9(3):e91326-e91326. doi:10.1371/journal.pone.0091326

307. Schaefer L, Sakai H, Mattei M, Lazdunski M, Lingueglia E. Molecular cloning, functional expression and chromosomal localization of an amiloride-sensitive Na(+) channel from human small intestine. *FEBS letters*. 2000;471(2-3):205-210. doi:10.1016/s0014-5793(00)01403-4

308. Yingjun G, Xun Q. Acid-sensing ion channels under hypoxia. *Channels (Austin, Tex)*. Jul-Aug 2013;7(4):231-237. doi:10.4161/chan.25223

309. Zha X-m. Acid-sensing ion channels: trafficking and synaptic function. *Molecular brain*. 2013;6:1-1. doi:10.1186/1756-6606-6-1

310. Lenzig P, Wirtz M, Wiemuth D. Comparative electrophysiological analysis of the bile acid-sensitive ion channel (BASIC) from different species suggests similar physiological functions. *Pflugers Archiv : European journal of physiology*. 2019;471(2):329-336. doi:10.1007/s00424-018-2223-z

311. Kenyon AP, Piercy CN, Girling J, Williamson C, Tribe RM, Shennan AH. Obstetric cholestasis, outcome with active management: a series of 70 cases. *BJOG : an international journal of obstetrics and gynaecology*. 2002;109(3):282-288. doi:10.1111/j.1471-0528.2002.01368.x

312. MacIntyre DA, Tyson EK, Read M, et al. Contraction in human myometrium is associated with changes in small heat shock proteins. *Endocrinology*. 2008;149(1):245-252. doi:10.1210/en.2007-0662

313. Wu WX, Derks JB, Zhang Q, Nathanielsz PW. Changes in heat shock protein-90 and -70 messenger ribonucleic acid in uterine tissues of the ewe in relation to parturition and regulation by estradiol and progesterone. *Endocrinology*. 1996;137(12):5685-5693. doi:10.1210/endo.137.12.8940400

314. Tang PZ, Gannon MJ, Andrew A, Miller D. Evidence for oestrogenic regulation of heat shock protein expression in human endometrium and steroid-responsive cell lines. *European journal of endocrinology*. 1995;133(5):598-605. doi:10.1530/eje.0.1330598

315. Worrall LJ, Wear MA, Page AP, Walkinshaw MD. Cloning, purification and characterization of the Caenorhabditis elegans small glutamine-rich tetratricopeptide repeat-containing protein. *Biochimica et biophysica acta*. 2008;1784(3):496-503. doi:10.1016/j.bbapap.2007.12.003

316. Paul A, Garcia YA, Zierer B, et al. The cochaperone SGTA (small glutamine-rich tetratricopeptide repeat-containing protein alpha) demonstrates regulatory specificity for the androgen, glucocorticoid, and progesterone receptors. *The Journal of biological chemistry*. 2014;289(22):15297-15308. doi:10.1074/jbc.M113.535229

317. Moritz A, Li Y, Guo A, et al. Akt-RSK-S6 kinase signaling networks activated by oncogenic receptor tyrosine kinases. *Science signaling*. 2010;3(136):ra64-ra64. doi:10.1126/scisignal.2000998

318. Kostyukova AS. Leiomodin/tropomyosin interactions are isoform specific. *Archives of biochemistry and biophysics*. 2007;465(1):227-230. doi:10.1016/j.abb.2007.05.012

319. Sahoo S, Meijles DN, Al Ghouleh I, et al. MEF2C-MYOCD and Leiomodin1 Suppression by miRNA-214 Promotes Smooth Muscle Cell Phenotype Switching in Pulmonary Arterial Hypertension. *PloS one*. 2016;11(5):e0153780-e0153780. doi:10.1371/journal.pone.0153780

320. Nanda V, Miano JM. Leiomodin 1, a new serum response factor-dependent target gene expressed preferentially in differentiated smooth muscle cells. *The Journal of biological chemistry*. 2012;287(4):2459-2467. doi:10.1074/jbc.M111.302224

