Pregnancy following Bariatric Surgery:

Maternal Considerations

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This thesis is dedicated to my husband, Tochukwu Kanu

Abstract

Background

The mechanism behind the perinatal complications associated with obesity in pregnancy is not fully understood. There is convincing evidence that pregnancies following bariatric surgery have a lower incidence of gestational diabetes (GDM), pre-eclampsia (PE), large for gestational age (LGA) neonates, higher incidence of small for gestational age (SGA) neonates and moderately preterm birth. The mechanism for this is also unknown, however, could be related to changes in maternal insulin resistance and other metabolic pathways involved in glucose and fat metabolism.

Aims

1. To investigate the effects of bariatric surgery on maternal insulin resistance, waist to hip ratio (WHR), blood pressure and components of fat and glucose metabolism such as adipokines, pro-inflammatory hormones, incretins and metabolites.

2. To compare the lipoprotein profile of obese women and women with a normal BMI in the third trimester, without previous bariatric surgery.

Method

We conducted a prospective, longitudinal study comparing pregnant women with previous bariatric surgery to those without surgery. The following were assessed:

1. Insulin, glucose, glycosylated haemoglobin (HbA1c), Homeostasis Model Assessment of insulin resistance (HOMA-IR) and the Matsuda Index were measured using fasting blood samples collected at 28 weeks gestation.

2. Maternal weight, height, waist to hip ratio (WHR) and blood pressure were measured at all antenatal visits.

3. Fasting blood samples at 28+0-30+0 weeks' gestation were used to measure peptide hormones, adipokines, pro-inflammatory hormones and incretins.

4. Untargeted metabolomics with proton Nuclear Magnetic Resonance (H1 NMR) was performed on samples obtained at six time points: 11+0-14+0 (T1), 20+0-24+0 (T2), 28+0-30+0 (T3), 30+0-33+0 (T4) and 35+0-37+6 (T5) weeks' gestation, and within 72 hours of delivery (T6).

H1 NMR lipoprotein profiling was performed for pregnant women recruited without previous bariatric surgery at 28+0-30+0 weeks' gestation. Results were compared between women with normal BMI and women who were obese (BMI ≥ 30kg/m2).

Results

The no surgery group had higher median insulin resistance (IR), [2.20 (IQR 1.53-3.38)] compared to the post bariatric surgery group [1.15 (IQR 1.04 -2.07); p <0.05] and post malabsorptive bariatric surgery group, [1.08 (0.99 – 1.23; p <0.05].

Pregnant women with previous bariatric surgery had significantly lower leptin levels at 28-30 weeks [13.3ng/ml (IQR 9.71-15.36)] compared to women with no surgery [20.84ng/ml (IQR 18.12-24.1); p<0.05].

Maternal adiponectin levels at 28-30 weeks of gestation were higher in the post bariatric women [4.9µg/ml (IQR 2.9-6.7)] compared to no surgery women [2.43 µg/ml (IQR 1.8-3.2); p <0.05].

Pregnant women with previous malabsorptive bariatric surgery had an altered serum metabolome by T4 (30-33 weeks) and T5 (35-37 weeks) compared to those without bariatric surgery (p=0.027 and p=0.006, respectively). There is a lower serum level of unsaturated lipids, isobutyrate, leucine, isoleucine and N-acetyl glycoprotein and higher level of glutamine and D-ß-hydroxybutyrate.

The lipoprotein profile of women at 28 weeks gestation without surgery showed that, compared to women with normal BMI, obese women have higher levels of HDL4 Triglyceride (p=0.02) VLDL1 Phospholipid (p=0.023) and VLDL1 Cholesterol (p=0.02) and lower levels of HDL, HDL1 cholesterol (p=0.02, 0.02), LDL2, LDL3 cholesterol (p=0.03, 0.02) and HDL1 phospholipid (p=0.03).

Conclusion

The study has demonstrated that women with previous bariatric surgery have a reduction in insulin resistance, especially post malabsorptive surgery. In the third trimester, they have a lower leptin and higher adiponectin level. These findings may explain the reduced incidence of GDM and LGA babies seen in this group.

Statement of Originality

I, Chidimma Chinyerem Kanu, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, this has been appropriately indicated and referenced.

Copyright Declaration

Acknowledgements

'Lord, you establish peace for us; all that we have accomplished you have done for us.' Isaiah 26:12. The Holy Bible, New International Version.

Almighty God, Heavenly Father, You have shown me once again that with you, all things really are possible. There are no words to express my gratitude for all that you have done and continue to do in my life. This work is a testament of your loving kindness and abundant grace. Thank you Lord.

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List of Conference presentations

1. Maric T, **Kanu C**, Johnson M, Savvidou M.
   Maternal insulin resistance and neonatal profile in pregnancies following bariatric surgery.
   Fetal Medicine 17th World Congress, Athens, Greece, June 2018

2. West K, **Kanu C**, Maric T, Johnson M, Holmes E, Savvidou M.
   Maternal and neonatal metabolomic profile in pregnancies following bariatric surgery.
   Fetal Medicine 17th World Congress, Athens, Greece, June 2018

3. Maric T, **Kanu C**, Muller D, Tzoulaki I, Savvidou M.
   Fetal growth and feto-placental circulation in pregnancies following bariatric surgery.
   BMFMS, Brighton, UK, April 2018

4. Maric T, **Kanu C**, Muller D, Tzoulaki I, Savvidou M.
   Birthweight, intra-uterine fetal growth pattern and feto-placental Dopplers of pregnancies following bariatric surgery.
   ISUOG, Vienna, Austria, September 2017

5. Maric T, **Kanu C**, Johnson M, Savvidou M.
   Bariatric Surgery: Effect on insulin Resistance
   Fetal Medicine 16th World Congress, Ljubljana, Slovenia, June 2017

6. Maric T, **Kanu C**, Muller D, Tzoulakis I, Savvidou M.
   Birthweight, intra-uterine fetal growth pattern and feto-placental Dopplers of pregnancies following bariatric surgery.
   Fetal Medicine World Congress, Ljubljana, Slovenia, June 2017

7. **Kanu C**, Maric T, Johnson M, Savvidou M
   Maternal Insulin Resistance post bariatric surgery.
   BMFMS, Amsterdam, The Netherlands, March 2017

8. Maric T, **Kanu C**, Johnson M, Savvidou M.
   Insulin Resistance in neonates of post bariatric surgery women
   BMFMS, Amsterdam, The Netherlands, March 2017

List of Publications

1. Fetal fractional limb volumes in pregnancies following bariatric surgery.
   Maric T, **Kanu C**, Mandalia S, Johnson MR, Savvidou MD.
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2. Longitudinal metabolic and gut bacterial profiling of pregnant women with previous bariatric surgery.
   West KA, **Kanu C (joint first authorship)**, Maric T, McDonald JAK, Nicholson JK, Li JV, Johnson MR, Holmes E, Savvidou MD.
   Gut. 2020 Aug;69(8):1452-1459.

3. Maternal, neonatal insulin resistance and neonatal anthropometrics in pregnancies following bariatric surgery.
   Maric T, **Kanu C**, Johnson MR, Savvidou MD.
   Metabolism. 2019 Aug;97:25-31.

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Abbreviations

ADP	Adenosine Diphosphate
AGB	Adjustable gastric band
Apo A1/A2/B100	Apolipoprotein A1/A2/B100
ART	Assisted reproductive techniques
AT1	Angiotensin type 1 receptor
ATP	Adenosine Triphosphate
BCAA	Branched chain amino acids
BPD/DS	Biliopancreatic diversion/duodenal switch
BMI	Body mass index
BPL	Extended biliopancreatic limb
BPD	Biliopancreatic diversion
BIB	Bio enteric intragastric balloon
BS	Bariatric Surgery
BW	Birth weight
CHD	Congenital heart defect
CMACE	Centre for Maternal and Child Enquiries
CRP	C reactive protein
CS	Caesarean Section
CVD	Cardiovascular Disease
DJB-SG	Duodenal-Jejunal Bypass with sleeve gastrectomy
DM	Diabetes Mellitus
DSIT	Diverted sleeve gastrectomy with ileal transposition

EFW	Estimated fetal weight
FFA	Free fatty acids
GB	Gastric Band
GBP	Gastric Bypass
G-6-P / DH	Glucose 6 phosphate / Dehydrogenase
GDM	Gestational Diabetes Mellitus
GH	Growth Hormone
GIT	Gastrointestinal tract
GLP -1	Glucagon-like peptide – 1
GLP - 2	Glucagon-like peptide – 2
GnRH	Gonadotrophin Releasing Hormone
GS	Gastric sleeve
GT	Glucose tolerance
HAPO	Hyperglycaemia and Adverse pregnancy Outcome
Hba1c	Glycosylated Haemoglobin
HDL	High density lipoprotein
HDL-C	High density lipoprotein - cholesterol
H1 NMR	Proton Nuclear Magnetic Resonance
HOMA – IR	Homeostatic Model Assessment of Insulin resistance
HOMA – B	Homeostatic Model Assessment of Beta cell function
HPLC	High performance liquid chromatography
HPO axis	Hypothalamic Pituitary Ovarian axis
HMW	High Molecular Weight
Hs-CRP	High sensitivity C reactive Protein
HT	Hypertension

ICAM-1	Intercellular adhesion molecule -1
IGF-1	Insulin growth factor - 1
IGFBP	Insulin growth factor binding protein
IGT	Impaired glucose tolerance
IL-6	Interleukin - 6
IOL	Induction of labour
IR	Insulin resistance
IUGR	In utero growth restriction
LAGB	Laparoscopic adjustable gastric band
LBW	Low birth weight
LCGP	Laparoscopic greater curvature plication
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein - cholesterol
LGA	Large for gestational age
LPS	Lipopolysaccharide
LSG	Laparoscopic sleeve gastrectomy
MAL	Malabsorptive bariatric surgery
MATLAB	MATrix LABoratory
MBRRACE	Mothers and Babies, Reducing Risk through Audits and Confidential Enquiries across the UK
MCP-1	Monocyte Chemoattractant Protein-1
MDC	Multidisciplinary diabetes care
MGB	Mini gastric bypass
MMP-9	Matrix metallopeptidase - 9
MMT	Mixed meal tolerance test

MO	Morbidly Obese
MS	Malabsorptive surgery
MS	Mass Spectrometry
NAD	Nicotinamide adenine dinucleotide
NBS	No bariatric surgery
NGT	Normal glucose tolerance
NICE	National Institute of Health and Clinical Excellence
NICU	Neonatal intensive care unit
NO	Nitric Oxide
NS	Non-significant
NTD	Neural tube defect
NVD	Normal vaginal delivery
OASI	Obstetric anal sphincter injury
OGTT	Oral glucose tolerance test
OPLS-DA	Orthogonal projections to latent structures discriminant analysis
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor type-1
PCA	Primary Component Analysis
PCOS	Polycystic ovarian syndome
PE / PET	Preeclampsia
PIH	Pregnancy induced hyperrtension
PPH	Postpartum haemorrhage
PROM	Preterm rupture of membranes
PTH	Parathyroid hormone
PTX3	Pentraxin-3

PYY	Peptide YY
RANTES	Regulated upon activation, normal T cell expressed and secreted
RES	Restrictive bariatric surgery
RBC	Red blood cell
RBP4	Retinol binding protein 4
RCOG	Royal College of Obstetricians and Gynaecologists
RYGB(P)	Roux en Y Gastric Bypass
SAT	Subcutaneous adipose tissue
SCBU	Special care baby unit
SFlt-1	Soluble fms (Feline McDonough Sarcoma) -like tyrosine kinase
SGA	Small for Gestational Age
SG	Sleeve gastrectomy
SHBG	Sex hormone binding globulin
sICAM	Soluble intracellular adhesion molecule-1
SIMCA	Soft Independent Modelling of Class Analogy
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
TNF-a	Tumour necrosis factor alpha
TRACP 5a	Tartrate-resistant acid phosphatase 5a
TSP	3-trimethyl-silyl-[2,2,3,3-2H4]propionic acid
VAT	Visceral adipose tissue
VLCD	Very low calorie diet
VLDL	Very low density lipoprotein
VSG	Vertical sleeve gastrectomy
VTE	Venous thromboembolism

CHAPTER 1

Introduction

1.1 OBESITY: GENERAL OVERVIEW

Obesity is defined as abnormal or excessive fat accumulation that presents a risk to health. (1) The World Health Organisation recommends the use of body mass index (BMI), which is weight (in kilograms) divided by the square height (in metres), as a population-level measure of obesity. A person with a BMI equal to or more than 25 is considered overweight and one with BMI of 30kg/m^2 or more is classified as obese (Table 1.1).

Table 1.1: World Health Organisation (WHO) classifications of obesity. (2)

WHO CLASSIFICATION	**BODY MASS INDEX (BMI), kg/m^2**
Underweight	<18.5
Normal Weight	18.5 – 24.9
Overweight	25.0 -29.9
Obese	≥ 30
Obese Class I	30.0 – 34.9
Obese Class II	35.0- 39.9
Obese Class III	≥ 40

In 2016, 650 million adults were obese worldwide, making this a global public health issue with a worldwide mortality rate of 2.8 million deaths per annum. (3) According to a report by the UK Health Forum, by 2034, 70% of adults are expected to be overweight or obese. (4)

In the UK, managing the consequences of obesity is estimated to cost the NHS more than £5 billion/annum, a major burden on a service already financially stretched. (5)

Obesity is a risk factor for chronic diseases such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) including hypertension, dyslipidaemia, stroke and coronary heart disease. (6) The aetiology of several cancers are linked to obesity

including; endometrial, breast (postmenopausal), oesophageal adenocarcinoma, colorectal, prostate, and renal. (7,8)

There is an increased incidence of gallstones, non-alcoholic fatty liver disease and gastro-oesophageal reflux disease in the obese population. In severe cases, the respiratory system is also compromised, resulting in obstructive sleep apnoea. (9,10)

Although not life-threatening, quality of life is greatly disrupted by chronic musculoskeletal conditions caused by obesity such as osteoarthritis, particularly in the knee. (11)

It has also proved detrimental to mental health with a higher prevalence of depression in the obese population. (12) This has led to additional demands on social care resources.

1.2 OBESITY IN PREGNANCY

The 2016-2018 report from Mothers and Babies, Reducing Risk through Audits and Confidential Enquiries across the UK (MBRRACE-UK) states that almost half of women who died (55%) were either overweight or obese. (13)

In England, 56% of women of childbearing age are either overweight (BMI 25–29.9 kg/m²) or obese (BMI ≥ 30 kg/m²). (14) The Royal College of Obstetricians and Gynaecologists (RCOG) have produced a joint guideline with the Centre for Maternal and Child Enquiries (CMACE) addressing the issue of obesity in the pregnant population stating that 1 in 3 pregnant women in the UK are overweight or obese. (15)

Obesity is strongly associated with subfertility. This detrimental effect on reproductive capability is well documented, however, the pathophysiology is not fully understood.

Proposed aetiology in obese women points to a disruption of the hypothalamic-pituitary-ovarian (HPO) axis, oocyte function and endometrium. (16) Increased peripheral aromatization of androgens to oestrogens causes reduced gonadotrophin releasing hormone (GnRH) production by negative feedback mechanisms. Increased insulin resistance (IR) and hyperinsulinaemia reduces liver production of sex hormone binding globulin (SHBG) resulting in hyperandrogenaemia. There is also a reduction in growth hormone (GH) and Insulin-like growth factor binding protein (IGFBP) levels and higher leptin levels. (16,17) These metabolic alterations have a negative impact

on the HPO axis and ovarian function. Clinically, this presents as irregular menstrual cycles, sub-fertility and polycystic ovarian syndrome. Despite this, some obese women have ovulatory cycles and the aetiology of their subfertility is still uncertain. (18)

Assisted reproductive techniques (ART) have highlighted possible endometrial dysfunction in obese women which may be responsible for lower pregnancy rates after embryo transfer compared to normal BMI women. (19) The exact mechanism is unknown and confounded by the influence of ovarian steroidogenesis. Obesity is associated with oocyte abnormalities including an altered ovarian follicular environment, with increased levels of insulin, glucose, C-reactive protein and lactate; increased androgen activity; and decreased human chorionic gonadotropin levels. (20) However, the impact of these differences is not yet known.

If a successful pregnancy is achieved it is classified as a high risk pregnancy requiring increased surveillance, monitoring and senior specialist input. The risks of obesity in pregnancy can be sub-divided into those encountered in the antenatal, intra-partum and postnatal period.

