Placenta 129 (2022) 111–118

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

The feto-placental metabolome of spontaneous labour is not reproduced following induction of labour

Katherine Alice Birchenall [a,b,*], Gavin Iain Welsh [b], Andrés López Bernal [a,b]

[a] Department of Obstetrics and Gynaecology, St Michael's Hospital, Southwell Street, Bristol, BS2 8EG, UK
[b] Translational Health Sciences, University of Bristol, Dorothy Hodgkin Building, Whitson Street, Bristol, BS1 3NY, UK

ARTICLE INFO

Keywords:
Fetoplacental unit
Labour
Cord
Intervillous blood
Metabolomics

ABSTRACT

Introduction: The mechanism for human labour remains poorly understood, limiting our ability to manage complications including spontaneous preterm birth and induction of labour (IOL). The study of fetal signals poses specific challenges. Metabolomic analysis of maternal blood, the cord artery (CA), and cord vein (CV), allows simultaneous interrogation of multiple metabolic pathways associated with different modes of labour onset and birth.

Methods: Global mass spectrometry metabolomics analysis was performed on serial samples collected from participants during pregnancy, in latent phase of labour, and following birth (CA, CV, and intervillous (IV) blood), from those who spontaneously laboured and birthed vaginally (SL group), had IOL and birthed vaginally (IOL group), or birthed via elective caesarean section (no labour; ECS group).

Results: There were clear differences in fetal and maternal steroid, arachidonate and sphingosine pathways between the SL and IOL groups, despite similar uterine contractions and vaginal birth. The CA/CV ratio for key steroids of the IOL group were more alike the ECS group than the SL group, including progesterone (CA/CV ratio for: SL group=3.5; IOL group=0.5; and ECS group=0.5), and oestriol (CA/CV ratio for: SL group=4.3; IOL group=0.4; and for ECS group=0.2). There were no such changes in the maternal samples.

Discussion: These findings indicate that IOL does not reproduce the pathways activated in spontaneous labour. The decreased placental progesterone production observed with spontaneous labour may represent a local intrauterine progesterone withdrawal, which, together with other signals, would activate parturition pathways involving arachidonate and sphingosine metabolism.

1. Introduction

The pathways involved in spontaneous human labour remain elusive [1–3], limiting our effectiveness at managing complications such as preterm labour (PTL) and failed induction of labour (IOL) [1,4,5]. Improved understanding of the mechanisms of human parturition would allow more effective strategies for PTL prevention and improved IOL. Although infection and inflammatory processes are implicated in some episodes of PTL, in most cases no clear cause is found, and there may be an early triggering of the physiological events of spontaneous parturition which would normally occur at term [6,7].

Placentally-derived hormones include progesterone, oestradiol, and oestriol. A substantial proportion of the steroid substrates required are produced by the fetus, involving an active exchange of molecules across the fetoplacental unit [8–10]. As with other mammals, progesterone appears essential for quiescence of the uterine myometrium during human pregnancy [11], while oestrogen promotes transcription of genes which encode proteins associated with myometrial excitability and contractility [12]. In many non-human mammals, there is a shift in the steroid balance from progesterone towards oestrogens at the time of labour [13–15]: for example, following luteolysis in rabbits and mice [16], and following fetal adrenal maturation in sheep [13,14]. These mechanisms are not present in humans [4,11,17], with no measurable fall in maternal progesterone until after completion of the third stage of labour [8,18]. Instead, a functional progesterone withdrawal may alter the steroid responses, involving local changes in activity and structure of progesterone [11,19] and oestrogen receptors [15,20].

Intrauterine paracrine and autocrine changes likely have important direct effects on the myometrium and cervix at the initiation of labour [2,4,21,22], however these molecular changes are difficult to detect by

* Corresponding author.Translational Health Sciences, University of Bristol, Dorothy Hodgkin Building, WhitsonStreet, Bristol, BS1 3NY, UK
E-mail addresses: katherine.birchenall@doctors.org.uk (K.A. Birchenall), G.I.Welsh@bristol.ac.uk (G.I. Welsh), ALopezBernal@bristol.ac.uk (A. López Bernal).

https://doi.org/10.1016/j.placenta.2022.10.005
Received 10 January 2022; Received in revised form 4 October 2022; Accepted 9 October 2022
Available online 19 October 2022

maternal peripheral blood sampling. Furthermore, ethical considerations limit invasive experimentation during pregnancy. Modern metabolomics methodologies allow simultaneous analysis of multiple biochemical pathways in relation to different clinical or physiological situations. In a previous study we compared the concentration of metabolites in cord and maternal (intervillous (IV)) plasma sampled from participants who had a vaginal birth following spontaneous labour at term with those sampled from participants who did not labour and had an elective caesarean section (elCS) at term. Of 826 metabolites measured, 26.9% were significantly changed between the different modes of labour (MOL) in maternal plasma, and 21.1% in cord plasma. We concluded that while a proportion of these differences are likely due to the metabolic challenges of labour itself, the findings present clues as to the biochemical pathways involved in spontaneous labour, including steroid, endocannabinoid, sphingolipid, and ceramide systems [23].

The present prospective study was designed to investigate the metabolomic changes of pregnancy and labour through serial sampling of maternal plasma during pregnancy, and of cord vein (CV), cord artery (CA), and IV plasma at birth, across different types of labour onset. CAs transport blood from the fetus to the placenta, while the CV transports blood from the placenta to the fetus. Performing metabolomics analysis on paired CA and CV samples was expected to provide a comprehensive assessment of multiple metabolites in the fetoplacental unit associated with labour and mode of birth [23]. There is much interest in the identification of relevant pathways for human parturition [23–30]. If any deviation from the observed metabolic profile of spontaneous labour was observed with either IOL or no labour, this would enable further elucidation of the physiological pathways of spontaneous labour. Here we report novel and significant changes in maternal metabolite concentrations associated with late pregnancy, and further changes in CA and CV steroid, sphingosine, and fatty acid metabolites between different MOL onset and birth.

Our specific questions were:

1. Are there associations between the maternal metabolomic profile at 28 weeks' gestation, 34 weeks' gestation, or latent phase, and the gestational age (GA) at the onset of spontaneous labour?
2. Are there differences in the metabolomic profiles of maternal blood in latent phase, and IV, CA and CV blood at birth, between different MOL onset, which could provide clues as to the physiological mechanisms of spontaneous labour?