321. Rehman KS, Yin S, Mayhew BA, Word RA, Rainey WE. Human myometrial adaptation to pregnancy: cDNA microarray gene expression profiling of myometrium from non‐pregnant and pregnant women. *Molecular Human Reproduction*. 2003;9(11):681-700. doi:10.1093/molehr/gag078

322. Hassan SS, Romero R, Tarca AL, et al. The transcriptome of cervical ripening in human pregnancy before the onset of labor at term: identification of novel molecular functions involved in this process. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*. 2009;22(12):1183-1193. doi:10.3109/14767050903353216

323. Cole AR, Causeret F, Yadirgi G, et al. Distinct priming kinases contribute to differential regulation of collapsin response mediator proteins by glycogen synthase kinase-3 in vivo. *The Journal of biological chemistry*. 2006;281(24):16591-16598. doi:10.1074/jbc.M513344200

324. Hohaus A, Person V, Behlke J, Schaper J, Morano I, Haase H. The carboxyl-terminal region of ahnak provides a link between cardiac L-type Ca2+ channels and the actin-based cytoskeleton. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2002;16(10):1205-1216. doi:10.1096/fj.01-0855com

325. Lomashvili KA, Wang X, Wallin R, O'Neill WC. Matrix Gla protein metabolism in vascular smooth muscle and role in uremic vascular calcification. *The Journal of biological chemistry*. 2011;286(33):28715-28722. doi:10.1074/jbc.M111.251462

326. Stetson B, Kellert BA, Zhao G, et al. 623: Decreased myometrial expression of matrix-Gla protein (MGP) is associated with preterm and term laboring state. *American Journal of Obstetrics & Gynecology*. 2018;218(1):S372. doi:10.1016/j.ajog.2017.11.151

327. Ma H, Zhang BL, Liu BY, et al. Vitamin K2-Dependent GGCX and MGP Are Required for Homeostatic Calcium Regulation of Sperm Maturation. *iScience*. 2019;14:210-225. doi:10.1016/j.isci.2019.03.030

328. Finney J, Moon H-J, Ronnebaum T, Lantz M, Mure M. Human copper-dependent amine oxidases. *Archives of biochemistry and biophysics*. 2014;546:19-32. doi:10.1016/j.abb.2013.12.022

329. Wei Y, Kim TJ, Peng DH, et al. Fibroblast-specific inhibition of TGF-β1 signaling attenuates lung and tumor fibrosis. *The Journal of clinical investigation*. 2017;127(10):3675-3688. doi:10.1172/JCI94624

330. Ween MP, Oehler MK, Ricciardelli C. Transforming growth Factor-Beta-Induced Protein (TGFBI)/(βig-H3): a matrix protein with dual functions in ovarian cancer. *International journal of molecular sciences*. 2012;13(8):10461-10477. doi:10.3390/ijms130810461

331. Thapa N, Lee B-H, Kim I-S. TGFBIp/betaig-h3 protein: a versatile matrix molecule induced by TGF-beta. *The international journal of biochemistry & cell biology*. 2007;39(12):2183-2194. doi:10.1016/j.biocel.2007.06.004

332. Wang Y, Zhao S, Loyd S, Groome LJ. Increased urinary excretion of nephrin, podocalyxin, and βig-h3 in women with preeclampsia. *American journal of physiology Renal physiology*. 2012;302(9):F1084-F1089. doi:10.1152/ajprenal.00597.2011

333. Ween MP, Lokman NA, Hoffmann P, Rodgers RJ, Ricciardelli C, Oehler MK. Transforming growth factor-beta-induced protein secreted by peritoneal cells increases the metastatic potential of ovarian cancer cells. *International journal of cancer*. 2011;128(7):1570-1584. doi:10.1002/ijc.25494

334. Uekita T, Kim Y-J, Yamanouchi K, Tojo H, Tachi C. Dynamics of betaig-h3 mRNA expression during pregnancy in the uterus and the placenta of the mouse: a possible regulatory factor for trophoblastic invasion. *The Journal of reproduction and development*. 2003;49(3):243-252. doi:10.1262/jrd.49.243

335. Yan W, Sheng N, Seto M, Morser J, Wu Q. Corin, a mosaic transmembrane serine protease encoded by a novel cDNA from human heart. *The Journal of biological chemistry*. 1999;274(21):14926-14935. doi:10.1074/jbc.274.21.14926