1.2.1 Antenatal Risks

Miscarriage

Women with a BMI of ≥ 25 kg/m^2 have significantly higher risk of miscarriage regardless of the method of conception. (21) Although this association between obesity and miscarriage is widely documented in the literature, the pathophysiology is not well defined. Proposed mechanisms focus on the negative impact of obesity on the endometrium affecting implantation or embryo quality affecting early pregnancy development or both. (22)

It is conceivable that insulin resistance (IR) plays a role since the incidence of spontaneous miscarriage has been reported to rise as IR increases. (23) Hyperinsulinaemia causes a reduction in GH and Insulin-like growth factor protein-1 (IGFBP) levels. IGFBP-1 appears to facilitate adhesion processes at the fetal-maternal interface therefore reduced levels of it would interfere with implantation. (24) Jakubowicz et al. adds that there is a reduction in Glycodelin which plays a role in inhibiting the endometrial immune response to the embryo, thus rendering the embryo vulnerable to the maternal immune system.

Increased production of inflammatory and pro-thrombotic agents produced by adipose tissue or released from the endothelium may also play an important part. It has been suggested that plasminogen activator inhibitor type 1 (PAI-1) is associated with increased rates of miscarriage in association with maternal obesity by inducing villous thrombosis. (25) Several studies have used metformin, which increases insulin sensitivity and decreases the PAI-1 levels successfully to reduce the high miscarriage rates in women with polycystic ovarian syndrome (PCOS); a condition also associated with obesity. (26,27,28)

Congenital malformations

Maternal obesity is strongly linked to a higher incidence of neural tube defects (NTD) and congenital heart defects (CHD). (29,30) Hyperglycaemia in pregnant diabetics is responsible for congenital defects probably due to altered lipid metabolism, oxidative stress and activation of apoptosis. (31)

Obese women should be informed that there is the potential for poor ultrasound visualisation of the baby and consequent difficulties in fetal screening and surveillance for anomalies, as per RCOG guidelines.

The RCOG recommends the use of high dose folic acid (5mg) in obese pregnant women starting at least one month before conception and continuing during the first trimester of pregnancy to reduce the risk of the first occurrence, as well as the recurrence, of NTDs (relative risk (RR) 0.28, 95% CI [confidence interval] 0.13–0.58). (32) Folate deficiency seen in obesity may be due to poor dietary choices and lack of compliance. Although not associated with congenital malformations, obese pregnant women are also at increased risk of vitamin D deficiency. The RCOG recommends preventing this with 10mcg vitamin D daily throughout pregnancy and while breast feeding.

Stillbirth

The risk of stillbirth is directly proportional to maternal BMI. A systematic review showed that women with BMI of 40 have twice the risk of stillbirth compared to women with normal BMI. (33) Observational studies including both overweight and obese women show that the risk of stillbirth in both groups can be as high as 40%. (34,35)

Yao et al conducted a retrospective population based study including 2,868,482 singleton births. Obesity was associated with nearly 25% of the stillbirths that occurred between 37 and 42 weeks' gestation. (36)

The pathophysiology for this increased risk is not known. Proposed mechanisms include placental dysfunction and inflammation, IR and hyperlipidaemia. (37,38)

Macrosomia

Macrosomia describes fetal growth greater than or equal to a high birthweight, regardless of gestation.(39) An internationally accepted definition is yet to be determined. Most studies define macrosomia as a birth weight ≥ 4000g and some use 4500g as the cut-off. (40)

Results from a meta-analysis in 2014 showed that maternal obesity is associated with macrosomia, defined as birth weight ≥ 4000 g (odds ratio [OR] 2.17, 95% CI 1.92, 2.45), birth weight ≥4500 g (OR 2.77,95% CI 2.22, 3.45) and birth weight ≥90th percentile for gestational age (OR 2.42, 95% CI 2.16, 2.72). (41) The main concern of macrosomia, similar to when it occurs in gestational diabetic mothers, is the increased risk of Caesarean section (CS) delivery, shoulder dystocia with concomitant obstetric anal sphincter injury (OASI), neonatal bone fracture and/or nerve palsy or perinatal death. (42)

Maternal obesity is associated with increased IR, which promotes fetal hyperglycaemia and hyperinsulinaemia, which in turn drives the excessive growth. (42,43) Also, placental lipases metabolise maternal triglycerides (TG) to free fatty acids (FFA) that cross the placenta in excess to the growing fetus. (45)

Gestational Diabetes

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance resulting in hyperglycaemia of variable severity with the onset of first recognition during pregnancy. (46) According to the National Institute of Health and Clinical Excellence (NICE), approximately 700,000 women give birth in England and Wales each year, and up to 5% (35,000) have either pre-existing or gestational diabetes. (47)

There is convincing evidence that obesity in pregnancy is associated with an increase in the incidence of GDM. Pregnancy alone is an insulin-resistant condition (40-50% rise in serum insulin levels) which is potentially compounded by increased pre-conceptual IR in obese women. (48) A meta-analysis by Chu et al. included 20 studies and showed the unadjusted odd ratios (ORs) of developing GDM were 2.14 (95% CI 1.82-2.53), 3.56 (3.05-4.21) and 8.56 (5.07-16.04) among overweight, obese and severely obese women respectively, compared with normal-weight pregnant women. (49) A retrospective cohort analysis of 22,351 women showed inter-pregnancy BMI gain was associated with an increased risk of GDM in the second pregnancy (OR 1.71 [95% CI 1.42-2.07] for gaining 1.0-1.9 BMI units; OR 2.46 [95% CI 2.00-3.02] for 2.0-2.9 BMI units; and OR 3.40 [95% CI 2.81-4.12] for 3.0 or more BMI units). It also showed that weight loss was associated with a reduced GDM risk in overweight and obese women (OR 0.26 [95% CI 0.14-0.47] for the loss of at least 2.0 BMI units).(50)

As mentioned previously, increased fetal insulinaemia is a growth factor leading to fetal macrosomia which can result in increased risk of CS and birth trauma (vaginal tears, shoulder dystocia and asphyxia). The Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study showed that maternal obesity and GDM combined have a greater adverse effect on pregnancy than either alone. The OR for birth weight >90th percentile for GDM alone was 2.19 (1.93–2.47), for obesity alone 1.73 (1.50–2.00), and for both GDM and obesity 3.62 (3.04–4.32). Results for primary CS delivery, preeclampsia, cord C-peptide and neonatal percent body fat >90th percentile were similar. (51) In view of the above, it is recommended that all pregnant women with a booking BMI ≥30 should undergo a 75g oral glucose tolerance test (OGTT) between 24-28wks gestation, to diagnose GDM. (52)

Pre - eclampsia

NICE defines pre-eclampsia (PE) as new onset hypertension (systolic bloods pressure [BP] ≥ 140mmHg and diastolic BP ≥ 90mmHg) presenting after 20 weeks gestation with significant proteinuria (urinary protein: creatinine ratio › 30mg/mmol or 24-hour urine protein › 300mg). (53)

According to the recent MBRRACE report the death rate from PE in the UK remains low at 0.18 per 100,000 maternities. However, it continues to be a significant contributor to maternal and perinatal morbidity and mortality worldwide. High pre-

pregnancy BMI is an independent risk factor for PE although the mechanisms are not clear. (54)

Mbah et al. conducted a large population-based retrospective study and included 854,085 singleton births. A stepwise increase in the rates of PE with increasing BMI class was reported (Figure 1.1). (55)

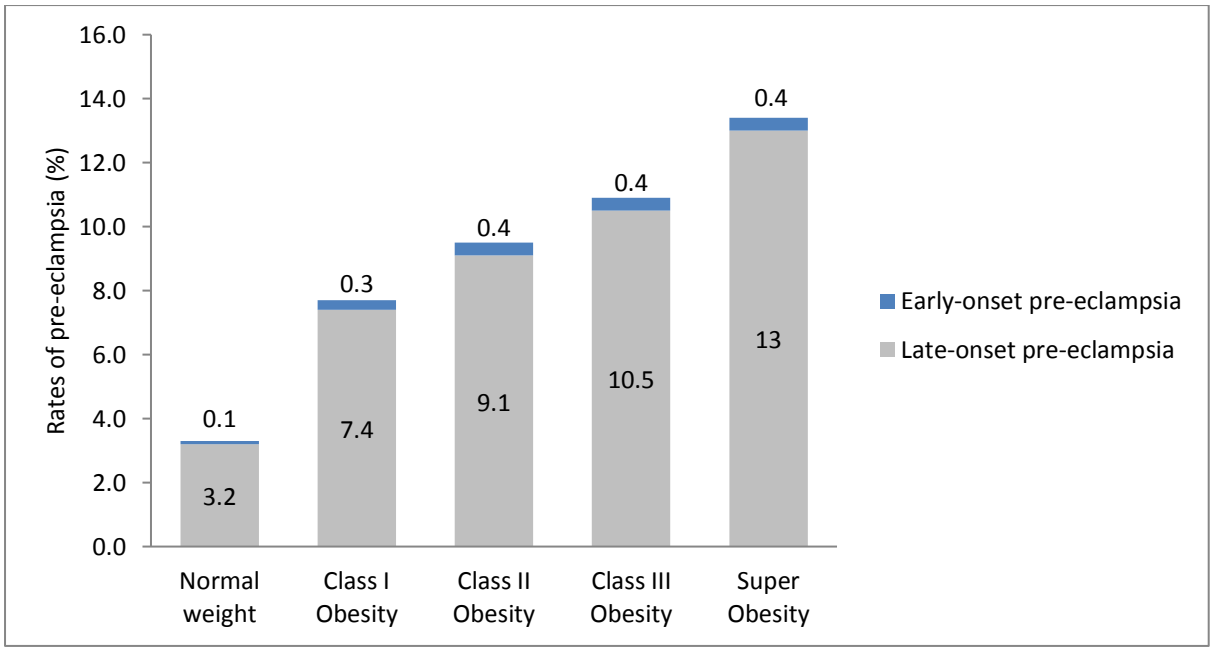


Figure 1.1: Increasing obesity increases the risk for PE. Grey bar (bottom) represents percentage incidence of late onset PE. Blue bar (top) represents percentage incidence of early-onset PE. In normal weight gravidas the incidence of late onset PE is 3.2%; In Class I obese gravidas the incidence of late onset PE is 7.4%; In class II obese gravidas the incidence of late onset PE is 9.1%; In class III obese gravidas the incidence of late onset PE 10.5%; In super-obese women the incidence of late onset is PE 13%. (55)

Obesity is a state of chronic inflammation and there is increasing evidence suggesting that abnormal maternal immune and inflammatory responses act as a mediator of pathological cascades leading to PE. Specifically, activated macrophages and natural killer cells within the uterus and placenta and activation in the peripheral T helper cells producing cytokines, such as tumour necrosis factor– alpha (TNF-α), Interleukin-6 and 17 (IL-6 and IL-17), and the anti-angiogenic factor soluble fms-like tyrosine kinase type-1 (sFlt-1) and B cells producing the agonistic autoantibodies to the angiotensin type 1 receptor (AT1-aa) have been implicated. (56) Other hormonal and biochemical pathways adversely affected by obesity such as IR and dyslipidaemia have also been implicated in the pathophysiology of PE. (56,57)

1.2.2. Intrapartum Risks

Slow labour

Obese nulliparous women have a prolonged first stage of labour. (59) Nuthalapaty et al conducted a secondary analysis of 360 nulliparous women enrolled in a prospective observational study of a labour induction protocol. Women in the highest weight quartile (103 to 193 kg) took 5 hours longer to reach full dilation after oxytocin initiation compared with women in the lowest weight quartile ($p < 0.001$). (60) Metabolic changes associated with obesity may be responsible for disrupting progression in labour. Zhang et al. proposed that hypercholesterolaemia, a common finding in obesity, changes intracellular calcium, thus affecting myometrial contractility. (61) Obesity is also associated with elevated levels of visfatin and leptin, released from adipose tissue, which can inhibit uterine contractions resulting in prolonged first stage of labour. (61,62)

Difficult Monitoring

Uterine contractions can be assessed by either manual palpation or using external toco-dynamometry. In obese women this can be a challenge due to the attenuating effect of excess subcutaneous fat. This issue is particularly important when assessing slow progress and considering oxytocin augmentation. Maternal obesity can also hinder adequate monitoring of the fetal heart rate in labour. NICE recommends that uncomplicated pregnancies do not require continuous external fetal monitoring, however, these women are at high risk of conditions such as GDM and PE that would warrant such monitoring.(64) The RCOG therefore recommends recourse to fetal scalp electrode or ultrasound assessment of the fetal heart activity if necessary.

Difficult regional Anaesthesia

General anaesthesia in obese women has the increased risk of failed intubation and gastric aspiration, which are both life threatening complications. (64,65) The loss of bony landmarks can lead to difficulties with regional anaesthesia resulting in multiple

attempts and can result in a patchy, uneven spread. (67) The RCOG advises an early epidural in obese women depending on the clinical scenario.

Shoulder Dystocia

The incidence of shoulder dystocia is significantly higher in obese women. (67,68) Avci et al. investigated 931 pregnant women and found that shoulder dystocia affected 0.4% of women with normal BMI and 6.8% of obese women (≥30 kg/m^2). (70) Diabetes and macrosomia, both more common in obese pregnant women, also increase the risk for shoulder dystocia.

Caesarean Section

Obese women face a higher risk of CS. As previously mentioned, prolonged first stage of labour and macrosomia are some of the factors contributing to this risk. A meta-analysis of 11 cohort studies showed the crude pooled OR (95% CI) for CS in overweight, obese and morbidly obese women were 1.53 (95% CI 1.48- 1.58), 2.26 (95% CI 2.04- 2.51) and 3.38 (95% CI 2.49- 4.57) respectively. The pooled OR of having an emergency CS were 1.64 (95% CI 1.55- 1.73) in overweight and 2.23 (95% CI 2.07-2.42) in obese women. (71)

Obesity is a risk factor for unsuccessful vaginal birth after Caesarean section with a higher risk for uterine rupture and neonatal injury. (72) Operative deliveries in obese women are technically more difficult requiring the presence of experienced clinicians. The post-operative period can also be challenging. Obese women have a higher risk of wound infection and dehiscence despite prophylactic antibiotics. (73) Negative pressure wound therapy, alternative skin closure techniques (interrupted sutures) and early post-operative mobilisation are all methods considered for combating this problem. (73,74)

Postpartum Haemorrhage

Obesity is an independent risk factor for major postpartum haemorrhage (PPH ≥1000ml). (76) Fyfe et al. performed a cohort study of 11,363 nulliparous singleton pregnancies and found PPH rates were increased in overweight and obese compared

with normal-weight women (n=255 [9.7%], n=233 [15.6%]), n=524 [7.2%]; p <.001) respectively. Being obese was a risk factor for major PPH following both caesarean (OR=1.73 (95% CI 1.32-2.28) and vaginal delivery (OR: 2.11 (95% CI 1.54-2.89)). (77) The RCOG recommends active management of the third stage of labour in obese women since there is strong evidence that it reduces the risk of PPH, postpartum anaemia and the need for blood transfusion.

1.2.3 Postnatal Risks

Thromboembolism

Pregnancy is a hyper-coagulable state due to increased clotting factors, venous stasis and vascular damage (Virchow's triad). (78) Obesity compounds the effects of pregnancy thus significantly increasing thrombotic risk. The MBRRACE-UK report states that thrombosis and thromboembolism continue to be the leading cause of direct maternal deaths occurring within 42 days of delivery with a rate of 1.13 per 100,000 maternities (95% CI 0.74-1.65).

Blondon et al. conducted a population-based, case-control study including 4,497 cases and found that, compared to women with a normal pre-pregnancy BMI, overweight and obese women had an 1.5- and 1.8-4 fold greater risk of postpartum venous thromboembolism, respectively, with greatest risks in women with class III obesity. (79) The RCOG recommends that all obese women should be considered for prophylactic low molecular weight heparin in doses appropriate for their weight for 10 days after delivery. (80)

Breast feeding

Obese women are less likely to initiate breastfeeding and more likely to breastfeed for shorter duration and introduce solid foods to their infants earlier than normal weight women. (80,81) Proposed reasons for this low uptake may relate to the type of delivery as many undergo an operative delivery requiring recovery time in a high dependency unit. Under those circumstances a lack of privacy, breastfeeding support and skin-to-skin initiation has been reported.(83) Breastfeeding reduces the risk of childhood obesity, which is between 2.4 and 2.7 times higher in the offspring of obese women and further increased by GDM. (83, 84,85,86)

The complications of obesity in pregnancy are addressed by a multidisciplinary team approach. This should consist of a consultant obstetrician, a consultant anaesthetist and senior midwives throughout the pregnancy. The RCOG recommends that women with a booking BMI ≥30 should be referred to a consultant obstetrician for the opportunity to discuss the risks and how they can be minimised. Women with a BMI ≥35 should give birth in a consultant-led obstetric unit with appropriate neonatal services as per the NICE guidelines.