2. Methods

2.1. Participants

Ethical approval for the study was granted by the National Research Ethics Service Committee South-West, Bristol (reference: E5431). Eligible individuals were approached at community health centres during their first trimester booking appointments. Individuals gave informed, written consent for participation in the study. Inclusion criteria were a low-risk singleton pregnancy, Body Mass Index (BMI) 18 kg/m^2-30 kg/m^2, age 20–40 years, and planning to birth at St Michael's Hospital (SMH), Bristol, UK. Exclusion criteria were BMI <18 kg/m^2 or >30 kg/m^2, age <20 or >40 years, multiple pregnancy, and development of maternal or fetal pathology such as diabetes, preeclampsia, or infection. Participants were excluded from the analysis of metabolites in relation to GA at spontaneous labour if their birth was iatrogenically expedited, either with IOL or elCS. Participants who remained low-risk but were induced for prolonged gestation (post-maturity) to reduce the risk of stillbirth [31] were included in the analysis of GA at birth. Clinical characteristics of included pregnancies for whom cord blood was sampled were collated according to previously described criteria for placental investigation [32] (Supplementary Table (ST)1).

At SMH, IOL involves intravaginal administration of prostaglandin (10 mg Dinoprostone (Propess), a prostaglandin PGE2 analogue [33], then 0.5–1 mg Dinoprostone (Prostin), if required), followed by artificial rupture of membranes (ARM). If labour does not establish within 2 h of ARM, an oxytocin infusion is commenced to promote uterine contractions.

2.2. Sample collection

Maternal peripheral blood samples were obtained at 28-weeks' (28WG) and 34-weeks' gestation (34WG) and in the latent phase (LP) of labour. For participants who remained low-risk and who spontaneously laboured (SL group), LP samples were obtained when participants attended hospital with a cervical dilatation between 1 and 4 cm; for participants who were induced (IOL group), the LP sample was obtained at the time of transfer to the Central Delivery Suite (CDS) for ARM. For participants who had an elCS (ECS group), the LP sample was obtained at the preoperative appointment attended the day before their elCS. Intervillous, CA, and CV blood samples were obtained from the placenta within 30 min of birth, following routine delayed cord clamping, as described previously [23]. Samples were collected into Vacutainer tubes containing EDTA and centrifuged at 1000 g for 10 min. 200 μl of the clear upper plasma layer was transferred into chilled propylene tubes and stored at −80 °C, all within 60 min of placental delivery.

2.3. Metabolomics analysis

Samples were transported on dry ice to Metabolon, Inc., (Morrisville, NC, USA) for ultrahigh performance liquid chromatography-tandem mass spectrometry, as described previously [23,34–36]. In brief, plasma samples were subjected to methanol extraction then split into aliquots for analysis by UHPLC/MS in the negative, positive (involving two methods, one optimised for hydrophobic compounds and the other for hydrophilic), or polar ion mode. Metabolites were then identified by automated comparison of ion features to a reference library of chemical standard [23,37]. Multiple water blanks were included on each plate of experimental samples to identify any compounds resulting from handling or storage. Compounds which were detected at a level at least three times 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 control and quality assurance, pooled quality control plasma replicates, as well as several internal standards, were assessed to determine instrument variability, with representative relative standard deviations of 3% for internal standards and 7% for endogenous biochemicals.

2.4. Statistical analysis

Metabolites were normalised to sample volume and then log-transformed. For comparisons between metabolites, each metabolite value was rescaled to set the median to be equal to 1.

2.4.1. Associations with GA at spontaneous labour

Spearman's rank correlation was used to assess potential associations between the GA at spontaneous labour (or gestation at IOL for post-maturity) and maternal metabolomic profile at 28WG, 34WG, and LP. Paired t-tests were conducted to assess changes in metabolites between each time point (28WG vs 34WG; 28WG vs LP; and 34WG vs LP).

2.4.2. Cord blood analysis

Welch's *t*-test was used to compare the mean value of each metabolite between the three MOL onset and birth groups (SL, IOL, and ECS) for the LP, IV, CA, and CV samples. Paired t-tests were used to compare the mean amount of each metabolite measured between each of the different samples (LP/CV/CA/IV) within the same mode of birth groups.

2.4.3. Over-representation analysis

Over-representation analysis (ORA) was performed using

MetaboAnalyst (version 4.0), a tool that maps common compound names to a range of database identifiers [38–41]. Human Metabolome Database (HMDB) identifiers for those metabolites which were significantly different ($p \leq 0.05$) between pregnancy time points or mode of birth groups were entered into MetaboAnalyst for each experimental comparison. Enrichment analysis using the hypergeometric test was performed to evaluate whether a specific metabolite group was represented more than would be expected by chance within the given compound list. Both raw one-tailed p-values and a p-value following adjustment for multiple testing using the Holm method were calculated [40,41].

3. Results

3.1. Participants

Between August 2018 and August 2019, 61 individuals with low-risk pregnancies were approached to take part in the study. Supplementary Figure (SF)1 presents the numbers of participants approached, recruited, and reasons for any withdrawal for those included in the association between maternal metabolome and GA at spontaneous labour analysis. Table (T)1 shows the maternal characteristics for these participants. SF2 presents the participants included in the MOL onset and birth analysis.

3.2. Associations between maternal metabolites during pregnancy and GA at onset of spontaneous labour

The pregnancies included in this part of the study either spontaneously laboured or were induced for post-maturity (SF2). The maternal metabolome was remarkably stable with advancing gestation, with only: 63 of the 1032 metabolites measured at 28WG significantly correlated with GA at spontaneous birth (49 negative association; 14 positive association) (ST2); 84 of the 1032 metabolites measured at 34WG significantly correlated with GA at spontaneous birth (75 negative association; 9 positive association) (ST3); and 53 of the 1032 metabolites measured in the LP (n = 11) or at the beginning of IOL (n = 3) significantly correlated with GA at spontaneous birth (50 negative association; 3 positive association) (ST4). Seven metabolites had a significant negative correlation with GA at spontaneous labour at all three maternal blood sampling time points (i.e., 28WG, 34WG and LP), these 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. 17alpha-hydroxypregnanolone glucuronide, 5alpha-androstan-3alpha,17beta-diol disulfate, oestriol 3-sulfate and oestriol 16-glucuronide were significantly correlated at 34WG and in LP. All were negatively correlated with GA at spontaneous labour except for DHEA-S and androstenediol (3beta, 17beta) monosulfate (1), which were positively correlated (Figure (F)1 and ST5).

Table 1
Maternal characteristics for the 17 participants included in the GA association analysis.