336. Yan W, Wu F, Morser J, Wu Q. Corin, a transmembrane cardiac serine protease, acts as a pro-atrial natriuretic peptide-converting enzyme. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97(15):8525-8529. doi:10.1073/pnas.150149097

337. Itoh H, Sagawa N, Hasegawa M, et al. Expression of biologically active receptors for natriuretic peptides in the human uterus during pregnancy. *Biochemical and biophysical research communications*. 1994;203(1):602-607. doi:10.1006/bbrc.1994.2225

338. Cui Y, Wang W, Dong N, et al. Role of corin in trophoblast invasion and uterine spiral artery remodelling in pregnancy. *Nature*. 2012;484(7393):246-250. doi:10.1038/nature10897

339. Cootauco AC, Murphy JD, Maleski J, Blakemore KJ, Slodzinski MK. Atrial natriuretic peptide production and natriuretic peptide receptors in the human uterus and their effect on myometrial relaxation. *American journal of obstetrics and gynecology*. 2008;199(4):429.e1-429.e4296. doi:10.1016/j.ajog.2008.05.019

340. Soloff MS, Jeng Y-J, Izban MG, et al. Effects of progesterone treatment on expression of genes involved in uterine quiescence. *Reproductive sciences (Thousand Oaks, Calif)*. 2011;18(8):781-797. doi:10.1177/1933719111398150

341. Pereira NL, Aksoy P, Moon I, et al. Natriuretic peptide pharmacogenetics: membrane metallo-endopeptidase (MME): common gene sequence variation, functional characterization and degradation. *Journal of molecular and cellular cardiology*. 2010;49(5):864-874. doi:10.1016/j.yjmcc.2010.07.020

342. Jin F, Qiao C, Luan N, Li H. Lentivirus-mediated PHLDA2 overexpression inhibits trophoblast proliferation, migration and invasion, and induces apoptosis. *International journal of molecular medicine*. 2016;37(4):949-957. doi:10.3892/ijmm.2016.2508

343. Wu W, Shi S-Q, Huang H-J, Balducci J, Garfield RE. Changes in PGRMC1, a potential progesterone receptor, in human myometrium during pregnancy and labour at term and preterm. *Molecular human reproduction*. 2011;17(4):233-242. doi:10.1093/molehr/gaq096

344. Wang R, Sheehan PM, Brennecke SP. Changes in myometrial expression of progesterone receptor membrane components 1 and 2 are associated with human parturition at term. *Reproduction, fertility, and development*. 2016;28(5):618-627. doi:10.1071/RD13430

345. Myhill N, Lynes EM, Nanji JA, et al. The subcellular distribution of calnexin is mediated by PACS-2. *Molecular biology of the cell*. 2008;19(7):2777-2788. doi:10.1091/mbc.e07-10-0995

346. Goto M, Piper Hanley K, Marcos J, et al. In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. *The Journal of clinical investigation*. 2006;116(4):953-960. doi:10.1172/JCI25091

347. Sun X, Deng W, Li Y, et al. Sustained Endocannabinoid Signaling Compromises Decidual Function and Promotes Inflammation-induced Preterm Birth. *The Journal of Biological Chemistry*. 02/2112/01/received02/09/revised 2016;291(15):8231-8240. doi:10.1074/jbc.M115.707836

348. Maccarrone M, Bisogno T, Valensise H, et al. Low fatty acid amide hydrolase and high anandamide levels are associated with failure to achieve an ongoing pregnancy after IVF and embryo transfer. *Mol Hum Reprod*. Feb 2002;8(2):188-95.

349. Park B, Gibbons HM, Mitchell MD, Glass M. Identification of the CB1 cannabinoid receptor and fatty acid amide hydrolase (FAAH) in the human placenta. *Placenta*. Nov 2003;24(10):990-5.

350. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. Feb 2003;32(1):1-22.

351. Burgess S, Timpson NJ, Ebrahim S, Davey Smith G. Mendelian randomization: where are we now and where are we going? *Int J Epidemiol*. 2015:379-88. vol. 2.

352. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Statistics in medicine*. 2008;27(8):1133-1163. doi:10.1002/sim.3034