Overall, eliminating obesity is the ultimate challenge and primary goal of all healthcare professionals. The MBRRACE-UK report has recommended further research into the most effective way to encourage obese women to normalise their weight before conception in order to reduce the risk associated with obesity in pregnancy.

1.3 BARIATRIC SURGERY

To date, the problem of obesity has been resistant to traditional weight management programmes. The non-surgical approach to weight loss is multifaceted involving dietary changes to reduce energy intake; behavioural therapy; increasing physical activity and occasionally pharmacotherapies. (88)

A recent UK Government initiative, supported by the Department of Health to tackle rising levels of Obesity, is called Change4Life and aims to improve diet and fitness levels amongst the UK population giving practical advice on the NHS website (http://www.nhs.uk/change4life). Despite these efforts, there is overwhelming evidence from meta-analysis of studies and Cochrane review of randomised controlled trials that bariatric surgical intervention is superior to non-surgical management. They conclude that bariatric surgery is a more cost effective and sustainable treatment of severe obesity than non-surgical measures after two years. (87,88,89)

1.3.1 Classification of Bariatric Surgery procedures

Bariatric surgery procedures are classified according to surgical technique and mechanism of action: restrictive, malabsorptive or a combination of both (Figure 1.2).

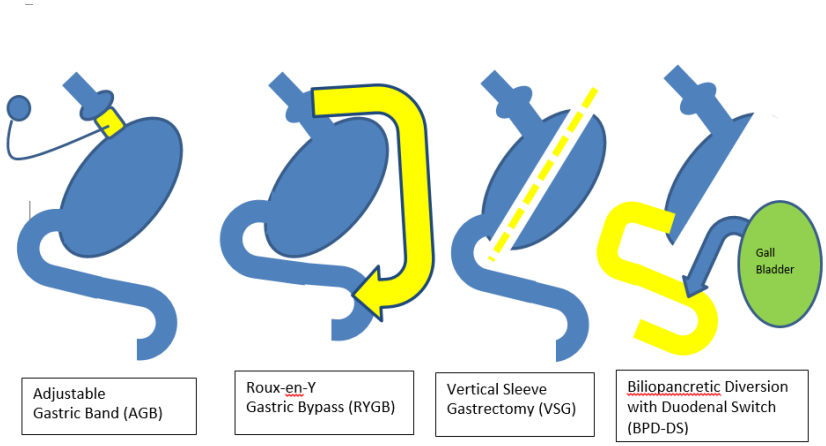


Figure 1.2: Types of Bariatric Surgery.

Restrictive Bariatric surgery procedures

This includes adjustable gastric band and vertical sleeve gastrectomy which limit food intake by reducing stomach capacity.

Adjustable Gastric band (AGB) surgery was first introduced in the 1970s. It involves the insertion of an inflatable silicone band around the fundus of the stomach creating a small pouch with an adjustable opening. Food entering this pouch signals the release of satiety signals transmitted to the satiety centres in the hypothlalmus via the vagus nerve. (91) This response is perpetuated by continuous pressure of the band and delayed passage of food to the lower part of the stomach. There is no increase in gastric emptying. Weight is lost as a result of increased satiety and appetite control with smaller amounts of food.

Advantages

The AGB has the advantage of being adjusted (non-surgically) as patients lose weight. This ensures that it is not too tight, preventing the passage of food or too loose

rendering it ineffective. A saline solution is infiltrated into a subcutaneous access port which is attached to the abdominal wall. Laparoscopic insertion (LAGB) is associated with a short inpatient stay and faster recovery. (92) It has a lower surgical complication risk and, since the rest of the gastrointestinal tract (GIT) is unaltered, malabsorption is rarely encountered. The band can be removed or replaced laparoscopically. The stomach returns to a normal pre-operative size and function such that patients often regain weight. However, the procedure is not entirely reversible due to residual adhesions and scarring.

Disadvantages

Compared to other types of bariatric surgery, the weight loss achieved is low, 40% of excess body weight. (92,93) Although the early complication rate is minimal, AGB can be associated with some late complications. There is a risk of band slippage or erosion requiring surgical repositioning or removal. (95) Dilatation of the gastric pouch and/or oesophagus caused by AGB can lead to obstruction or dysmotility. (96) Access ports can become painful, leak and be displaced. (97) Both the AGB and access port can act as a nidus for infection and be associated with an intra-abdominal abscess.

Follow up

Regular follow-up is required, especially in the first few months postoperative. These assessments ensure that the band is appropriately filled and the patient is adhering to a modified diet and exercise regime. There are no standardised dietary guidelines for patients post operatively. (98) In general, patients are encouraged to eat a balanced, healthy diet of solid foods that require a degree of chewing. This food would pass through the stomach pouch slowly and avoid the high calorie intake a liquid diet could cause.

Vertical Sleeve Gastrectomy (VSG) describes the surgical removal of 80% of the stomach. Laparoscopic surgeons resect along the greater curvature, starting from the antrium (5-6cm from the pylorus) to the fundus, close to the cardia. (99) A tubular, banana-shaped stomach pouch or sleeve now acts as the conduit for food intake. It is approximately 150ml in size after the procedure. (100) There is evidence that gastro-intestinal hormones such as ghrelin, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are affected by VSG as a result of the reduction in gastric tissue (especially the

fundus), increase in gastric emptying and intestinal transit. (101) There is a decrease in ghrelin, responsible for appetite, and a rise in GLP-1 which promotes insulin secretion, inhibits gastric emptying, glucagon secretion and hepatic glucose production. (101,102) A significant increase in PYY is reported in the literature which promotes satiety, inhibits gastric and pancreatic secretion, attenuates gallbladder contraction and also slows gastric emptying. (104) PYY also causes increased absorption of nutrients in the ileum. (101) These hormonal effects promote more weight loss than reduced calorie intake alone.

Advantages

A greater weight loss is achieved compared to the AGB (>50% of excess body weight) which is similar to bypass procedures. (105)

Disadvantages

The main drawbacks are the irreversible nature of the procedure and higher surgical risks e.g. sleeve leakage (1-3%), strictures or torsion.(106) There is a higher incidence of gastro-oesophageal reflux,(107) which is rarely experienced in AGB and occasionally managed by recourse to gastric bypass.

Follow up

Like AGB, the remaining gastro-intestinal tract is unaltered but there is still potential for vitamin deficiencies such as zinc, vitamin D3, folic acid, iron and vitamin B12. (108) It is therefore advised that the nutritional status is assessed pre- and post-operatively to enable targeted multivitamin therapy. (108)

Malabsorptive Bariatric surgery procedures

Biliopancreatic Diversion with Duodenal Switch (BPD/DS) predominantly works by malabsorption although there is a mild restrictive component. This makes it the most effective bariatric surgery procedure for weight reduction and combating diabetes. (105) Despite this, it only accounts for 2% of bariatric surgeries worldwide. (109) The surgical procedure has two parts: the first is almost identical to the VSG procedure

resulting in a tubular stomach pouch. The duodenum is then divided distal to the pyloric sphincter and the distal portion of the small intestine attached to the outlet of the newly modified stomach. Bile and pancreatic enzymes are transported via the bypassed section of small intestine to the distal small bowel where it is reattached. (110)

Advantages

Since two-thirds of the small intestine is by-passed, significant weight loss is achieved (≥ 60 – 70% excess weight) by malabsorption of calories and nutrients, particularly protein and fat. (111) BDP/DS also enhances the levels of GLP-1 and PYY altering appetite, increasing satiety and improving glycaemic control. (112)

Disadvantages

BDP/DS has lost popularity due to concerns about the high risk of malnutrition and deficiencies in essential vitamins, particularly fat-soluble vitamins (A, D, E & K). Bloating and frequent, loose stools are common adverse side effects. The need for parenteral/enteral nutritional supplementation is greater in this bariatric surgery group. (113) It is a complex operation with a higher surgical complication rate e.g. sleeve and duodenal anastomosis leakage (1.5%) and a longer inpatient stay compared to other bariatric surgery procedures. (114)

Follow up

Specialist dietician support and nutritional follow up is of paramount importance. Patients require lifelong, daily supplementation of minerals and fat soluble vitamins. (115) Adherence to a high protein diet is advised and compliance is reinforced at each assessment, guided by regular blood tests. (116)

Combination Bariatric surgery procedures

Roux en Y gastric bypass (RYGB) is the most commonly performed bariatric surgery worldwide and considered the gold standard in bariatric surgery. Like BPD/DS there are two parts to the procedure: The stomach is divided and the upper portion creates

a small gastric pouch (< 30mL in volume). Then, the proximal small intestine is divided and the lower portion anastomosed to the gastric pouch (proximal gastro-jejunal anastomosis). Gastric acid and digestive enzymes from the bypassed stomach enter the upper portion of the divided small intestine. This is re-anastomosed further down (distal jejuno-jejunal anastomosis) to enable digestive fluid to mix with food.

Advantages

The gastric pouch restricts food intake and the bypass promotes malabsorption achieving a significant weight loss (60-80% excess weight loss) which is sustained long-term. Increased GLP-1 secretion occurs post RYGB which improves meal-related glycaemia, resulting in improved glycaemic control and reduction in diabetes. Post-operative alterations in leptin, GLP-1, PYY, and ghrelin also reduce appetite and increase satiety. (116,117)

Disadvantages

RYGB is associated with a longer hospital inpatient stay and higher surgical risk. Early complications include anastomotic or staple line leaks (0.4 -5.2%), post-operative haemorrhage (1.9-4.4%), internal hernia (1-9%) and small bowel obstruction. Late complications include gastro-jejunostomy anastomotic strictures (2.9 – 23%), marginal peptic ulceration (1-16%) and gastro-gastric fistulae (1.5-6%). (119)

Follow up

As with BDP/DS, malabsorption, particularly of vitamin B12, iron, calcium, and folate must be actively countered by adherence to strict dietary advice, supplements and regular follow up.

Table 1.2: Comparing different types of bariatric surgical procedures.

	Adjustable Gastric Band (AGB)	**Roux en Y Gastric Bypass (RYGB)**	**Vertical Sleeve Gastrectomy (VSG)**	**Biliopancreatic Diversion with a Duodenal Switch (BPD-DS)**
Classification	Restrictive	Combination	Restrictive	Malabsorptive (Mild restrictive component)
Surgical Procedure	Laparoscopic insertion of inflatable silicone band at the fundus of the stomach.	Laparoscopic division of stomach (upper and lower pouch) and small intestine. Lower portion of small intestine anastomosed to gastric pouch.	Laparoscopic removal of 80% of stomach, along the greater curvature from antrum to the fundus.	Laparoscopic stomach reduction similar to VSG method. The duodenum is divided. The distal small bowel is attached to the outlet of the stomach and the bypassed section of small bowel (duodenum) acts a a conduit for bile and pancreatic enzymes to the distal small bowel where it is reattached.
Mechanism leading to weight loss	↓Food intake. ↑ Satiety	↓Food intake ↑Satiety Malabsorption	↓Food intake. ↑ Satiety ↑ Gastric emptying ↑ Intestinal transit	Malabsorption ↓Food intake ↑Satiety
GI Hormone Changes	↓ Ghrelin ↑ Leptin	↓ Ghrelin ↓ Leptin ↑GLP-1	↓ Ghrelin ↑ GLP-1 ↓ Glucagon	↑ GLP-1, PYY
Advantages	Reversible Adjustable ↓Inpatient stay ↓Recovery time ↓Complication ↓Malnutrition	↑Glycaemic control High weight loss	↑Glycaemic control High weight loss Low malnutrition risk	↑Glycaemic control
Weight Loss	40% excess body weight.	60-80% excess body weight.	50-80% excess body weight.	70% excess body weight
Disadvantages & Complications	Band slippage Band erosion Infection Obstruction	↑Inpatient stay ↑ Surgical risk Infection Obstruction Dumping syndrome Haemorrhage Leaks, Strictures Hernia, fistulae Nutrient deficiencies Reflux & Ulcers	↑ Surgical risk Irreversible Leaks, Strictures Torsion Reflux	↑Inpatient stay ↑ Surgical risk Complex surgery Sleeve and Duodenal leak High malnutrition risk Diarrhoea

1.3.2 Pregnancy following bariatric surgery

There is a recognised increase in the number of women opting for bariatric surgery. During 2014-15, NHS records showed that 6,030 bariatric surgery procedures were recorded in total with 4,590 procedures carried out on women, and approximately *70%* of these women were of childbearing age. (120)(121)

Bariatric surgery is known to reverse the detrimental effects of obesity on fertility and pregnancy outcomes. The dramatic weight loss achieved often removes the need for fertility treatment (which has previously been denied because of obesity) with the majority of women being able to conceive spontaneously after the operation.(122) Weight loss after bariatric surgery improves ovulation and according to work by Jain et al, there is partial recovery of luteal function.(123) As a result, there is a rising cohort of women entering pregnancy following bariatric surgery.

The effect of bariatric surgery on pregnancy outcomes is summarised in the appendix (Supplementary Table 1).

Crucially, there is convincing evidence that pregnancy following bariatric surgery is associated with a significant reduction in the incidence of GDM, large for gestational age (LGA) neonates and PE but higher risk of small for gestational age (SGA) neonates and moderately preterm birth (between 32 completed weeks and 36 weeks and 6 days gestation) compared to women with similar pre-surgery BMI. (124–126)

Yi et al. conducted a meta-analysis of 11 cohort studies. Compared with obese women who had not undergone bariatric surgery, women who had undergone bariatric surgery had significantly lower OR of GDM (OR 0.31; 95% CI 0.15-0.65), hypertensive disorders (OR 0.42; 95% CI 0.23-0.78), and macrosomia (OR 0.40; 95% CI 0.24-0.67). However, their OR of SGA neonates were increased (OR 2.16; 95% CI 1.28-3.66). (127) The subgroup analysis in Galazis et al. meta-analysis reported that in the bariatric surgery group, compared to pre-surgery BMI of the same women as controls, there was a higher incidence of preterm birth (OR 1.51, 95% CI 1.33-1.72; $p<0.001$) and small neonates (OR 2.30, 95% CI 1.53-3.44; $p<0.001$). (128)

The exact mechanisms of these alterations of pregnancy following bariatric surgery are yet unknown.

To date, there are no evidence-based clinical guidelines for health professionals caring for these women nor any standardised pre-conception advice post bariatric surgery. Current practice is based on expert opinion. (129)

1.3.3 Benefits of bariatric surgery

Bariatric surgery has proved to be a popular solution to the obesity epidemic in the non-pregnant population. It has been shown to reduce the incidence of T2DM and CVD as well as achieving dramatic, sustained weight loss, thus improving quality of life. (129,130,131,132) The International Diabetes Federation recommends bariatric surgery for management of T2DM in selected obese patients. (134)

The NICE Obesity guideline states that bariatric surgery should be offered to individuals with a BMI ≥ 40 when other options have failed. (47) It further advises that individuals with BMI 30-35 or more with T2DM diagnosed within 10 years should be considered for assessment. South Asian individuals with T2DM diagnosed at a lower BMI, should also be considered.

1.4 BIOCHEMICAL CHANGES FOLLOWING BARIATRIC SURGERY

1.4.1 Metabolic changes following bariatric surgery

The aetiology of health benefits following bariatric surgery are multifactorial and not credited to altered digestion alone. It is accepted that a metabolic component is responsible for much of the health improvements previously mentioned e.g. reduction in T2DM, HT and hyperlipidemia before any weight is lost. For this reason, bariatric surgery is also known as metabolic surgery.

The physiological effects associated with bariatric surgery have been summarised in the mnemonic BRAVE: **B**ile flow alteration; **R**eduction of gastric size, **A**natomical gut rearrangement and altered flow of nutrients **V**agal manipulation and **E**nteric gut hormone modulation. (135)

An improvement in IR is also likely to play a pivotal role. The role of peptides, adipokines, pro-inflammatory cytokines and incretins in this process is summarised in Figure 1.3.