Characteristic	(range)
Age at Recruitment (years)	31.6[a] (24–40)
BMI (weight (Kg)/height (m^2))	**24.5** [a] (20–28.8)
Past Medical History	**100% (17/17) had no relevant past medical history**
Gravida	**2**[b] (1–4)
Parity	**0** [b] (0–1)
Alcohol during pregnancy (units)	**0% (0/17)**
Ethnicity	**94% (16/17) WE** **6% (1/17) WE/Chinese**
Smoking during pregnancy (cigarettes per day)	**6% (1/17) 5/day** **94% (16/17) 0/day**
Type of labour onset	**82% (14/17) SP** **18% (3/17) IOL PT**
Gestational age at birth (days)	**287** [b] (240–295)
Maternal complications	**100% (17/17) None**
Apgar scores at 1, 5 and 10 min	**1 min: 9** [b] **(7–9)** **5 min: 10** [b] **(8–10)** **10 min: 10** [b] **(9–10)**
Birthweight (Kg)	**3.620** [a] (2.44–4.18)
Baby sex	**41.2% (7/17) M** **58% (10/17) F**

[a] Mean.
[b] Median; Kg = kilograms; m = meters; BMI=Body Mass Index; WE=White European; PT=Post-Term; F=Female; M = Male; SP = Spontaneous; IOL PT = Induction of labour for post-term.

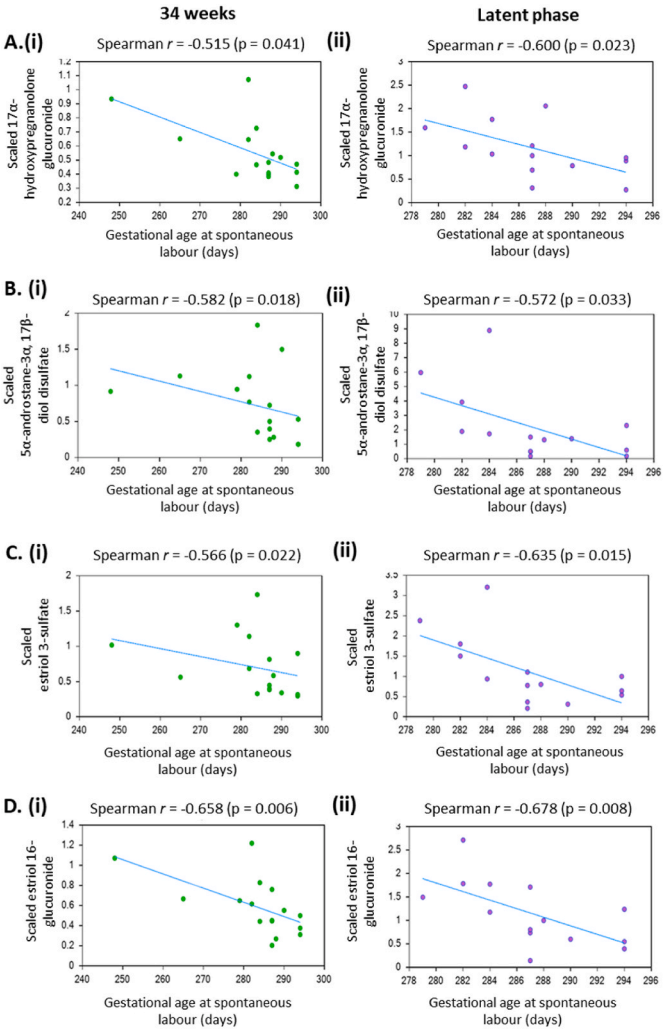

Fig. 1. Scatter plots showing correlation between gestational age at birth and scaled metabolite at 34 weeks' gestation and latent phase, for: A. 17α-hydroxypregnanolone glucuronide at (i) 34 weeks' gestation and (ii) latent phase; B. 5α-androstan-3α, 17β-diol disulfate at (i) 34 weeks' gestation and (ii) latent phase; C. estriol 3-sulfate at (i) 34 weeks' gestation and (ii) latent phase; and D. estriol 16-glucuronide at (i) 34 weeks' gestation and (ii) latent phase. Spearman's correlation with p-value 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.

3.3. Changes in the metabolomic profile of the different vascular components between the different onsets of labour

Latent phase, CA, CV, and IV samples were obtained from four participants with SL, four with IOL, and four who birthed via elCS, all at term. One-way ANOVA analysis indicated no statistical differences between the three groups regarding age, BMI, gravidity, parity, estimated-blood-loss at birth, and birthweight. There was a significant difference between GA at birth ($p = 0.03$), expected as participants who spontaneously laboured did so after 40 weeks' gestation, whereas all elCSs occurred prior to 40 weeks', as per national guidance [31].

Ten percent (104/1032) of the metabolites measured were significantly ($p \leq 0.05$) different between the CA of the SL and IOL groups (ST6), and 11.0% (113/1032) significantly different between the CV samples (ST7). This is in comparison with only 3.1% (32/1032) significantly different between the LP samples (ST8), and 2.9% (30/1032) significantly different between the IV samples (ST9). The greatest difference for all was a higher mean concentration of Ranitidine in the IOL group: two IOL participants had epidurals and administered Ranitidine to reduce aspiration risk in case of general anaesthetic, as per local protocol. Fig. 2 presents these results as volcano plots, and ST10 and ST11 present those metabolites which significantly at least doubled or halved with SL as the comparison group. Most differences were seen between the ECS and SL groups, which is not surprising given the physiological difference between labour and no labour. Interestingly, although both the IOL and SL groups experienced uterine contractions and vaginal births, there were differences in more than 10% of the metabolites measured in the CA and CV.

3.4. Metabolite differences in the fetoplacental circulation between induction of labour, elective caesarean section (no labour), and spontaneous labour

3.4.1. Latent phase

Regarding the LP samples, between the IOL and SL groups, only 1.8% (19/1032) were significantly lower (1.3% (14/1032) at least halved), and 1.3% (13/1032) significantly increased (0.9% at least doubled), in the IOL group compared with the SL group. Between the ECS and SL groups, 1.1% (11/1032) metabolites significantly increased in the ECS group (0.2% at least doubled), and 7.4% (76/1032) were significantly lower (5% at least halved) (Fig. 2, ST10 and ST11).

3.4.2. Intervillous blood

Regarding the IV samples, only Ranitidine and its metabolite Ranitidine N-oxide were significantly increased in the IOL group compared with the SL group; and 2.7% (28/1032) metabolites were significantly lower (1.4% at least halved). Between the ECS group and the SL group, 1.7% (18/1032) were significantly higher in the ECS group (1.3% at least doubled), and 9.0% (93/1032) were significantly lower (4.9% at least halved), including corticosterone, cortisone, estriol-3-glucuronide, and testosterone sulfate (Fig. 2, ST10 and ST11).