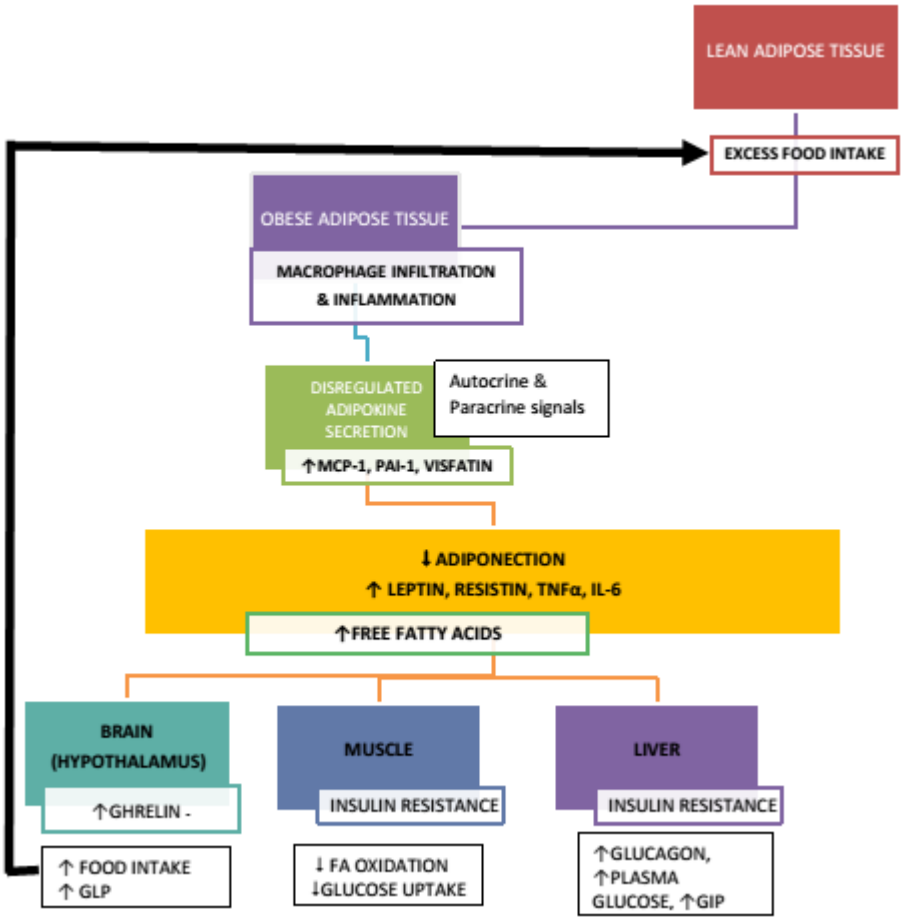


Figure 1.3: Insulin Resistance in Obesity.

Peptides

The main peptide biomarkers in this pathway are C-peptide, insulin, glucagon and ghrelin. The effect of bariatric surgery on peptide hormones is summarised in the appendix (Supplementary Table 2).

C-peptide: A 31 amino acid polypeptide that links the A and B chains of insulin allowing correct folding and inter-chain disulfide bond formation. It is enzymatically cleaved off and co-secreted in equimolar proportion with insulin. (136) C-Peptide has an insulin-mimetic effect and has been used as a surrogate marker of insulin secretion and resistance in studies. The level of C-peptide increases with age, BMI, gestation and parity. (137,138,139) Its levels decrease following any type of bariatric surgery. (140,141,142)

Insulin: A 51 amino acid peptide hormone produced by the beta cells of the pancreas. (143) It regulates the uptake and utilization of glucose and is also involved in protein synthesis and triglyceride storage. Studies show a rise of insulin levels with BMI and decrease with rising parity and following bariatric surgery. (144,145,146,147)

Both insulin and c-peptide levels increase throughout pregnancy. (148) They are linked to maternal complications such as gestational diabetes (GDM), PE and gestational hypertension.(149,150,151) Their levels correlate to fetal complications of poorly controlled GDM which include intrauterine death, neonatal hypoglycaemia and macrosomia. (152)

Glucagon: A 29 amino acid single chain polypeptide hormone produced in the hypothalamus and pancreatic alpha-islet cells. (153) Pancreatic islet glucagon is secreted in response to hypoglycemia with resultant increases in blood glucose concentration by stimulating hepatic glycogenolysis and gluconeogenesis. Glucagon secretion is also stimulated by the incretin hormone glucose-dependent insulinotropic peptide (GIP) and epinephrine and is suppressed by insulin, leptin, amylin and glucagon-like peptide -1 receptor agonist GLP-1. (154) Studies have found that following bariatric surgery, the fasting levels of ghrelin, GLP-1, glucagon, leptin and plasminogen activator inhibitor-1 (PAI-1) are all significantly decreased compared to pre-operative baseline levels. (155)

In pregnancy, glucagon levels rise from 16 to 28 weeks gestation. (156) It is associated with GDM and maternal levels have been linked to the incidence of T2DM in adult offspring. (157)

Ghrelin: A peptide hormone consisting of 28 amino acids with a fatty acid chain modification (octanoyl group) on the third amino acid.(158) It is produced by ghrelinergic cells in the GIT and secreted by A cells in the oxyntic glands of the stomach fundus.(159) There is a pre-prandial (when stomach is empty) rise and postprandial (when stomach is stretched) decrease in ghrelin. The mechanism controlling ghrelin secretion from the stomach is unknown. It acts on hypothalamic

brain cells increasing hunger, gastric acid secretion and GI motility to prepare the body for food intake. (160) Ghrelin also plays a role in regulating the distribution and rate of use of energy. Studies have shown an inverse correlation between ghrelin and advancing age, BMI and following bariatric surgery. (161)

In pregnancy, the level of Ghrelin peaks at the second trimester and decreases at third trimester. (162) It is associated with gestational hypertension and intricately involved with in utero fetal development.(164,165)

Adipokines

There are four adipokines of importance in this metabolic pathway: adiponectin, leptin, visfatin and resistin. The effect of bariatric surgery on adipokines is summarised in the appendix (Supplementary Table 3).

Adiponectin: A fat cell derived hormone with insulin-sensitizing properties (modulating glucose metabolism, anti-diabetic) and a role in lipid metabolism (anti-atherogenic adipokine) in insulin sensitive tissues. (165) Low plasma adiponectin levels are associated with IR as found in obesity (Figure 1.3). Adiponectin is exclusively secreted from adipose tissue. Insulin, amino acids, naicin (vitamin B3) and interleukin 15 (IL-15) cytokine stimulate adiponectin secretion from studied adipocytes. (166) The primary mechanisms by which adiponectin enhance insulin sensitivity appears to be through increased fatty acid oxidation and inhibition of hepatic glucose production. There is a strong negative correlation between plasma adiponectin concentration in humans and fat mass, with the exception of severe cases of undernutrition and in the newborn. (167) Adiponectin increases with age, black ethnicity and bariatric surgery but decreases with smoking and rising BMI. (168,169,170)

In pregnancy, adiponectin decreases from the second trimester with the lowest levels in the third trimester. (171) There is an inverse association with maternal BMI which is linked to maternal complications such as GDM, LGA babies and increased risk of future T2DM. (172,173)

Leptin: A 167–amino acid hormone secreted mainly by adipocytes. (174) It is also derived from the gastric mucosa and placenta. Insulin is believed to regulate leptin secretion through a post-transcriptional mechanism in the short term, and via glucose metabolism in the long term. Glucocorticoids, serotonin, insulin and oestrogen have also been reported to stimulate leptin secretion. Plasma leptin levels decrease during fasting or energy restriction and increase during re-feeding, overfeeding, and surgical stress. It acts on receptors in the arcuate nucleus of the hypothalamus to regulate appetite to achieve energy homeostasis. (175)

Leptin is a mediator of long-term regulation of energy balance, suppressing food intake and thereby inducing weight loss. Studies suggest that intrahepatic and intramyocellular lipid lowering effects of leptin are mediated mostly by a reduction in energy intake, as opposed to a leptin-induced increase in energy expenditure, although a lipolytic effect of leptin on these tissues cannot be ruled out. (176) It can cause an approximately twofold increase in insulin suppression (i.e. increase insulin sensitivity) of hepatic glucose production and an almost twofold increase in insulin-stimulated peripheral glucose disposal. (177)

Leptin levels increase with rising BMI, age, smoking cessation and restrictive bariatric surgery (AGB). However, its levels are found to decrease after RYGB. (178) Leptin concentrations are known to vary according to gender. (179)

Its levels increase throughout pregnancy.(180) There is growing evidence that it has a key role in the pathogenesis of pregnancy complications such as PE, GDM, macrosomia and FGR. (181)

Visfatin: A 52-kDa protein found in living species from bacteria to humans and is produced by the visceral adipose tissue. (182) The expression of visfatin is increased in individuals with abdominal obesity and T2DM. It has been shown that glucose stimulates visfatin release, which in turn is a modulator of insulin sensitivity and inflammatory status in humans. (183) Visfatin has been reported to be a beneficial adipocytokine with insulin mimicking/sensitizing effects and may contribute to inflammatory progression by evoking cytokine production and nuclear factor-kappa B (NF-κB) activation. (184) Visfatin is inversely correlated with BMI and is increased after bariatric surgery. (185, 186)

In pregnancy, Visfatin levels increase, reaching a peak between 19 and 26 weeks, then decrease to its lowest level between 27 and 34 weeks of gestation. (187) There are conflicting reports regarding its role in GDM, PE and SGA babies.(188, 189)

Resistin: An adipocyte-derived signalling cysteine-rich molecule made up of 114 amino acids and detected in tissues like placenta, skeletal muscle, small intestine, spleen, stomach, thymus, thyroid gland and uterus. (190) Resistin levels rise with age, BMI, smoking and Black ethnic group but decrease after bariatric surgery. (191,192) It is named because of its resistance to the action of insulin and is considered a pro-inflammatory molecule, which plays an important role in the pathogenesis of diabetes and its complications. It has a direct positive correlation with HbA1c. The release of resistin is often stimulated by inflammatory processes, IL-6, hyperglycaemia and hormones such as growth hormone and gonadal hormones. (193)
Resistin levels rise during pregnancy, reaching maximum levels in the third trimester. (194) There is uncertainty regarding the link with PE and GDM.

Pro-inflammatory factors

Interleukin-6 (IL-6), Monocyte Chemotactic Protein -1 (MCP-1), PAI-1 and C-C motif Ligand 5 mediate the inflammatory response in this pathway. The effect of bariatric surgery on pro-inflammatory biomarkers is summarised in the appendix (Supplementary Table 4).

Interleukin 6: This is secreted by T cells and macrophages to stimulate an immune response. (195) Interleukin 6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-a and IL-1, and activation of IL-1 receptor agonist (IL-1ra) and IL-10. In muscle and fatty tissue, IL-6 stimulates energy mobilization that leads to increased body temperature. It increases with age, BMI, smoking. Following bariatric surgery its levels have been found to decrease. (196,197, 198,199, 200)

In pregnancy, its levels increase with gestational age, emergency CS, labour and spontaneous vaginal delivery. It is associated with maternal complications such as PE, gestational hypertension, PPROM, preterm labour. (201, 202, 203, 204)

Monocyte Chemotactic Protein -1/ chemokine (C-C motif) ligand 2 (MCP-1/CCL2): This is an inflammatory marker and potent chemotactic factor for monocytes. (205) It is composed of 76 amino acids and 13 kDa in size. White adipose tissue is infiltrated with macrophages in response to adipocyte hypertrophy and increased MCP-1 expression. (206) It is produced by a variety of cell types, either constitutively or after induction by oxidative stress, cytokines or growth factors. Increased circulating concentrations of MCP-1 are found to be predictive of both diabetes risk, independent of other traditional risk factors, and atherosclerosis. Its levels increase with age and BMI but decreases following bariatric surgery. (207)

In pregnancy, it is elevated in the 2nd and 3rd trimester and postpartum. Associated maternal complications include GDM, morbid obesity and PE with the related fetal sequelae. (208, 209)

Plasminogen activator inhibitor-1 (PAI-1): This is a serine protease inhibitor (serpin) that is prothrombotic and functions as the principal inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA), the activators of plasminogen and hence fibrinolysis. (210) PAI-1 is mainly produced by the endothelium but also secreted by other tissue types, such as adipose tissue. It is higher in smokers and individuals with raised BMI but lower in the Black ethnic group and following bariatric surgery. (211, 212)

PAI-1 promotes thrombogenesis and is an independent risk factor for adverse pregnancy outcomes including recurrent first trimester miscarriage, PE, intrauterine growth restriction, abruption and stillbirth. (213) Its levels are increased in the 2nd trimester reaching a maximum rise by 32 to 40 weeks. It is reduced to pre-pregnant levels within 5 to 8 weeks postnatal. (214)

C-C motif Ligand 5 (CCL5) or RANTES (regulated on activation, normal T cell expressed and secreted): This is a key pro-inflammatory cytokine, which is chemotactic to monocytes and T-lymphocytes. Activated macrophages and T cells within white adipose tissue produce increased levels of inflammatory chemokines such as CCL2/MCP-1, CCL5/RANTES,TNF-α, interleukin-1β (IL-1β), and IL-6. These have been proposed as mediators of obesity-related pathology such as hypertension,

atherosclerotic plaque formation, liver steatosis and pancreatic -cell degeneration, which lead to T2DM. (215) Its levels increase with BMI, Black ethnic group and decrease following bariatric surgery. (216, 218)

In pregnancy, levels rise in the first trimester consistent with its role in placental implantation. It has been linked to pathology of PE and rising levels in amniotic fluid have been implicated in the aetiology of preterm labour. (219, 220)

Incretins

Glucose-dependent Insulinotropic Polypeptide (GIP) and GLP-1 are incretins, that is, metabolic hormones that stimulate a decrease in blood glucose levels. The effect of bariatric surgery on peptide hormones is summarised in the appendix (Supplementary Table 5).

GIP, also known as the glucose-dependent insulinotropic peptide: This is an inhibiting hormone of the secretin (incretin) family of hormones. (220) It is synthesized by K cells, which are found in the mucosa of the duodenum and the jejunum of the GI tract and is stimulated primarily by hyperosmolarity of glucose in the duodenum, and nutrients including, proteins and fats. It enhances insulin secretion glucose-dependently to exert glucose-lowering effects. In addition to its insulinotropic activity, GIP exerts a number of additional actions including promotion of growth and survival of the pancreatic beta-cell and stimulation of adipogenesis. The brain, bone, cardiovascular system and GI tract are additional targets of GIP. In adipose tissue, GIP interacts with insulin to increase lipoprotein lipase activity and lipogenesis. It decreases with age and BMI but increases following bariatric surgery. (221)

GLP-1 is secreted by intestinal ileal L cells and is dependent on the presence of nutrients in the lumen of the small intestine. (222) It is a potent antihyperglycemic hormone, inducing the β-cells of the pancreas to release insulin in response to rising glucose, while suppressing glucagon secretion. GLP-1 no longer stimulates the β-cells to release more insulin when blood glucose levels are in the fasting range. Additionally, GLP-1 appears to restore the glucose sensitivity of pancreatic β-cells. Its levels are increased in black obese compared to white obese individuals and following bariatric surgery. (223, 224)

There is no change in fasting and postprandial GIP levels during pregnancy. (225) Fasting GIP-1 levels increase from the second to third trimester when it negatively correlates to fetal abdominal circumference and birth weight. (226) Abnormal fasting levels of both GIP and GLP-1 correlate to GDM risk. (227)

1.4.2 Metabolomic changes following bariatric surgery

Metabolomics is a relatively new field whereby the object of study is the metabolome, which is the full complement of small molecules (<1500Da) called metabolites within an organism, cells or tissue. These dynamic metabolites act as either substrates or products of metabolism at any given time. In order to profile metabolic pathways, metabolomics quantitatively identifies metabolites at given time points. It is a powerful tool because metabolites and their concentrations, directly reflect the underlying biochemical activity and state of cells or tissues which are influenced by genetic and environmental factors. (228)

Metabolomics profiling methods include the use of high resolution proton nuclear magnetic resonance (^{1}H NMR) or mass spectrometry (MS). Both have the ability to analyse several hundred metabolites in a single measurement. They can be used to perform non-targeted profiling, measuring as many metabolites as possible, or targeted profiling, where a selection of known metabolites is measured.

Metabolomic profiling following bariatric surgery

In the last 5 years there have been over 100 metabolomic studies gaining insight into the mechanism by which bariatric surgery procedures led to health improvements, particularly resolution of T2DM. The outcomes of seven of these studies are summarised below.

A longitudinal observational study to assess metabolic alterations associated with severe obesity and bariatric surgery was conducted by Gralka E et al. (1)H-nuclear magnetic resonance-based global, untargeted metabolomics was used on serum samples collected before and repeatedly ≤1 year after bariatric surgery (VSG, proximal and distal RYGB). A metabolomic fingerprint in obese subjects was clearly discriminated from that of normal-weight subjects. Metabolites that contributed to this

were higher levels of aromatic and branched-chain amino acids, metabolites related to energy metabolism (pyruvate and citrate; elevated) and metabolites suggested to be derived from gut microbiota (formate, methanol, and isopropanol; all elevated). Bariatric surgery (VSG and proximal and distal RYGB) reversed most of the metabolic alterations associated with obesity and was also associated with profound changes in gut microbiome-host interactions. (229)

Sarosiek K et al conducted a non-targeted, global metabolomic pilot study including nondiabetic and T2DM patients who underwent VSG or RYGB. Their results suggested that bariatric surgery might promote antioxidant defence and insulin sensitivity through both increased heme synthesis and heme oxygenase (HO) activity or expression. Changes in histidine and its metabolites following surgery might be an indication of altered gut microbiome ecology or liver function.(230)

Narath SH et al also used an untargeted metabolomics approach with mass spectrometry (MS) and identified relevant metabolic changes one year after RYGB in serum of 44 patients (24 patients with T2DM). Metabolites identified included trimethylamine-N-oxide, alanine, phenylalanine and indoxyl-sulfate which are known markers for cardiovascular risk. In addition they found a significant decrease in alanine after one year in the group of patients with diabetes remission relative to non-remission. (231)

A systematic review of 32 studies by Tulipani et al showed that the metabolic adaptations shared by surgical and dietary interventions mirrored a state of starvation: ketoacidosis (increase of circulating ketone bodies), an increase of acylcarnitines and fatty acid β-oxidation, a decrease of specific amino acids including branched-chain amino acids (BCAA) and (lyso) glycerophospholipids previously associated with obesity, and adipose tissue expansion. The metabolic profile post bariatric surgery was characterized by an increase of bile acid, a decrease of ceramide levels, a greater perioperative decline in BCAA, and the rise of circulating serine and glycine, mirroring glycaemic control and inflammation improvement. 3-hydroxybutyrate was identified in one study as an early metabolic marker of long-term prognosis after surgery. (232)

Lopes TI et al conducted a mixed-meal tolerance test on subjects before and 12 months after RYGB. The outcomes were investigated by time-resolved hydrogen

nuclear magnetic resonance ((1)H NMR)-based metabolomics. They showed a significant decrease in glucose levels after bariatric surgery (from 159.80 ± 61.43 to 100.00 ± 22.94 mg/dL), demonstrating T2DM remission ($p < 0.05$). The metabolic profile indicated lower levels of lactate, alanine, and branched chain amino acids for the operated subject at fasting state after the surgery. However, soon after food ingestion, the levels of these metabolites increased faster in operated than in non-operated subjects. (233)

Yao et al used metabolomic profiling to quantify insulin-mediated glucose, amino acid, and lipid metabolism in eleven morbidly obese non-diabetic Asian individuals undergoing VSG and nine non-obese controls. They demonstrated that impairment in the regulatory actions of insulin on glucose, amino acid, and lipid metabolism in the morbidly obese subjects improved significantly 6 months after VSG. (234)

And finally, the serum lipidome in obese subjects undergoing restrictive (VSG) vs malabsorptive (BPD) was the focus of a study by Ramos-Molina et al. VSG restored fatty acids and glycerolipids nonobese levels. It also increased phospholipid and sphingolipid levels. BPD led to an overall reduction in circulating fatty acids, glycerolipids, phospholipids and sphingolipids and a substantial increase of bile acids. (235)

1.4.2.1 Metabolomic changes in pregnancy

Due to its novel status, there is a paucity of studies using metabolomics in pregnancy. There are on-going efforts in the literature to use metabolomics to discover pregnancy biomarkers to predict preterm delivery, fetal growth restriction, PE and placental abruption.(236,237)

Pinto et al, used NMR spectrometry metabolomic studies of maternal plasma and urine measured serially in normal pregnancy. The group confirmed a decrease in circulating amino acids early in pregnancy and newly observed changes in citrate, lactate, and dimethyl sulfone suggested early adjustments in energy and gut microflora metabolisms. Alterations in creatinine levels were also noted, in addition to creatinine variations reflecting alterations in glomerular filtration rate.(238)

A study by Virgiliou C et al. applied holistic and targeted metabolomics approaches for the assessment of the metabolic content of prospectively collected amniotic fluid (AF)

and paired maternal blood serum samples from 35 women who delivered preterm (29^{+0}-36^{+5} weeks of gestation) and 35 women who delivered at term. Untargeted and targeted profiling showed differentiations in certain key metabolites in the biological fluids of the two study groups. In AF, intermediate metabolites involved in energy metabolism (pyruvic acid, glutamic acid, and glutamine) were found to contribute to the classification of the two groups. In maternal serum, increased levels of lipids and alterations of key end-point metabolites were observed in cases of preterm delivery. Overall, the metabolic content of second-trimester AF and maternal blood serum shows potential for the identification of biomarkers related to fetal growth and preterm delivery.

Orczyk-Pawilowicz et al, applied NMR-based metabolic profiling to track metabolic changes occurring in AF and plasma of healthy mothers over the course of pregnancy. From the second to third trimester increasing plasma levels of glycerol, choline and ketone bodies (3-hydroxybutyrate and acetoacetate) were recorded while pyruvate concentration was significantly decreased. Lactate to pyruvate ratio was decreased in AF and conversely increased in plasma during these time points. They concluded that metabolomic profiling enabled better understanding of complex physiological changes between the mother, the placenta and the fetus. (239)

There has also been a lot work using metabolomics technology to investigate the pathophysiology of PE. Austdal et al, managed to phenotype the pre-eclamptic placenta. (240) Principal component analysis showed inherent differences in placental metabolic profiles between PE and normotensive pregnancies. Significant differences in metabolic profiles were found between placentas from severe and non-severe PE, but not between PE pregnancies with fetal growth restricted versus normal weight neonates. The placental metabolites correlated with the placental stress marker sFlt-1 and triglycerides in maternal serum, suggesting variation in placental stress signalling between different placental phenotypes.

1.4.2.2 Effect of Bariatric Surgery on the maternal and neonatal metabolome.

Accumulating literature supports metabolomics as a viable tool to understand biochemical response to external factors including bariatric surgery.

In 2018, a review of 30 studies demonstrated that amino acids were the metabolites whose levels were most affected by bariatric surgery. (241) For example, branched chain amino acids (BCAA) decreased following surgery, especially Isoleucine, leucine and valine and this may correlate with decreased insulin resistance. (242, 243) There are lower levels of aromatic amino acids: methionine, alanine, and lysine following Roux-en-Y gastric bypass (RYGB) while sleeve gastrectomy was associated with increased serum concentrations of serine and glycine. (244)

As previously discussed, there is strong evidence that bariatric surgery is associated with altering maternal and neonatal outcomes. In particular, a lower incidence of GDM, pre-eclampsia, LGA neonates and a higher risk of SGA and moderately preterm birth compared to women with similar BMI. (245)

In summary, metabolomics technology is an ideal approach to characterize the maternal metabolic milieu, which could shed light on the mechanisms underlying the alterations in pregnancy outcomes following bariatric surgery.

1.5 LIPOPROTEIN PROFILING

Obesity in pregnancy has many serious consequences to both mother and fetus. The exact mechanisms underlying this are not fully understood. Alterations in the lipid and lipoprotein profiles may play a role.

Lipidomics is an important branch of omics involving the large scale analysis of the lipidome which consists of hundreds to thousands of lipid species. (246) This research tool requires a high throughput and uses analytical chemistry methods to identify lipid structures; quantify lipid levels in biological fluid samples and elucidate metabolic pathways involving different lipid classes with other lipids, proteins, and metabolites in vivo. (247)

Lipidomics has been applied to studies on obesity, atherosclerosis, the metabolic syndrome and cancers outside the context of pregnancy. (248) Data obtained from lipidomics can identify lipid disorders and help inform the impact on various metabolic processes. (249) It can also be used to identify biomarkers which can be applied to

determine prognosis, prevention, diagnosis and treatment of various metabolic diseases. (250)

To date, there are only 23 lipoprotein studies in pregnant women in the literature. The most recently published work is by Youssef et al who demonstrated altered lipid profiles in women with PE with and without growth-restricted fetuses. Compared to controls, they had a more atherogenic lipid profile with higher levels of triglycerides, very low density lipoproteins and intermediate density lipoproteins. (251)

Lipoprotein profiling, the analysis of lipid and lipoprotein classes is in contrast to conventional lipidomic studies where Mass Spectrometry is used to quantify lipid molecules. Lipoprotein profiling uses H1 NMR analysis which is becoming a valuable tool in lipidomics analyses, and could give valuable insight on the effect of obesity in pregnancy at a molecular level. (252)

Hypothesis

Previous bariatric surgery has a positive, advantageous effect on the biophysical and biochemical profile of pregnant women, compared to women with no surgery and similar BMI. This could be mediated by an improvement in maternal insulin resistance and activation of other metabolic pathways involved in glucose and fat metabolism.

Obesity in pregnancy may result in a negative, disadvantageous effect on the lipid and lipoprotein profile as pregnancy advances, compared to women with normal BMI.

Aims

1. To compare the maternal insulin resistance (IR), as assessed by the homeostatic model assessment (HOMA), glycosylated haemoglobin (HbA1c) and other biochemical markers, involved in glucose and fat metabolism, measured at 28 weeks of gestation in pregnant women following bariatric surgery to those without surgery.
2. To compare the maternal biophysical, biochemical and anthropometric profile of pregnant women following bariatric surgery to those without surgery.
3. To compare the maternal metabolome post bariatric surgery with those without surgery.
4. To compare the lipoprotein profile in obese and normal BMI pregnant women without previous bariatric surgery.

CHAPTER 2

Materials and Methods

2.1 ETHICS STATEMENT

The study was approved by the research ethics committee of the West London Local Research Ethics Committee (REC number 14/LO/0592) and all women provided written consent form.

2.2 STUDY DESIGN

This was a prospective, longitudinal, observational study.

Recruitment

Pregnant participants were recruited voluntarily from the antenatal clinic at Chelsea & Westminster Hospital, West Middlesex University Hospital and Hillingdon Hospital NHS trusts from May 2015 until April 2017.

Informed written consent was obtained and patients were put into two groups: pregnant women post bariatric surgery or pregnant women without surgery.

Inclusion criteria:

1. Women with singleton pregnancy and previous bariatric surgery
2. Women with singleton pregnancy without previous bariatric surgery

Exclusion criteria:

1. Women that do not belong to any of the above groups
2. Women that are less than 18 years of age
3. Women with multiple pregnancy e.g. twins/triplets
4. Women that had a miscarriage, termination of pregnancy or intrauterine death
5. Women diagnosed with fetal anomaly in the index pregnancy.

The participants were seen at six time points during the pregnancy at 12-14, 20-24, 28-30, 30-32 and 35-37 weeks of gestation and at delivery. Information on maternal age, racial group, smoking status, method of conception, parity, previous obstetric history and previous medical history was obtained at the first research visit and recorded on our electronic database. Maternal biophysical measurements and biological (blood and urine) samples were collected at each visit, including the delivery.

2.3 MATERNAL BIOPHYSICAL MEASUREMENTS

At each visit maternal weight, height, waist to hip ratio (WHR) and blood pressure were measured.

Maternal weight was measured in kilograms and obtained using calibrated Marsden weighing scales with the women in light clothing without shoes.

Maternal height was measured in centimeters with the women standing in a vertical position, without shoes in front of a manual Stadiometer. A horizontal headpiece was adjusted to rest on the top of their head prior to the measurement being recorded. Body mass index was calculated as weight (kg)/ height $(m)^2$.

The WHR was measured in centimeters with a tape measure. The landmarks used were:

1. The apex of the iliac crest which determined the waist circumference.

2. The greatest protuberance of the buttocks at the level of the pubic symphysis which indicated the widest area for the hip measurement.

Maternal blood pressure (BP) was measured using a fully automated upper arm blood pressure device validated in pregnancy (Microlife WatchBP, Taipei, Taiwan). Microlife monitors follow European Society of Hypertension recommendations for conventional, ambulatory and home BP measurement. (253) The monitor was calibrated before and at regular intervals during the study. A normal (22-32cm) or large (33-42cm) adult cuff was used, depending on the mid-arm circumference. The BP was measured twice at 5 minute intervals with the women in a seated position, relaxed, silent (not speaking) and using their left arm resting on a table at the level of the heart. The average BP result was recorded.

2.4 MATERNAL SAMPLE COLLECTION

Maternal blood and urine samples were taken at each antenatal visit and within 72 hours of delivery. Blood samples were centrifuged (4600RPM for 10 mins) and the serum or plasma removed and stored immediately at -80°C. Urine samples were also stored at -80°C within 10 mins of collection.

At 28-30 weeks of gestation, a 2h 75gr full oral glucose tolerance test, OGTT (0 and 120 minutes) was performed as a diagnostic test for gestational diabetes (GDM).

A diagnosis of GDM was confirmed as per NICE guidelines:(52)

- A fasting plasma glucose level ≥ 5.6 mmol/litre or
- A 2-hour plasma glucose level ≥ 7.8 mmol/litre

It soon became apparent both clinically and in research published at the time, that a full OGTT following gastric bypass surgery, in particular, was associated with dumping syndrome. (253,254) This presented as a combination of all or some of the following symptoms: bloating, nausea, vomiting, diarrhoea, abdominal cramps, hot flushes, dizziness and palpitations. The altered gastro-intestinal transit time responsible for dumping syndrome called into question the accuracy of the OGTT in the literature. (256)

For this reason, in early 2017, the GDM screening method was modified for women post gastric bypass surgery. Only a fasting blood sample was taken for the study and then women were asked to do home glucose monitoring for 14 days. Blood glucose values were reviewed by the diabetic team, which was independent of the research team, and decisions for further management were made.

2.5 MATERNAL SAMPLE MEASUREMENTS

Maternal blood samples were used for measurement of insulin, glucose, insulin resistance, glycosylated haemoglobin, biomarkers involved in glucose and fat metabolism, metabolomics and lipoprotein profiling (together with maternal urine).

2.5.1 Insulin, glucose, insulin resistance and glycosylated haemoglobin

Maternal fasting blood samples at 28-30 weeks were used for the measurements of

maternal glucose, insulin, insulin resistance and glycosylated haemoglobin (Hba1c). Measurements were performed at Charing Cross Hospital Biochemistry Laboratories using the Architect cSystem assay:

Insulin

Chemiluminescent microparticle immunoassay (CMIA) on the Abbott Architect System ™ (Abbott Laboratories) was used for serum insulin measurement. Insulin binds to the anti-insulin coated microparticles and anti-insulin acridinium-labelled conjugate. A chemiluminescent reaction results from the addition of pre-trigger and trigger solutions. This reaction is measured as relative light units (RLUs) detected by the ARCHITECT immunoassay (*i)* optical system. The RLUs of the reaction is directly proportional to the quantitative insulin content of the sample. The ARCHITECT Insulin assay has a coefficient of variation of ≤ 7%. (257)

Glucose

Plasma glucose content was determined using the Glucose assay on the ARCHITECT *c* Systems™ (Abbott Laboratories). In the assay, glucose is phosphorylated by enzyme hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Enzyme Glucose-6-phosphate dehydrogenase (G-6-PDH) specifically oxidizes G-6-P to 6-phosphogluconate with the concurrent reduction of nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide reduced (NADH). NADH acts as a surrogate marker for glucose content on a 1:1 ratio since one micromole of NADH is produced for each micromole of glucose consumed. NADH absorbs light at 340 nm and can be detected spectrophotometrically as an increased absorbance by the ARCHITECT *c* Systems™. The ARCHITECT glucose assay has a coefficient of variation of ≤ 5%. (258)

Maternal fasting glucose and insulin levels were used to calculate insulin resistance as Homeostasis Model Assessment of IR (HOMA-IR) using the formula: (259)

Fasting Insulin (microU/L) x fasting glucose (nmol/L) / 22.5

Maternal insulin sensitivity was calculated using the formula for the Matsuda index: (260)

$$10{,}000 \div \sqrt{(\mathrm{GF} \times \mathrm{IF}) \times (\mathrm{Gmean} \times \mathrm{Imean})}$$

I_F – Fasting plasma insulin concentration (mIU/l),

G_F – Fasting plasma glucose concentration (mg/dl),

Gmean – Mean plasma glucose concentration during OGTT (mg/dl),

Imean – Mean plasma insulin concentration during OGTT (mU/l),

10,000– Simplifying constant to get numbers from 0 to 12.

√– Correction of the nonlinear values distribution.

Glycosylated Haemoglobin (Hba1c)

The Automated Glycohemoglobin Analyzer HLC-723G8 (Tosoh Corporation © 2018, Japan) uses non-porous ion exchange High Performance Liquid Chromatography (HPLC) to determine Hba1c content of the plasma samples. HbA1c is separated from other haemoglobin fractions by using differences in ionic interactions between the cation exchange group on the column resin surface and the haemoglobin components. The separated haemoglobin components pass through the LED photometer flow cell where the analyzer measures changes in absorbance at 415 nm. The analyser integrates and reduces the raw data, and calculates the relative percentages of each haemoglobin fraction. Hba1c has a coefficient of variation of ≤ 5%. (261)

2.5.2 Methods for biomarker assays

Maternal blood samples were also used to measure biomarkers involved in glucose and fat metabolism. Plasma samples from post-bariatric pregnant women and those with no surgery but similar early pregnancy BMI were analysed using Bio-Plex Pro Human Diabetes Panel 10-Plex, IL6 and Adiponectin Assay and Bio-Plex Pro Cytokine Assay (BIO-RAD USA). These panels were used to measure the following biomarkers that play a key role in fat and glucose metabolic pathways:

- **Peptide hormones:** C-Peptide, Ghrelin and Glucagon
- **Adipokines:** Adiponectin, Leptin, Visfatin and Resistin
- **Pro-inflammatory hormones:** Interleukin 6 (IL-6), Monocyte chemoattractant protein 1 / C-C Motif ligand 2 (MCP-1/CCL2), Plasminogen activator inhibitor - 1 (PAI-1), Regulated on Activation, Normal T Cell Expressed and Secreted / Chemokine ligand 5 (RANTES / CCL5)
- **Incretins:** Glucose dependent Insulinotropic Polypeptide (GIP) and Glucagon-like Peptide-1 (GLIP-1)

Assays were performed by Dr Chidimma Kanu under the supervision of Dr Bronwen Herbert (Research Associate, Imperial College London).