3.4.3. Cord artery

Regarding the CA samples, between the IOL and SL groups, 8.5% (88/1032) metabolites were significantly lower (with 4.4% at least halving), and 1.5% (16/1032) significantly higher (with 0.7% at least doubling), in the IOL group compared with the SL group. Those metabolites which significantly at least halved in the IOL group compared with the SL group include 1-stearoyl-GPS (18:0), inosine 5′-mono phosphate, 1-stearoyl-2-oleoyl-GPS (18:0/18:1), androsterone sulfate, and sphinganine-1-phosphate. The seven metabolites which at least doubled in the CA of the IOL group compared with the SL group were five fibrinogen peptide metabolites, adenosine 5′-monophosphate (AMP), and Ranitidine. Between the ECS and SL groups, 1.9% (20/1032) metabolites were significantly higher (1.6% at least doubled) and 20.5% (212/1032) were significantly lower (15.4% at least halved) in the ECS group (Fig. 2, ST10 and ST11).

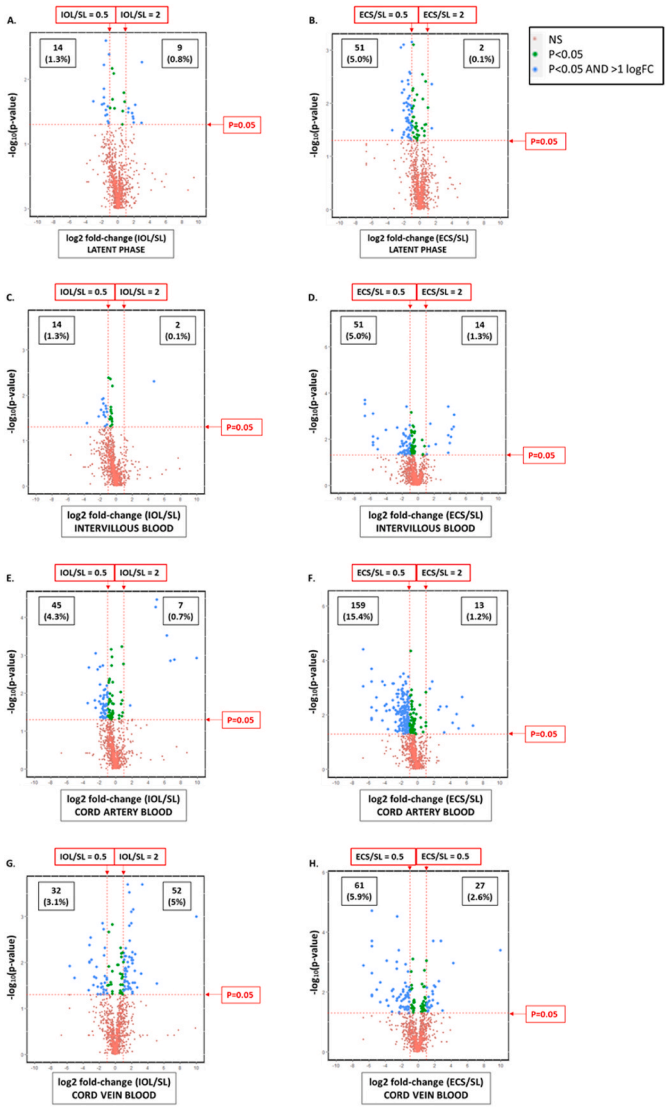

Fig. 2. Volcano plots illustrating the mean $\log_2$-fold change for each of the 1032 metabolites between mode of labour onset for each of the vascular compartments measured: **A.** Induction of labour (IOL) compared with spontaneous labour (SL) for maternal latent phase (LP) samples; **B.** elective caesarean section (ECS) compared with SL for maternal LP samples; **C.** IOL compared with SL for intervillous (IV) blood samples; **D.** ECS compared with SL for IV blood samples; **E.** IOL compared with SL for cord artery (CA) samples; **F.** ECS compared with SL for CA samples; G. IOL compared with SL for cord vein (CV) samples; H. ECS compared with SL for CV samples. Horizontal dashed red line indicates $p = 0.05$, above which differences between the two groups are significant; two vertical red dashed lines indicate halving and doubling between the two groups. Dot colour scheme: red = non-significant; green = significant difference smaller than a doubling or halving; blue = significant doubling or halving.

3.4.4. Cord vein

Regarding the CV samples, 4.1% (42/1032) of the metabolites were significantly lower (3.0% at least halved), and 6.9% (71/1032) higher (5.0% at least doubled), in the IOL group compared with the SL group. Those that at least doubled include oestriol and progesterone, 17alpha-hydroxyprogesterone, and arachidonate (20:4n6). Those that were significantly at least halved in the CV of the IOL group compared with the SL group included corticosterone and cortisol, sphingosine, sphinganine, adenosine 3′,5′-cyclic monophosphate, and cytidine 5′-

monoposphate. Between the ECS and SL groups, 4.8% (50/1032) metabolites were significantly increased (2.6% at least doubled) in the ECS samples compared with the SL samples, and 7.7% (79/1032) were significantly lower (5.9% at least halved), including cortisol, corticosterone, estrone 3-sulfate, sphingosine and sphinganine (Fig. 2, ST3, ST10, and ST11).

3.4.5. Comparing steroids and fatty acids between vascular compartments

The heatmaps of Table 2 illustrate differences in the normalised concentrations of progesterone, oestriol, 17α-hydroxyprogesterone, DHEA-S, sphingosine, sphingosine 1-phosphate, arachidonate and linoleate, in the LP, IV, CA and CV samples across the different MOL onset studied. For all, the LP and IV values were similar for each of the MOL onset. Interestingly, while the CV/CA ratio for each of these metabolites were similar between the IOL and ECS groups, they were reversed for the SL group. For example, while progesterone was significantly lower in the CV than CA in the SL group, with a CV/CA ratio of 0.28, it was significantly higher in the CV than CA of the IOL group, with a CV/CA ratio of 2.23, and non-significantly twice as high in the CV sample than the CA sample in the ECS group, with a CV/CA ratio of 2.23.

3.5. Over-representation analysis

The alpha linolenic acid and linoleic acid metabolic pathway was significantly over-represented in comparisons between the CV of the IOL group and CV of the SL group (Fig. 3). The raw p indicated that this pathway tended to be overrepresented between the CA of the ECS and SL group, and between the CA of the IOL group and SL group, although not statistically significant.

4. Discussion

This study provides the first detailed analysis of the maternal, CA and CV metabolome with different MOL onset and birth, demonstrating specific metabolite changes within the fetoplacental circulation associated with spontaneous labour.