The standard operating procedure is summarized below:

- The stored maternal plasma from pregnant women with previous bariatric surgery and those with no previous bariatric surgery but similar early pregnancy BMI, were retrieved from the -80ºC freezer. The samples were thawed at room temperature and centrifuged at 13000rpm for 10mins at 4ºC to remove any precipitate.
- A 10-plex BIORAD Bio-Plex Pro Human Diabetes Panel containing C-peptide, ghrelin, GIP, GLP-1, glucagon, leptin, PAI-1, resistin and visfatin and supplemented with IL6, was performed according to manufacturer's instructions. A second and third separate adiponectin and cytokine assay containing RANTES/CCL5 and MCP-1/CCL2 (Tables 2.1, 2.2 and Figure 2.1) was performed according to manufacturer's instructions.
- A fourfold standard diluent series and blank were prepared using the provided diabetes standard combined with a cytokine standard to quantify IL6.
- The adiponectin and cytokine plate used only the diabetes standard. The standard was prepared using the provided standard diluent for each assay.
- A test plate was run prior to ensure appropriate serum dilutions were used.
- For adiponectin, plasma sample dilution was 1:400 with the provided appropriate sample diluent. For cytokine and diabetes panel with IL6, plasma sample dilution was 1:4 which was diluted with the appropriate sample diluent.
- Diluted microbeads were vortexed and added to each well of assay plate. Plates were washed and then samples, standards and blanks were added to

each well of a 96-well plate containing antibodies that were chemically attached to fluorescent-labelled microbeads.

- The adiponectin plate was covered, incubated and agitated on a shaker at 850rpm for 1 hour at room temperature. The cytokine and diabetes & IL6 panel plate was covered and incubated on a shaker at 850 rpm for 30 mins at room temperature.
- The plates were washed with 100µL wash buffer and then the diluted secondary (detection) antibodies were added to each well.
- The plates were further covered and incubated on a shaker at 850 rpm for 30 mins at room temperature. They were then washed 3 times with 100 µL wash buffer. Streptavidin Phycoerythrin conjugate (SA-PE) was added to each well and the plates were covered and incubated for 10 minutes at 850rpm for 10 minutes. The plates was washed three times with 100 µL wash buffer.
- The beads were re-suspended in 125 µL assay buffer and then the plate was covered and shaken at 850rpm for 30 seconds.

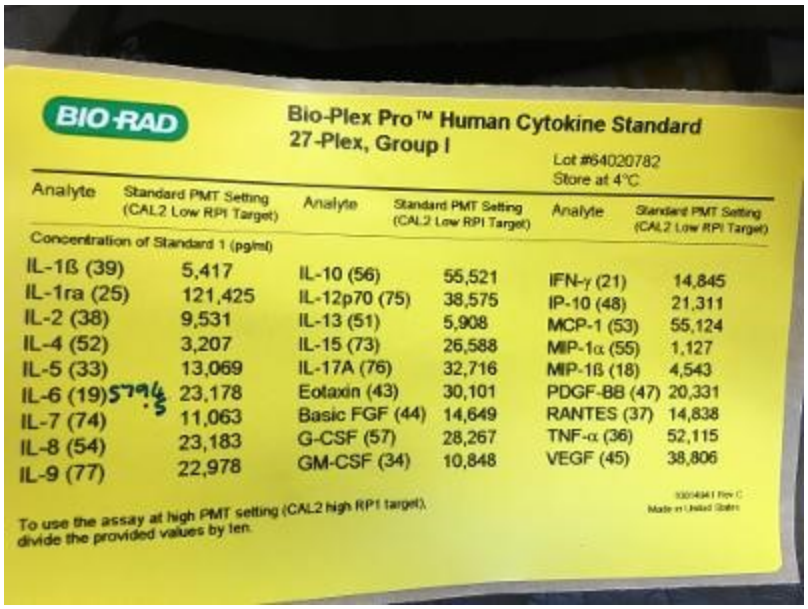
The plates were then read using the (Bio-Rad) MAGPIX instrument. The data was automatically analysed and processed using BIO-PLEX Manager version 6.1 software (Bio-Rad Laboratories).

Table 2.1: Diabetes and IL-6 panel dilutions

BEADS	**DIABETES BEADS**	**IL 6 BEADS**	**ASSAY BUFFER**	**TOTAL VOLUME**
TRIAL (µL)	50	100	850	1000
PLATE (µL)	262.5	525	4462.5	5250
SECONDARY ANTIBODY (AB)	**DIABETES AB**	**IL 6 AB**	**AB DILUENT**	**TOTAL VOLUME**
TRIAL (µL)	25	50	425	500
PLATE(µL)	131.25	262.5	2231.25	2625
PE	**PE**	**ASSAY BUFFER**		**TOTAL VOLUME**
TRIAL(µL)	10	990		1000
PLATE(µL)	52	5198		5250

Table 2.2: Adiponectin panel dilutions

BEADS	**ADIPONECTIN BEADS**	**ASSAY BUFFER**	**TOTAL VOLUME**
TRIAL (µL)	50	*956*	*1000*
PLATE (µL)	*262.5*	*4987.5*	*5250*
SECONDARY ANTIBODY (AB)	**ADIPONECTIN AB**	**AB DILUENT**	**TOTAL VOLUME**
TRIAL (µL)	25	475	250
PLATE (µL)	131.25	2493.75	2625
PE	**PE**	**ASSAY BUFFER**	**TOTAL VOLUME**
TRIAL (µL)	10	990	1000
PLATE (µL)	52	5198	5250



BIO-RAD

Bio-Plex Pro™ Human Cytokine Standard
27-Plex, Group I

Lot #64020782
Store at 4°C

Analyte	Standard PMT Setting (CAL2 Low RP1 Target)	Analyte	Standard PMT Setting (CAL2 Low RP1 Target)	Analyte	Standard PMT Setting (CAL2 Low RP1 Target)
Concentration of Standard 1 (pg/ml)					
IL-1ß (39)	5,417	IL-10 (56)	55,521	IFN-γ (21)	14,845
IL-1ra (25)	121,425	IL-12p70 (75)	38,575	IP-10 (48)	21,311
IL-2 (38)	9,531	IL-13 (51)	5,908	MCP-1 (53)	55,124
IL-4 (52)	3,207	IL-15 (73)	26,588	MIP-1α (55)	1,127
IL-5 (33)	13,069	IL-17A (76)	32,716	MIP-1ß (18)	4,543
IL-6 (19)	23,178	Eotaxin (43)	30,101	PDGF-BB (47)	20,331
IL-7 (74)	11,063	Basic FGF (44)	14,649	RANTES (37)	14,838
IL-8 (54)	23,183	G-CSF (57)	28,267	TNF-α (36)	52,115
IL-9 (77)	22,978	GM-CSF (34)	10,848	VEGF (45)	38,806

To use the assay at high PMT setting (CAL2 high RP1 target), divide the provided values by ten.

Figure 2.1: Bio-Plex Human Cytokine Standards containing RANTES/CCL5 and MCP-1/CCL2 used in assay plates.

2.5.3 Methods for metabolomic profiling

Metabolomics profiling technology is based on two main analytical platforms: high resolution nuclear magnetic resonance spectroscopy (H^1 NMR spectroscopy, Figure 2.2) and mass spectrometry (MS), whereby the latter is typically coupled with chromatographic separation technologies. (262) The high reproducibility of NMR-based techniques and the high sensitivity and selectivity of MS-based techniques mean that these tools are superior over other analytical techniques. (263)

Nuclear magnetic resonance spectroscopy (H^1 NMR spectroscopy)

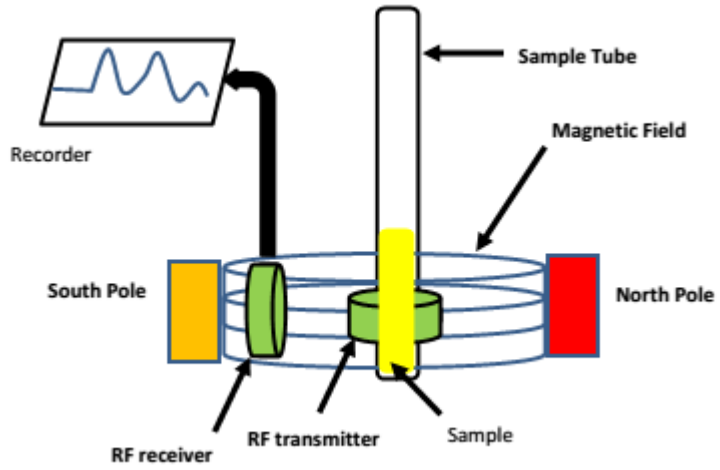


Figure 2.2: Schematic presentation of NMR spectroscopy.

NMR uses the magnetic properties of the atom nuclei to obtain information about molecules.

Atoms with nuclei that contain an odd number of protons and/or neutrons, such as Hydrogen (^1H) and Carbon (^{13}C), have nuclei that are electrically charged and rotate with a spin. (264) This spin produces an electromagnetic field. When placed in an external magnetic field (B_o), the nuclei either align with or against the direction of the applied magnetic field. (265) Nuclei that are aligned in the opposite direction to the applied external magnetic field have a higher energy level than those in alignment with a lower or base energy level (Figure 2.3). (265) The energy gap generated is in the range of energies found in radio waves (60-100MHz). (266) The exact size of the energy gap is proportional to the strength of the magnetic field.

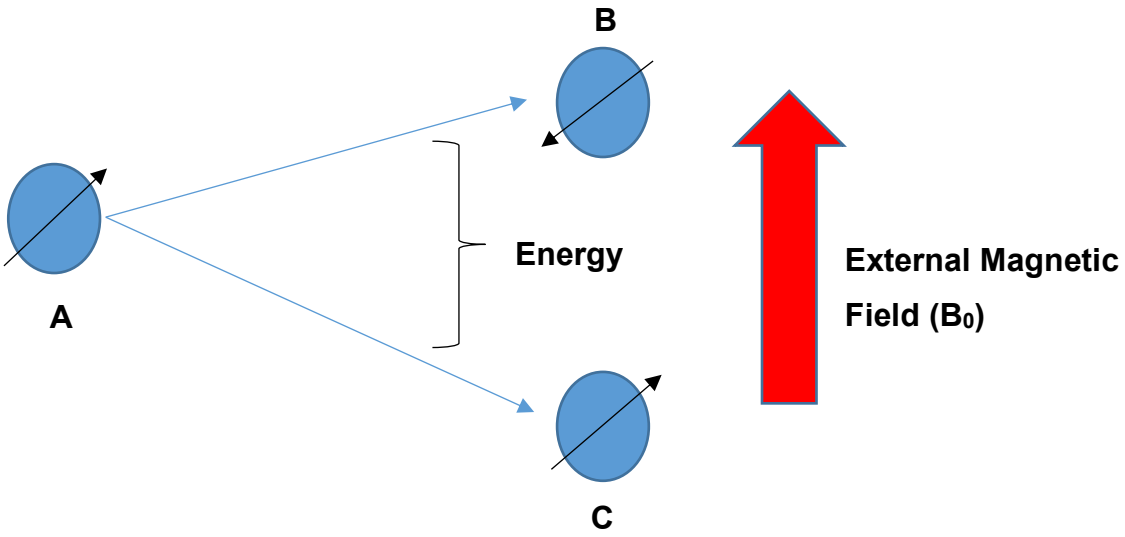


Figure 2.3: Effect of external magnetic field (B_0) on nuclei spin.
A: Electrically charged nuclei rotating with spin that produces an electromagnetic field.
B: Nuclei aligning against the external magnetic field (high energy)
C**:** Nuclei aligning with the direction of external magnetic field (low energy)

Nuclei are conventionally assigned a spin number which describes how many symmetrical facets a particle has in one full rotation; for example, a spin of ½ means that the particle must be fully rotated twice (through 720°) before it has the same configuration as when it started.

A fundamental equation of spectroscopy is $\Delta E = h\nu$ (264)

ΔE: Difference in energy between two states of a system.

h: A proportionality constant.

ν : Symbolizes frequency of electromagnetic radiation

The equation says that in order to flip the nuclei from base or low energy state to high energy state requires an energy transfer with a radiation frequency matching the difference in energy between two energy states (the energy gap).

The condition where $\Delta E = h\nu$ is referred to as resonance. When the spin returns to its base level this is called relaxation. Energy is emitted at the same radio frequency (RF), and the signal is detected by an RF receiver and recorded as a peak on a graph, so creating an NMR spectra for that particular nucleus. (264) The ability of the nuclei of

interest (e.g. ^{1}H) to respond to the external magnetic field depends on surrounding electrons. This is related to the molecule the nuclei resides in. Thus the strength of the external magnetic field is adjusted to compensate for the effect of surrounding electrons and enable a resonance to occur. (267) The NMR resonance frequencies caused by the different positions of the same nuclei within the molecule, are called the chemical shifts (δ). They are measured in parts per million (ppm) with respect to a reference sample. (268) 3-trimethyl-silyl-[2,2,3,3-2H4]propionic acid (TSP) is a reference sample used in aqueous media with the methylene groups deuterated to avoid giving rise to peaks in the ^{1}H NMR spectrum. (269)

Advantages

NMR spectroscopy is reproducible and non-destructive. This makes it ideal for metabolite profiling of bio-fluids and tissues.(270) Another advantage is its efficiency and high throughput. An NMR spectra is typically very quick to produce (within 5-10 minutes) allowing detection of a high number of metabolites simultaneously. (271) There is minimal sample preparation which would include a buffering agent and addition of chosen standard for a reference frequency (e.g. TSP). Suppression of water signals causing interference is easily achieved either with solvent suppression methods (e.g. Deuterium oxide, D2O). (272)

There is also superior metabolite identification with the use of multivariate statistical analysis combined with chemometric methods. (273)

Limitations

NMR is highly sensitive, able to detect metabolites at low-level nanograms, 10^{-9} making it very useful in biochemical investigations however, MS does have a higher sensitivity within the picomolar range (10^{-12}). (274) There are less known identifiable metabolites detected by NMR (>200) compared to MS (>4000), allowing scope for detection of novel metabolites. (275) The cost of an NMR machine is much greater than a Mass Spectrometer, however, the overall cost of analysis is lower.

<u>Mass Spectrometry (MS)</u>

MS measures the masses of molecules and their fragments. It determines their identity by using the mass-to-charge ratio (m/z) of ions formed by inducing the loss or gain of a charge from a neutral species. (276) It is usually preceded by a separation technique

(e.g. liquid or gas chromatography) since this reduces the complexity of the mass spectra, provides isobar separation, and delivers additional information on the physico-chemical properties of the metabolites. (277)

A mass spectrometer contains an ion source, a mass analyser and an ion detector (Figure 2.4). Samples are introduced into the mass spectrometer in liquid or gas form, then vaporized and ionized by the ion source. (278) The ions are accelerated through the remainder of the system to generate the same kinetic energy. (279) Electric and/or magnetic fields from mass analysers deflect the paths of individual ions based on their mass-to-charge ratio (*m/z*). The accelerated ions hit the detector (an electron multiplier or microchannel plates) causing an emission of electrons. This electron cascade is amplified for improved sensitivity.(280) The entire process occurs under vacuum which removes contaminating gases, neutral atoms or molecules and non-sample ions. Such contaminants can collide with sample ions and alter their paths. (281)Mass spectrometers are connected to computers with integrated software that analyses the ion detector data and produces spectra that organize the detected ions by their individual *m/z* values and relative abundance (Figure 2.5). These ions can then be compared with available databases and libraries to predict their molecular identities based on their *m/z* values.