4.1. Steroids

There were no differences in maternal steroid concentrations in the LP samples among the different MOL onset, confirming the previously described lack of detectable steroid changes in maternal blood prior to parturition. Further, it is well known that progesterone is a major placental product [8,18], and for all MOL onset and birth, progesterone concentrations were consistently higher in the IV samples than the CA and CV samples. Importantly, while the CV/CA progesterone ratios of the IOL and ECS groups reflect a passive gradient of progesterone from the placenta, this ratio was reversed in the SL group, suggesting active fetal production, and oestriol concentrations followed the same pattern. These findings indicate differences in placental and fetal production of steroids associated with spontaneous labour which do not occur with IOL, despite the intervening events of uterine contractions and cervical dilatation of both spontaneous and induced labour. Thus, iatrogenic IOL does not replicate the physiological signals that originate in the fetus with spontaneous labour, and this may explain the higher incidence of complications, such as failure to progress in labour and emergency caesarean section, associated with IOL [42]. Moreover, the actions of progesterone on the myometrium include relaxation, inhibition of gap junction formation, and blockage of oxytocin responses [43]. The shift in

Table 2
Heat maps comparing scaled normalised mean values of progesterone, oestriol, 17α-hydroxyprogesterone, DHEA-S, sphingosine, sphingosine 1-phosphate, arachidonate (20:4n6) and linoleate (18:2n6) between the spontaneous labour 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..

A.	SL		IOL		ECS	
Sample	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
DHEAS	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
Arachidonate (20:4n6)	1.2	6.6	1.5	4.4	0.8	6.6
Linoleate (18:2n6)	2.2	2.3	2	2.3	1	2.4

B.	SL		IOL		ECS	
Sample	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
DHEAS	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
Arachidonate (20:4n6)	0.6	1.8	1.6	1.1	1.6	0.8
Linoleate (18:2n6)	0.2	1.1	1	0.4	0.5	0.2

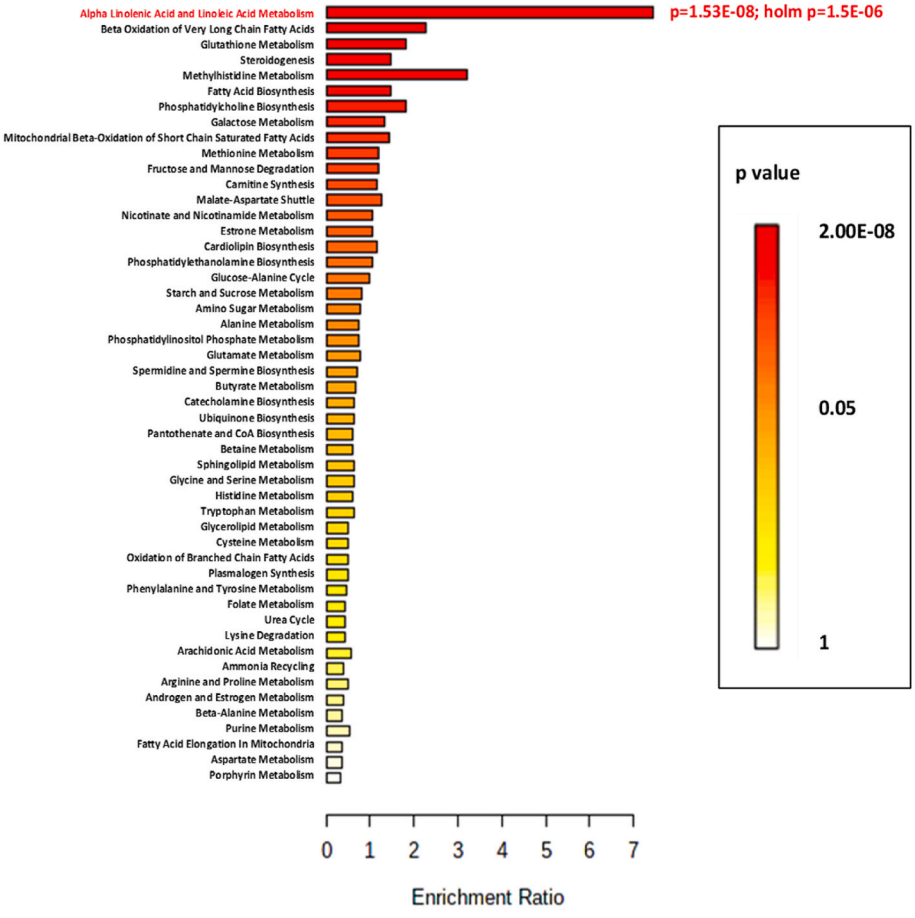

Fig. 3. Over-representation enrichment analysis using MetaboAnalyst software [38] for metabolites significantly different ($p \leq 0.05$) between the cord vein (CV) of the induction of labour (IOL) group and the CV of the spontaneous labour (SL) group, with metabolite pathways on the y-axis, and enrichment ratio on the x-axis. The colour of the bar represents the unadjusted p value (see key). Only the Alpha Linolenic Acid and Linoleic Acid Metabolism pathway analysis reached significance ($p = 1.53E-08$; Holm p 1.5E-06), with 11 out of the 19 metabolites of the pathway significantly different between the two groups (expected 1.48/19): linoleic acid (HMDB00673), arachidonic Acid (HMDB01043), docosapentaenoic acid (22n-6) (HMDB01976); eicosapentaenoic acid (HMDB01999); tetracosahexaenoic acid (HMDB02007); docosahexaenoic acid (HMDB02183); adrenic acid (HMDB02226); docosapentaenoic acid (HMDB01976); and stearidonic acid (HMDB06547).

progesterone CV/CA ratios between the SL group and the IOL and ECS groups presented here may reflect a reduction in progesterone production by the placenta associated with spontaneous labour. This could be perceived as a local progesterone withdrawal by the myometrium, even if maternal progesterone concentrations remain high.

Oestriol is formed via 16 hydroxy DHEA-S, produced by the fetal adrenal glands and liver upon stimulation with ACTH and placental corticotrophin releasing hormone (CRH). In contrast, oestradiol is produced from conversion of oestrone produced from DHEA-S from both the maternal and fetal adrenal glands [10,44]. Oestriol is an inhibitor of oestradiol at low concentrations but becomes an agonist when the ratio of oestriol to oestradiol exceeds 10 to 1, creating an oestrogenic environment suggested to favour the onset of spontaneous labour [45]. ERα appears to be the oestrogen receptor through which the uterotonic actions of oestrogen are driven, and the spliced ERα receptor variant ERΔ7 dominantly suppresses the uterotonic actions of oestrogen and is withdrawn at term in an oestrogen-dependent manner, proposed to contribute to the loss of myometrial quiescence at term [15,44]. Our findings show that while the mean concentrations of maternal oestriol did not significantly change during pregnancy, there were changes in the ratio of oestrogen to progesterone between the different types of labouring groups, and between the different vascular compartments, with an increase in oestriol in CA in the SL group that may produce a localised oestrogenic environment required for spontaneous labour.

4.2. Sphingosine and ceramide

Both sphingosine and sphingosine 1-phosphate concentrations were greater in the CA of the SL and the IOL groups than the ECS group, with no such difference detected in the LP or IV samples. Sphingosine 1-phosphate and ceramide play essential but often opposing roles in cellular signalling and metabolism: sphingosine 1-phosphate stimulates proliferation, cell survival and tissue regeneration while ceramide is involved in apoptosis and stress-related responses [46–49]. In smooth muscle cells, Ca^{2+} mobilised via sphingosine 1-phosphate promotes an increase in cAMP and protein kinase A activation (a different response to that in other cells), resulting in disassembly of actin and relaxation. Moreover, protein kinase C activates sphingosine kinase which increases levels of sphingosine 1-phosphate [49]. Drugs which increase cAMP temporarily suppress myometrial contractions, although this effect wears off after approximately 24–48 h [50]. In the present study, sphingosine concentrations were greater in the CV of the SL group than the CV of both the IOL and ECS groups, which could indicate that the placenta produces more sphingosine with spontaneous labour than with IOL. The higher concentrations of sphingosine and sphingosine 1-phosphate observed in the SL group suggest a role for this pathway in the regulation of uterine activity during labour. This could be via fine-tuning of the contraction/relaxation cycles in myometrial cells through changes in Ca^{2+} mobilization from internal stores, cAMP production, and actin assembly [49,51]. Some of these changes may result from 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 IOL or no labour (elCS). Increased sphingosine 1-phosphate production could be one of the fetal signals for spontaneous labour through stimulating uterine smooth muscle contractions.

4.3. Linoleic acid

The alpha linolenic acid and linoleic acid pathway was significantly over-represented among those metabolites which were different between the CV of the IOL and SL groups. Linoleic acid is a polyunsaturated fatty acid (PUFA) and has become the most common n-6 PUFA in the human diet. Linoleate is the conjugate base of linoleic acid [52,53]. Elongation and desaturation of linoleic acid results in production of arachidonic acid [53], a precursor to prostaglandins as well as other eicosanoid mediators such as leukotrienes and thromboxanes, which in turn are associated with inflammatory processes [53]. Arachidonic acid concentrations within the amniotic fluid increases with labour [54], and there is enhanced conversion by amnion cells resulting in an increase in prostaglandin E2 [55] with labour. This process is stimulated by exposure to fetal surfactant, a key indicator of fetal lung maturation [56]. Our results show that arachidonate levels increased between 28WG and latent phase, and were significantly lower in the LP samples of the ECS group than the SL group. Further, both arachidonate and linoleate were higher in the CA than CV in the SL group compared to both the IOL and ECS groups. The availability of these precursors will be important for activation of prostaglandin pathways in spontaneous labour.

4.4. Limitations

Although the number of participants is relatively small, clear differences in metabolite concentrations across MOL groups are identified, demonstrating suitability and reliability of the methodological approach. Ethical and clinical considerations, including not disturbing mother-baby bonding, make sampling of maternal and fetal circulations around birth challenging. While future minimally invasive sampling technologies may improve options, IV sampling currently provides non-invasive access to maternal blood immediately following birth; however, IV may not exactly represent circulating maternal blood as syncytiotrophoblast and fetal diffusion/transport products will temporarily be at higher local concentrations until redistributed [23,57]. Differences in medication among MOL groups may also influence the metabolome.

4.5. Summary

In summary, this study highlights important differences in the ratio of key metabolites between the CA and CV sampled from participants who spontaneously laboured and those who were either induced or had an elCS. We provide detailed information about the maternal metabolome in late pregnancy and at birth which indicates that fetal maturation and changes in steroid metabolic pathways in the fetoplacental unit are important for human parturition. Importantly, our findings indicate that IOL does not reproduce the pathways activated in spontaneous labour. This may contribute to the higher risk for adverse pregnancy outcomes associated with IOL, as without the appropriate signals from the fetus, the myometrium may not contract as efficiently, and the uterine cervix may not undergo the essential changes necessary for spontaneous labour.

Author contributions

KB, GW, and ALB conceived and designed the study. KB performed the study, including recruitment, collection and processing of samples, analysis, and writing first draft. GW and ALB revised and contributed to subsequent drafts. All approved final manuscript.

Sources of funding

This work was supported by Above and Beyond, Bristol [2018-Spr-08].

Declaration of competing interest

The authors declare that they participated in the design, analysis, writing and editing of the study and that all have seen and approved the final version. They have no conflicts of interest.

Acknowledgements

We gratefully acknowledge the participants for taking part. We thank Metabolon, Inc., including Greg Michelotti, Brian Ingram, and Jose Martins.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2022.10.005.