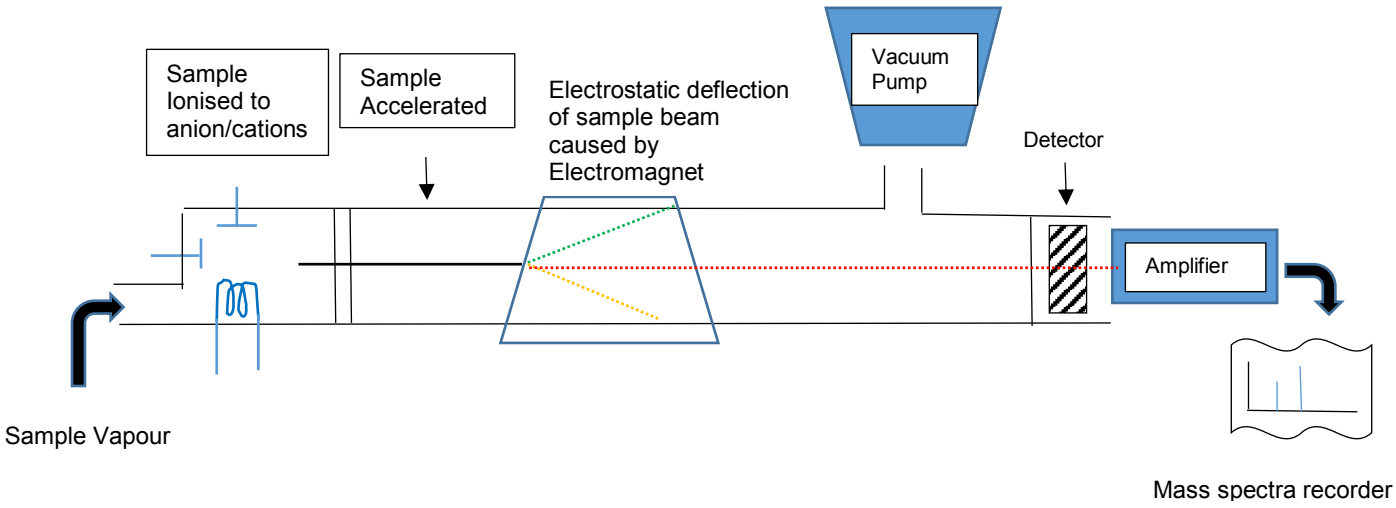


Figure 2.4: Schematic diagram of a Mass Spectrometer

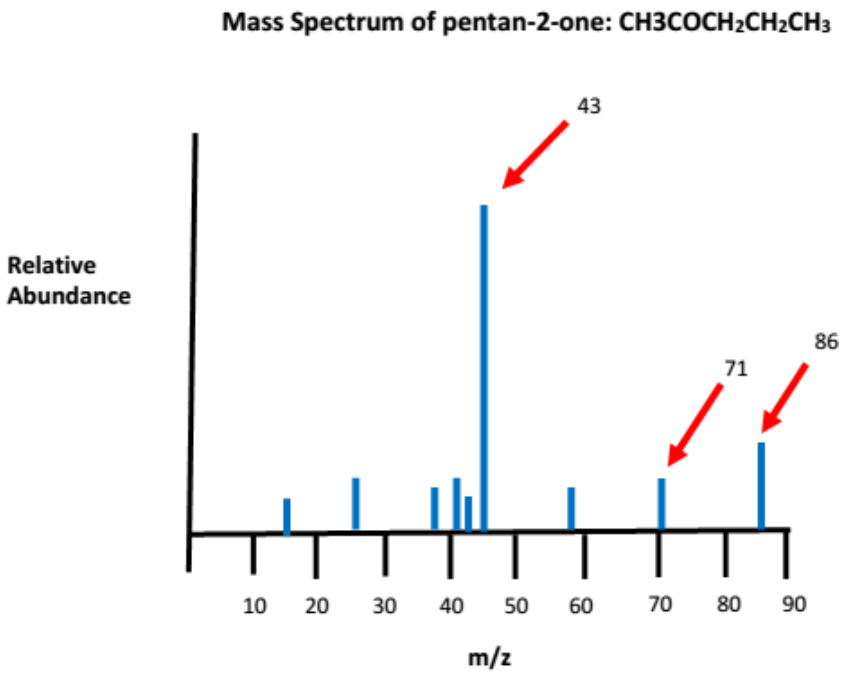


Figure 2.5: Example of a Mass Spectra with the arrows indicating the m/z values.

Adapted from Spectral Database for Organic Compounds (SDBS) at the National Institute of Materials and Chemical Research in Japan, https://sdbs.db.aist.go.jp. Accessed 1/12/2018.

Advantages

The high specificity of MS enables it to determine accurate mass and isotope distribution patterns and determine elemental structures and formulae. (282) This analytical tool can also identify chemicals by using spectral matching to original compound data and elucidate comparative concentration levels of different chemicals in mixed samples. (283) MS is a very high-throughput process that can routinely analyse hundreds of compounds in a single sample and run.(284) It offers quantitative analyses with high selectivity and sensitivity. This allows the unique ability to detect and measure many primary and secondary metabolites at picomole (pmol) to femtomole (fmol) levels.(285)

Limitations

MS cannot detect metabolites that do not ionize with particular ionisation methods or distinguish between isomers of a compound having the same charge-to-mass (m/z)

ratio. (263) Thermal stability of metabolites and their derivatives limit the coverage of gas chromatography MS (GC-MS). (286) Several metabolites can only be analysed by GC-MS after a process called derivatization that modifies an analyte's functionality in order to enable chromatographic separations. This can introduce variability or produce sample artifacts. (282)

2.5.3a NMR Sample Preparation

Maternal serum was used for metabolomics studies to identify differences in the metabolomic profile of pregnant women who had undergone bariatric surgery compared to those who had not with similar early pregnancy BMI.

Sample preparation and analytical techniques used in this study were in accordance with an untargeted approach. This was performed by Dr Chidimma Kanu with the supervision of postgraduate researcher Dr Kiana West and Dr Frances Jackson a post-doctoral researcher both at Imperial College, London.

An untargeted method measures as many metabolites as possible in a biological specimen, regardless of the chemical class of metabolites. This differs from the targeted approach where specific metabolites are quantified. An untargeted approach provides only relative quantification.

High resolution ^{1}H NMR spectroscopy was used as the metabolomics profiling method at the MRC-NIHR National Phenome Centre led by Imperial College London. It detects more abundant metabolites present at micromolar or greater concentrations and provides structural information as well as high reproducibility and throughput, compared with mass spectrometry.

The standard operating procedure (SOP) for the NMR sample preparation is outlined below:

- The maternal serum samples were thoroughly thawed at room temperature and the work bench was cleaned using 1% Vikron solution.
- An NMR serum buffer solution was pre-prepared by co-researchers at Imperial College using a standard protocol.(287) It included:
    - Potassium dihydrogen phosphate (NaH2PO4) as buffering agent.
    - 3-trimethyl-silyl-[2,2,3,3-2H4]propionic acid (TSP) as chemical shift

reference.

- Sodium azide (NaN3) and Deuterium oxide (D2O) as water suppression agent. Suppression of the prominent water NMR peak in bio-fluids is required to identify the peaks of the metabolites of interest.(269)

- 350 µL of serum buffer solution was added to 350 µL of sample in an empty 1.5 ml Eppendorf tube.
- The eppendorf tubes were centrifuged at 12000*g* at 4°C for 5 min, and 600µL of the supernatant transferred into a Sample Jet NMR tube (avoiding bubbles).
- In order to prevent solvent hydrogenation and evaporation, the NMR tube caps with preparation holes were sealed with polyoxometalate (POM) balls. (288)
- The NMR tubes were placed into racks with 3 to 4 composite quality control (QC) samples. These QC samples were made of equal parts of all specimens, included in each of the 10 racks (Figure 2.6). This was done as per protocol to rule out intra-study variation in preparation and analysis.
- The samples were then subjected to 1-dimensional and 2-dimensional NMR experiments to generate global metabolite profiles and identify metabolites of interest.

Rack2	Prep: Tuesday 10 Jan 2016											
	1	2	3	4	5	6	7	8	9	10	11	12
A	QC	93	94	95	96	97	98	99	100	101	102	103
B	104	105	106	107	108	109	110	111	112	113	114	QC
C	115	116	117	118	119	120	121	122	123	124	125	126
D	127	128	129	130	131	132	133	134	135	136	137	QC
E	138	139	140	141	142	143	144	145	146	147	148	149
F	150	151	152	153	154	155	156	157	158	159	160	QC
G	161	162	163	164	165	166	167	168	169	170	171	172
H	173	174	175	176	177	178	179	180	181	182	183	184

Figure 2.6: NMR Rack 2 samples.

NMR Racks are divided into rows (A-H) and columns (1-12) for reference. The numbers within the rack correspond to the number of serum samples analysed (e.g. 104 is the 104th sample analysed) and QC refers to the quality control samples.

2.5.3b ^{1}H NMR Spectroscopy

Bruker 600 MHz spectrometer (Bruker BioSpin, Karlsruhe, Germany) was used to obtain NMR spectra using published protocol.(287) Two experiments were performed on the samples to obtain ^{1}H NMR spectra. These both followed the Bruker Biospin nomenclature pulse sequences:

(i) Carr-Purcell-Meiboom-Gill sequence (CPMG) experiment *(Bruker pulse program: cpmgpr1d)*

After the radio frequency (RF) pulse has been applied in NMR, there is an exponential decay of the magnetization vector of nuclei which is perpendicular or transverse to the applied magnetic field (B_0).(289) The CPMG experiment measures the transverse (T2 or spin-spin) relaxation time of the nuclei, excluding signals from relatively immobile, high molecular weight macromolecules.(290) It is therefore used to select resonance signals from small, low molecular weight metabolites. (291) CPMG is ideal for blood serum or plasma samples to attenuate the large and broad signals that result from high concentrations of macromolecules such as proteins and lipids. (292)

In this study, the CPMG experiment was performed with water pre saturation and samples run at a temperature of 310 Kelvin (K) as per protocol. This generated a one dimensional (1D) NMR spectra. In addition to transverse relaxation, magnetic field inhomogeneities also have relaxation times. The dephasing time or T2 star (T2*) is a combination of these two relaxation times. (293) The CPMG experiment (or spin-echo pulse sequence) also measures the half bandwidth of the metabolite signals using the principle of the following equation (294):

$$\mathbf{d = 1/ (pi*T_2^*)}$$

d = Half bandwidth of NMR signal

T_2^* = Dephasing time

The half bandwidth also called the half-height NMR spectral line width of a given resonance, relates to the real transverse relaxation time of the nuclei (H1) responsible

for that signal. This gives more information about the structure and dynamics of metabolites. (295)

(ii) Two-dimensional J-Resolved experiment (2D JRES)

This experiment produces a two-dimensional (2D) NMR spectrum. It was used in this study because it is particularly helpful at deciphering the identity and structure of molecules whose signals are obscured by overlapping multiplets. The signal resolution is improved by separating the coupling constant and chemical shift into two frequency dimensions. (296)

NMR spectral data pre-processing Bruker TopSpin 3.1 software automatically performed spectral data pre-processing.

This included:

- Automatic referencing to signal of TSP used in this study.
- Shimming the homogenization of the magnetic field.(297)
- One of the composite QCs was used to set up the shimming file.
- Receiver gain adjustment, acquisition and automatic processing (apodization, Fourier transformation, phasing and baseline correction).

2.5.4 Lipoprotein Profiling

The lipoprotein profiling of the maternal samples was performed by the *Bruker Biospin GmbH* Laboratory in *Germany using* 1H Nuclear Magnetic Resonance (NMR) spectroscopy.

The lipid composition of lipoprotein classes was determined by NMR spectral measurement of the terminal methyl groups. The lipoprotein classes compared between BMI groups were: Very low density lipoproteins (VLDL), Intermediate density lipoprotein (IDL) and Low density lipoprotein (LDL) and High density Lipoproteins (HDL). Other lipid parameters that were measured included: Triglyceride (TG), Cholesterol and Apolipoprotein-A1, A2 and B100.

Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA^{TM}) prediction algorithm calculated the particle number in the different lipoprotein sub fractions directly from the ^{1}H NMR spectra of serum samples.

2.6 STATISTICAL ANALYSES

The Kolmogoroff–Smirnoff test was used to assess normal distribution of the data. The mean and standard deviation were used to describe continuous (dependent) variables with normal distribution. The median and interquartile range (IQR) described continuous variables without normal distribution. Categorical (independent) variables were represented by percentage or frequency counts. Analysis of the differences between the groups required the use of the T-test, Mann-Whitney U test, one-way analysis of variance (ANOVA) and chi-squared test as appropriate.

It was difficult to calculate the sample size accurately as there were no previous studies assessing prospectively insulin resistance in pregnant women with previous bariatric surgery. However, a sample of 30 post bariatric surgery pregnant women and 30 non bariatric pregnant women with similar early pregnancy BMI, would have been able to detect a difference of 1.58 points in insulin resistance, as calculated by HOMA-IR; a difference similar to that found in post-bariatric surgery subjects outside the setting of pregnancy (power >90% at alpha=0.05). (298)

Statistical NMR data pre-processing

Raw data were processed in MATLAB (MATrix LABoratory, version R2016b; The MathWorks, Inc., Natwick, MA) a multi-paradigm numerical computing environment. All data processing commands used were scripted by Dr. T. Ebbels at Imperial College.

Spectra were aligned using a recursive segment-wise peak alignment (RSPA) method. This algorithm reduces variability in peak positions across NMR spectra. (299) In order to remove systematic variation across samples, the NMR spectra were normalised. The probabilistic quotient normalisation (PQN) function was used where each NMR spectrum is adjusted based on a calculated dilution factor. (300)

Multivariate statistical analysis for metabolomics studies was performed in Soft Independent Modelling of Class Analogy (SIMCA) software, version 14.1 (MKS Umetrics, Umeå Sweden).

Univariate statistical analyses was performed using SPSS Software version 25.0 (IBM, Chicago, IL, USA). Differences were considered statistically significant at p value < 0.05.

NMR spectral peak analysis

Positive OPLS-DA models were used to generate S-line plots in SIMCA. This aids the identification of discriminating variables (metabolites). It has a form that resembles the NMR spectra, displaying the predictive loading, p1 (ctr) and is colour-coded according to the correlation scaled loading, p1 (corr).(301) The S-line peaks for metabolites that strongly discriminate sample classes have high loading values (p1 (ctr) ≥ 0.5) and a red to orange-red colour. A Mann-Whitney U test was used to assess statistical significance of metabolite discrimination between the two classes at each time point.

Identification of discriminatory metabolites

S-line plots were generated from OPLS-DA models in SIMCA to highlight areas of NMR spectra related to the discriminating metabolites between two classes. S-line plots appear as pseudospectrums generated from NMR spectral data in SIMCA. The areas of the spectrum that correlate to class-separation data are colour-coded depending on correlation scaled loading, p_1(corr) e.g. red/orange colour indicate strongly discriminating variables, green/yellow colour indicate moderate discriminator.(302)

Statistical Total Correlation Spectroscopy

Discriminatory NMR peaks represented in the S-line plots, were used as driver peaks for the statistical total correlation spectroscopy (STOCSY®) which was developed by the Nicholson group at Imperial College, London UK. It is a method for determining which NMR signals arise from the same molecule. STOCSY correlates the intensities of the peaks across samples .(303) Colour-coding was used to show the degree of correlation between the driver peak and each variable in the spectrum. A strong

correlation indicates that variables are likely to belong to the same molecule. A weak correlation may indicate molecules belonging to the same metabolic pathway as metabolite of interest. Highly correlated NMR peaks represent a molecular fingerprint for the metabolite of interest which can then be used to identify the molecule.(304)

The peaks in the Glucose spectrum from a high BMI maternal serum in Figure 2.7 represent the different environments of ^{1}H atomic nuclei within the molecule. The peaks are at different points since electrons surrounding the hydrogen nuclei in the molecule attenuate the effect of the external magnetic field, and the radio frequency to achieve resonance.

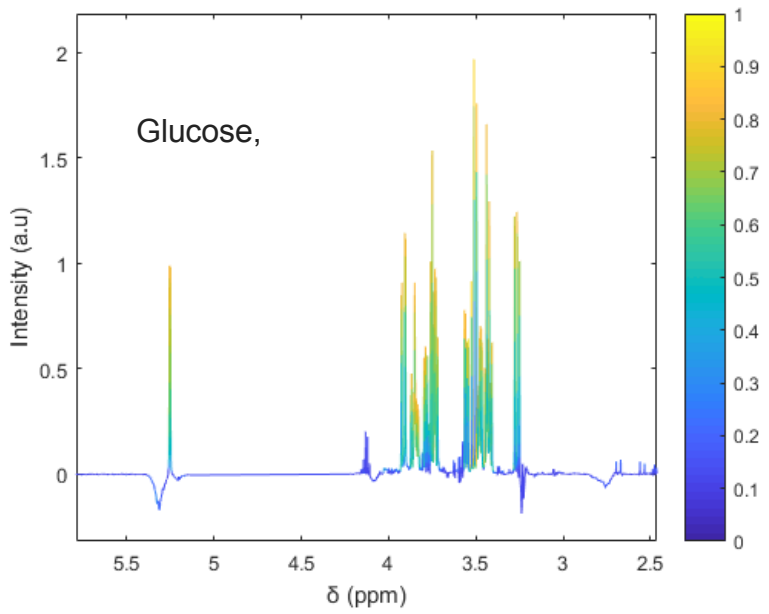


Figure 2.7: 1H NMR plot of Glucose following a STOCSY analysis.