References

[1] C.K. Ratajczak, L.J. Muglia, Insights into parturition biology from genetically altered mice, Pediatr. Res. 64 (6) (2008) 581–589.
[2] A. Lopez Bernal, Overview. Preterm labour: mechanisms and management, BMC Pregnancy Childbirth 7 (Suppl 1) (2007) S2.
[3] G.C. Sharp, J.L. Hutchinson, N. Hibbert, T.C. Freeman, P.T.K. Saunders, J. E. Norman, Transcription analysis of the myometrium of labouring and non-labouring women, PLoS One 11 (5) (2016), e0155413 e0155413.
[4] A. Lopez Bernal, Mechanisms of labour–biochemical aspects, BJOG An Int. J. Obstet. Gynaecol. 110 (Suppl 20) (2003) 39–45.
[5] R. Smith, Parturition, N Engl J Med 356 (3) (2007) 271–283.
[6] B.F. Mitchell, M.J. Taggart, Are animal models relevant to key aspects of human parturition? Am. J. Physiol. Regul. Integr. Comp. Physiol. 297 (3) (2009) R525–R545.
[7] R.L. Goldenberg, J.F. Culhane, J.D. Iams, R. Romero, Epidemiology and causes of preterm birth, Lancet 371 (9606) (2008) 75–84.
[8] M.A. Costa, The endocrine function of human placenta: an overview, Reprod. Biomed. Online 32 (1) (2016) 14–43.
[9] S. Mesiano, Yen and Jaffe's Reproductive Endocrinology (Eighth Edition): Chapter 11 - Endocrinology of Human Pregnancy and Fetal-Placental Neuroendocrine Development, eighth ed., Elsevier, Philadelphia, 2019.
[10] E. Diczfalusy, Endocrine functions of the human fetoplacental unit, 2005, Am. J. Obstet. Gynecol. 193 (6) (1964) 2024. discussion 2025.
[11] T. Zakar, F. Hertelendy, Progesterone withdrawal: key to parturition, Am. J. Obstet. Gynecol. 196 (4) (2007) 289–296.
[12] Z. Stanfield, P.F. Lai, K. Lei, M.R. Johnson, A.M. Blanks, R. Romero, M.R. Chance, S. Mesiano, M. Koyuturk, Myometrial transcriptional signatures of human parturition, Front. Genet. 10 (2019) 185.
[13] G.C. Liggins, Premature parturition after infusion of corticotrophin or cortisol into foetal lambs, J. Endocrinol. 42 (2) (1968) 323–329.
[14] G.C. Liggins, Premature delivery of foetal lambs infused with glucocorticoids, J. Endocrinol. 45 (4) (1969) 515–523.
[15] P. Anamthathmakula, C. Kyathanahalli, J. Ingles, S.S. Hassan, J.C. Condon, P. Jeyasuria, Estrogen receptor alpha isoform ERdelta7 in myometrium modulates uterine quiescence during pregnancy, EBioMedicine 39 (2019) 520–530.
[16] Y. Sugimoto, A. Yamasaki, E. Segi, K. Tsuboi, Y. Aze, T. Nishimura, H. Oida, N. Yoshida, T. Tanaka, M. Katsuyama, K. Hasumoto, T. Murata, M. Hirata, F. Ushikubi, M. Negishi, A. Ichikawa, S. Narumiya, Failure of parturition in mice lacking the prostaglandin F receptor, Science (New York, N.Y.) 277 (5326) (1997) 681–683.
[17] W.J. Honnebier, D.F. Swaab, The influence of anencephaly upon intrauterine growth of fetus and placenta and upon gestation length, J. Obstet. Gynaecol. Br. Commonwealth 80 (7) (1973) 577–588.
[18] A.I. Csapo, E. Knobil, H.J. van der Molen, W.G. Wiest, Peripheral plasma progesterone levels during human pregnancy and labor, Am. J. Obstet. Gynecol. 110 (5) (1971) 630–632.
[19] J.C. Condon, D.B. Hardy, K. Kovaric, C.R. Mendelson, 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. 20 (4) (2006) 764–775.
[20] S. Mesiano, E.C. Chan, J.T. Fitter, K. Kwek, G. Yeo, R. Smith, Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium, J. Clin. Endocrinol. Metabol. 87 (6) (2002) 2924–2930.
[21] A. López Bernal, The regulation of uterine relaxation, Semin. Cell Dev. Biol. 18 (3) (2007) 340–347.
[22] C.A. Hudson, A. Lopez Bernal, Phosphorylation of proteins during human myometrial contractions: a phosphoproteomic approach, Biochem. Biophys. Res. Commun. 482 (4) (2017) 1393–1399.
[23] K.A. Birchenall, G.I. Welsh, A. Lopez Bernal, 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. Publ. Health 16 (9) (2019).
[24] S. Travers, L. Martinerie, P. Boileau, M. Lombès, E. Pussard, Alterations of adrenal steroidomic profiles in preterm infants at birth, Archives of disease in childhood, Fetal and neonatal edition 103 (2) (2018) F143–F151.
[25] S.-Y. Oh, R. Romero, S.-S. Shim, J.S. Park, J.K. Jun, B.H. Yoon, Fetal plasma cortisol and dehydroepiandrosterone sulfate concentrations in pregnancy and term parturition, J. Matern. Fetal Neonatal Med. : Offl. J. Europ. Assoc. Perinatal Med. Feder. Asia Oceania Perinatal Soc. Int. Soc. Perinatal Onstetricians 19 (9) (2006) 529–536.
[26] B.E. Murphy, 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, Am. J. Obstet. Gynecol. 144 (3) (1982) 276–282.
[27] M.-C. Alexandre-Gouabau, F. Courant, T. Moyon, A. Küster, G. Le Gall, I. Tea, J.-P. Antignac, D. Darmaun, Maternal and cord blood LC-HRMS metabolomics reveal alterations in energy and polyamine metabolism, and oxidative stress in very-low birth weight infants, J. Proteome Res. 12 (6) (2013) 2764–2778.
[28] U.E. Rolle-Kampczyk, J. Krumsiek, W. Otto, S.W. Röder, T. Kohajda, M. Borte, F. Theis, I. Lehmann, M. von Bergen, Metabolomics reveals effects of maternal smoking on endogenous metabolites from lipid metabolism in cord blood of newborns, Metabolomics 12 (2016), 76-76.
[29] R.G. Farquharson, A.I. Klopper, Progesterone concentrations in maternal and fetal blood, Br. J. Obstet. Gynaecol. 91 (2) (1984) 133–137.
[30] E.J. Shaxted, V.M. Heyes, M.P. Walker, P.V. Maynard, Umbilical-cord plasma progesterone in term infants delivered by caesarean section, Br. J. Obstet. Gynaecol. 89 (1) (1982) 73–76.
[31] National Institute for Clinical and Health Excellence, Inducing Labour Clinical Guideline CG70, 2008.
[32] D.M. Nelson, G.J. Burton, A technical note to improve the reporting of studies of the human placenta, Placenta 32 (2) (2011) 195–196.
[33] A. Grignaffini, E. Soncini, S. Anfuso, E. Ronzoni, Dinoprostone: slow release vaginal insert (Propess) and intracervical gel (Prepidil) for the induction of labour with unriped cervix, Minerva Ginecol. 56 (5) (2004) 413–418.
[34] D.C. Montrose, X.K. Zhou, L. Kopelovich, R.K. Yantiss, E.D. Karoly, K. Subbaramaiah, A.J. Dannenberg, Metabolic profiling, a noninvasive approach for the detection of experimental colorectal neoplasia, Cancer Prev. Res. 5 (12) (2012) 1358–1367.
[35] T.P. Hiltunen, J.M. Rimpela, R.P. Mohney, S.M. Stirdivant, K.K. Kontula, Effects of four different antihypertensive drugs on plasma metabolomic profiles in patients with essential hypertension, PLoS One 12 (11) (2017), e0187729.
[36] S.Y. Shin, E.B. Fauman, A.K. Petersen, J. Krumsiek, R. Santos, J. Huang, M. Arnold, I. Erte, V. Forgetta, T.P. Yang, K. Walter, C. Menni, L. Chen, L. Vasquez, A. M. Valdes, C.L. Hyde, V. Wang, D. Ziemek, P. Roberts, L. Xi, E. Grundberg, M. Waldenberger, J.B. Richards, R.P. Mohney, M.V. Milburn, S.L. John, J. Trimmer, F.J. Theis, J.P. Overington, K. Suhre, M.J. Brosnan, C. Gieger, G. Kastenmuller, T.D. Spector, N. Soranzo, An atlas of genetic influences on human blood metabolites, Nat. Genet. 46 (6) (2014) 543–550.
[37] C.D. Dehaven, A.M. Evans, H. Dai, K.A. Lawton, Organization of GC/MS and LC/MS metabolomics data into chemical libraries, J. Cheminf. 2 (1) (2010) 9.
[38] A. Marco-Ramell, M. Palau-Rodriguez, A. Alay, S. Tulipani, M. Urpi-Sarda, A. Sanchez-Pla, C. Andres-Lacueva, Evaluation and comparison of bioinformatic tools for the enrichment analysis of metabolomics data, BMC Bioinf. 19 (1) (2018), 1-1.
[39] T. Nguyen, C. Mitrea, S. Draghici, Network-Based approaches for pathway level analysis, Curr. Protoc. Bioinformatics 61 (1) (2018), 8.25.1-8.25.24.
[40] A. Cambiaghi, M. Ferrario, M. Masseroli, Analysis of metabolomic data: tools, current strategies and future challenges for omics data integration, Briefings Bioinf. 18 (3) (2017) 498–510.
[41] J. Chong, O. Soufan, C. Li, I. Caraus, S. Li, G. Bourque, D.S. Wishart, J. Xia, MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis, Nucleic Acids Res. 46 (W1) (2018) W486–W494.
[42] N.M. Mealing, C.L. Roberts, J.B. Ford, J.M. Simpson, J.M. Morris, Trends in induction of labour, 1998-2007: a population-based study, Aust. N. Z. J. Obstet. Gynaecol. 49 (6) (2009) 599–605.
[43] L.R. Brancazio, A.P. Murtha, R.P. Heine, Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate, N. Engl. J. Med. 349 (11) (2003) 1087–1088.
[44] R. Smith, T. Butler, E.-C. Chan, Do estrogen receptor variants explain the enigma of human birth? EBioMedicine 39 (2018) 25–26.
[45] R. Smith, J.I. Smith, X. Shen, P.J. Engel, M.E. Bowman, S.A. McGrath, A.M. Bisits, P. McElduff, W.B. Giles, D.W. Smith, Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor, J. Clin. Endocrinol. Metabol. 94 (6) (2009) 2066–2074.
[46] F. Parveen, D. Bender, S.-H. Law, V.K. Mishra, C.-C. Chen, L.-Y. Ke, Role of ceramidases in sphingolipid metabolism and human diseases, Cells 8 (12) (2019) 1573.
[47] V. Filippov, M.A. Song, K. Zhang, H.V. Vinters, S. Tung, W.M. Kirsch, J. Yang, P. J. Duerksen-Hughes, Increased ceramide in brains with Alzheimer's and other neurodegenerative diseases, J. Alzheimers Dis. 29 (3) (2012) 537–547.
[48] R. Laaksonen, K. Ekroos, M. Sysi-Aho, M. Hilvo, T. Vihervaara, D. Kauhanen, M. Suoniemi, R. Hurme, W. März, H. Scharnagl, T. Stojakovic, E. Vlachopoulou, M.-L. Lokki, M.S. Nieminen, R. Klingenberg, C.M. Matter, T. Hornemann, P. Jüni, N. Rodondi, L. Räber, S. Windecker, B. Gencer, E.R. Pedersen, G.S. Tell, O. Nygård, F. Mach, J. Sinisalo, T.F. Lüscher, Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol, Eur. Heart J. 37 (25) (2016) 1967–1976.
[49] S. Spiegel, O. Cuvillier, L.C. Edsall, T. Kohama, R. Menzeleev, Z. Olah, A. Olivera, G. Pirianov, D.M. Thomas, Z. Tu, J.R. Van Brocklyn, F. Wang, Sphingosine-1-phosphate in cell growth and cell death, Ann. N. Y. Acad. Sci. 845 (1998) 11–18.
[50] P.F. Lai, R.M. Tribe, M.R. Johnson, Differential impact of acute and prolonged cAMP agonist exposure on protein kinase A activation and human myometrium contractile activity, J. Physiol. 594 (21) (2016) 6369–6393.
[51] K. Itagaki, C.J. Hauser, Sphingosine 1-phosphate, a diffusible calcium influx factor mediating store-operated calcium entry, J. Biol. Chem. 278 (30) (2003) 27540–27547.
[52] C.M. Mulligan, G.C. Sparagna, C.H. Le, A.B. De Mooy, M.A. Routh, M.G. Holmes, D. L. Hickson-Bick, S. Zarini, R.C. Murphy, F.Y. Xu, G.M. Hatch, S.A. McCune, R. L. Moore, A.J. Chicco, Dietary linoleate preserves cardiolipin and attenuates mitochondrial dysfunction in the failing rat heart, Cardiovasc. Res. 94 (3) (2012) 460–468.
[53] N. Shrestha, S.L. Sleep, T.J. Helman, O.J. Holland, J.S. Cuffe, A.V. Perkins, A. J. McAinch, J.P. Headrick, D.H. Hryciw, Maternal diet high in linoleic acid alters offspring fatty acids and cardiovascular function in a rat model, Br. J. Nutr. (2021) 1–37.
[54] M.J. Keirse, B.R. Hicks, M.D. Mitchell, A.C. Turnbull, Increase of the prostaglandin precursor, arachidonic acid, in amniotic fluid during spontaneous labour, Br. J. Obstet. Gynaecol. 84 (12) (1977) 937–940.
[55] P.R. Bennett, D. Slater, M. Sullivan, M.G. Elder, G.E. Moore, Changes in amniotic arachidonic acid metabolism associated with increased cyclo-oxygenase gene expression, Br. J. Obstet. Gynaecol. 100 (11) (1993) 1037–1042.
[56] A. Lopez Bernal, G.E. Newman, P.J. Phizackerley, A.C. Turnbull, Surfactant stimulates prostaglandin E production in human amnion, Br. J. Obstet. Gynaecol. 95 (10) (1988) 1013–1017.
[57] H. Kaipe, J. Raffetseder, J. Ernerudh, M. Solders, E. Tiblad, MAIT cells at the fetal-maternal interface during pregnancy, Front. Immunol. 11 (2020) 1788.