As previously discussed, the 2D NMR JRES experiment was used to improve signal resolution to aid metabolite identification when results were equivocal. The 2D ^{13}C, ^{1}H Heteronuclear Single Quantum Correlation (HSQC) NMR Spectroscopy experiment was also used for metabolite identification. It provides chemical shifts for the carbon nuclei attached to each proton, providing further information on molecular structure of each metabolite of interest. The nuclei are attached via one bond carbon-proton coupling (^{1}JC-H).(305) Spiking in known internal standards also helped to confirm debated metabolite assignments.

Each metabolite assignment was cross-referenced with an in-house reference database. External databases were also used to assist with metabolite identification, including the Chenomx NMR Suite software (Chenomx, Edmon-ton, Canada) and the Human Metabolome Database version 4.0 (www.hmdb.ca).

Statistical analyses were performed by Dr Chidimma Kanu under the supervision of Miss Makrina Savvidou. The statistical analyses of the metabolomics and lipoprotein data were performed by Dr Chidimma Kanu under supervision of Dr Kiana West and Dr Frances Jackson.

CHAPTER 3

Maternal Biophysical Profile and Insulin Resistance at 28 weeks gestation in pregnancy following Bariatric Surgery.

ABSTRACT

Objective: To evaluate the maternal insulin resistance at 28 weeks of gestation in pregnancies following bariatric surgery compared to those without such surgery.

Methods: The study included 123 pregnant women at 28-30 weeks of gestation recruited from antenatal clinics at Chelsea and Westminster Hospital, London, from May 2015 until April 2017. 41 women had undergone a previous bariatric surgery (19 with restrictive and 22 with a malabsorptive) and 82 had no history of bariatric surgery but had similar early pregnancy body mass index (BMI). Maternal blood samples collected at the time of the oral glucose tolerance test (OGTT) were used for measurements of insulin, glucose, glycosylated haemoglobin (HbA1c), Homeostasis Model Assessment of insulin resistance (HOMA-IR) and the Matsuda Index.

Results: The median fasting glucose levels were higher in the no surgery group, [4.58mmol/L (IQR 4.22-4.92)] compared to the post bariatric surgery (malaborptive and restrictive) group [4.25mmol/L (IQR 4.12-4.61); $p < 0.05$] and post malabsorptive bariatric surgery group [4.14mmol/L (IQR 4.02- 4.30); $p < 0.05$]. The median fasting insulin levels were higher in the no surgery group [11.10u/mL (IQR 8.34-15.92)] compared to post bariatric surgery [6.30mu/mL (IQR 5.57-9.24); $p < 0.05$] and post malabsorptive bariatric surgery [5.90mu/mL (IQR 5.35-6.30); $p < 0.05$]. The no surgery group also had higher median insulin resistance (IR), [2.20 (IQR 1.53-3.38)] compared to the post bariatric surgery group [1.15 (IQR 1.04 -2.07),;$p < 0.05$] and post malabsorptive bariatric surgery group, [1.08 (0.99 – 1.23;, $p < 0.05$].

There was no significant difference in the HbA1c and Matsuda index between groups.

Conclusion: Our study has demonstrated that pregnancy following bariatric surgery is associated with improvement in maternal fasting insulin, glucose and IR, compared to BMI-matched no surgery group. This suggests that the positive effects of bariatric surgery extends beyond what would be expected from weight loss alone.

3.1 INTRODUCTION

In the general adult population, bariatric surgery has proven to be of tremendous benefit in obese T2DM patients; improving sensitivity to insulin, correcting glycaemic control and leading to diabetes remittance. (306)

There is strong evidence that pregnancy following bariatric surgery is associated with improved perinatal outcomes including a significant reduction in maternal complications such gestational diabetes (GDM) and pre-eclampsia (PE). Neonatal outcomes are also affected with a lower incidence of large for gestational age (LGA) infants. However, there is a reported higher risk of small for gestational age (SGA) infants and moderately preterm birth compared to women with similar BMI. (307) The aetiology behind these changes in pregnancy outcomes is still unknown.

There are several studies in post bariatric participants, outside the context of pregnancy, that show a significant reduction in post-operative insulin resistance. (Supplementary table 2).

Our study asks this question: Is the improvement in insulin resistance (IR) seen after bariatric surgery sustained in pregnancy? If so, this may provide a plausible explanation for the alterations in pregnancy outcomes.

The Homeostasis Model Assessment of IR (HOMA-IR) and the Matsuda index are indices used in research for the assessment of insulin sensitivity. HOMA IR is calculated from the level of fasting plasma glucose and insulin. It reflects hepatic insulin sensitivity, since the liver is responsible for most (75%) of endogenous glucose. (308)

In contrast, the Matsuda index calculation includes the postprandial plasma insulin and glucose levels, following an oral glucose load. This index reflects both hepatic and skeletal muscle insulin sensitivity since both are responsible for glucose uptake. (309)

The aim of the current study was to evaluate maternal IR, using both the HOMA-IR and the Matsuda index at 28 weeks of gestation in pregnancies following bariatric surgery compared to those without such surgery but similar early pregnancy BMI.

3.2 MATERIALS AND METHODS

This is a prospective study investigating the effect of bariatric surgery on maternal and fetal/neonatal outcomes. Women were recruited from Chelsea and Westminster Hospital from May 2015 until April 2017.

Written informed consent was obtained from all women. All data collected was recorded on our electronic database. Maternal biophysical profile was obtained, as previously described in Chapter 2. In light clothing and no shoes, maternal weight (in Kilograms) and height (in Centimetres) were measured using Calibrated Marsden weighing scales and a manual Stadiometer. BMI was calculated as weight (kg)/ height $(m)^2$. The waist to hip ratio (WHR) was measured in centimeters with a measuring tape using the following landmarks:

1. Waist: The apex of the iliac crests.

2. Hips: The greatest protuberance of the buttocks at the level of the pubic symphysis.

Maternal blood pressure (BP) was measured seated, using the left arm with an appropriately sized cuff. An automated BP device validated in pregnancy (Microlife WatchBP, Taipei, Taiwan) was used. (310) The average of two BP readings taken at 5 minute intervals was recorded.

Women without previous bariatric surgery then underwent a 75gr oral glucose tolerance test (OGTT) at 28-30 weeks of gestation. The GDM screening method was modified for women post gastric bypass surgery. This was in line with current research which highlighted inaccuracy in OGTT results following bariatric surgery. (311) Instead, these women were asked to do home glucose monitoring for 14 days.

A diagnosis of GDM was confirmed as per NICE guidelines:(312)

- A fasting plasma glucose level ≥ 5.6 mmol/litre or
- A 2-hour post prandial plasma glucose level ≥ 7.8 mmol/litre

All maternal fasting plasma and serum samples were collected, centrifuged and stored at -80°C, within 30mins of collection. Maternal blood (serum and plasma) was used for measurements of insulin, glucose and glycosylated haemoglobin (HbA1c), as described in Chapter 2.

The fasting glucose and insulin levels were used to calculate the Homeostasis Model Assessment of IR (HOMA-IR) using the formula:(313)

Fasting Insulin (microU/L) x fasting glucose (nmol/L) / 22.5

The mean plasma glucose and insulin level during the OGTT was used to calculate the Matsuda index using the formula: (314)

$$10{,}000 \div \sqrt{(\mathrm{GF} \times \mathrm{IF}) \times (\mathrm{Gmean} \times \mathrm{Imean})}$$

I_F – Fasting plasma insulin concentration (mIU/l),

G_F – Fasting plasma glucose concentration (mg/dl),

Gmean – Mean plasma glucose concentration during OGTT (mg/dl),

Imean – Mean plasma insulin concentration during OGTT (mU/l),

10,000– Simplifying constant to get numbers from 0 to 12.

√– Correction of the nonlinear values distribution.

Neonatal birth weight was measured immediately at birth (grams). The birth weight percentile formula was calculated using the gestational age and birth weight in Microsoft Excel. (315)

Statistical Analysis

The Kolmogoroff–Smirnoff test was used to assess normality of the data distribution. Statistics were used to summarize maternal characteristics. Quantitative variables were reported as mean +/- standard deviation or median (interquartile ranges). Qualitative variables were expressed as frequencies and percentages.

Unpaired t-test, Mann Whitney, and Chi-squared tests were used to compare the differences between values obtained from the no surgery and bariatric surgery group. Values, that were not normally distributed, were $\log_{10}$ transformed to make their distribution approximately Gaussian. The Pearson correlation coefficient was used to examine correlations between variables. Factors found to be significant predictors of maternal HOMA-IR on univariate analyses were entered into a multiple regression and those that remained significant were then used to calculate the adjusted maternal

HOMA-IR. The statistical software package SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA) was used for data analyses. Statistically significant differences had a p value < 0.05.

3.3 RESULTS

The study included 123 pregnant women at 28-30 weeks of gestation; 41 had undergone a previous bariatric surgery (19 with a gastric band or sleeve gastrectomy and 22 with a gastric bypass) and 82 had no history of bariatric surgery but had similar early pregnancy BMI. The maternal demographic, biophysical characteristics and pregnancy outcomes are given in Table 3.1.

Table 3.1: Maternal demographics, biophysical characteristics and pregnancy outcomes of the study participants according to bariatric surgery.

Characteristics	**No surgery (N=82)**	**Post-bariatric surgery (N=41)**	**Post-Restrictive (N=19)**	**Post-Malabsorptive (N=22)**
Maternal age (yrs)	30.29 ± 4.94	33.70 ± 4.72*	33.31 ± 4.33 *	34.04 ± 5.12 *
Parity, n (%)				
Nulliparous	52 (63.4)	20 (48.8)	10 (52.6)	10 (45.5)
Multiparous	30 (36.6)	21 (51.2)	9 (47.4)	12 (54.5)
Ethnic group, n (%)				
White	64 (78)	33 (80.5)	16 (84.2)	17 (77.3)
Other	18 (22)	8 (19.5)	3 (15.8)	4 (22.7)
Conception, n (%)				
Spontaneous	79 (96.3)	37 (90.2)	17 (89.5)	18 (81.8)
Assisted reproductive techniques	3 (3.7)	4 (9.8)	2 (10.5)	4 (18.2)
Smoking, n (%)				
No	78 (95.1)	35 (85.4)	17 (94.7)	19 (86.4)
Yes	4 (4.9)	6 (14.6)	1 (5.3)	3 (13.6)*
Time interval surgery-conception (months)	-	56.97 ± 31.80	45.63 ± 29.83	66.77 ± 30.77†
Body Mass Index prior to surgery (kg/m^2)	-	46.85 ± 8.20	42.48 ± 7.07	50.62 ± 7.28†
Booking Body Mass Index	33.90 ± 6.90	33.00 ± 5.21	33.30 ± 6.49	32.74 ± 3.94
Gestational age at OGTT	28.46 ± 0.73	28.32 ± 0.88	28.33 ± 1.01	28.32 ± 0.78
Body mass index at OGTT	36.25 ± 6.42	35.52 ± 5.10	35.88 ± 6.67	35.23 ± 3.52
Waist to Hip ratio at OGTT	0.95 ± 0.06	0.91 ± 0.07*	0.91 ± 0.09	0.91 ± 0.06*
Systolic blood pressure at OGTT (mmHg)	113.78 ± 12.23	108.63 ± 10.09*	109.23 ± 12.02	108.1 ± 8.51*
Diastolic blood pressure at OGTT (mmHg)	71.33 ± 9.54	67.97 ± 8.33	69.17 ± 9.32	67.00 ± 7.52
Gestational age at delivery (weeks)	39.41 ± 1.47	38.63 ± 2.25*	38.75 ± 2.14	38.53 ± 2.38 *
Mode of delivery				
Vaginal	27 (32.9)	13 (31.7)	7 (36.8)	6 (27.3)
Emergency Caesarean section	45 (54.9)	21 (51.2)	7 (36.8)	14 (63.6)
Elective Caesarean section	10 (12.2)	7 (17.1)	5 (26.3)	2 (9.1)
Birth weight (gr), median± IQR	3460 ± 812.5	3120 ± 733.5*	3100 ± 820*	3150 ± 720*
Birth weight percentile	57.88 ± 32.38	41.41 ± 30.62*	45.50 ± 32.85	37.88 ± 28.86 *
<10th percentile, n (%)	6 (7.31)	8 (19.51)	4 (21.05)	4 (18.18)
>90th percentile, n (%)	20 (24.39)	5 (12.19)	3 (15.78)	2 (9.09)
Gestational diabetes, n (%)				
No	74 (90.2)	37 (90.2)	15 (78.9)	22 (100)
Yes	8 (9.8)	4 (9.8)	4 (21.1)	0 (0)

Data are given as mean ± SD, n (%) or median ± IQR. All comparisons were made with the no surgery group. *p<0.05, † denotes comparison between the malabsorptive and restrictive group.

The post bariatric surgery group were older than the no surgery group. There was no significant difference between the groups with regard to other maternal demographic characteristics. The group of women who had undergone a malabsorptive procedure had higher pre-surgery BMI and longer surgery-to conception interval than women who had undergone a restrictive procedure. Post bariatric surgery women, and especially those following a malabsorptive procedure, had lower systolic BP and mean arterial pressure and lower waist to hip ratio compared to the no surgery group, at the time of OGTT (Figures 3.1-3.3) . Women in the bariatric surgery group, and especially those following a malabsorptive procedure delivered smaller babies, slightly earlier compared to women without surgery, as expected.

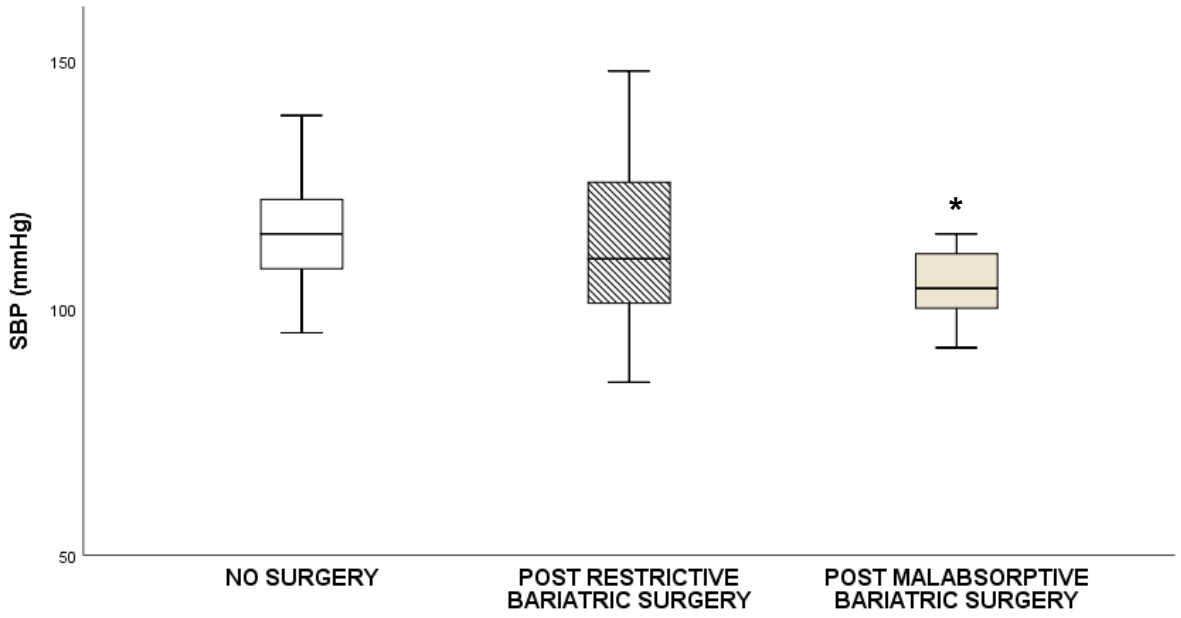


Figure 3.1 Maternal systolic blood pressure (SBP) at 28-30 weeks gestation in women with and without different types of bariatric surgery. Box plots represent interquartile range with the middle line indicating the median value of SBP. The whiskers represent minimum and maximum SBP. White box, no-surgery group; striped box, restrictive bariatric surgery and grey box, malabsorptive bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p < 0.05$).

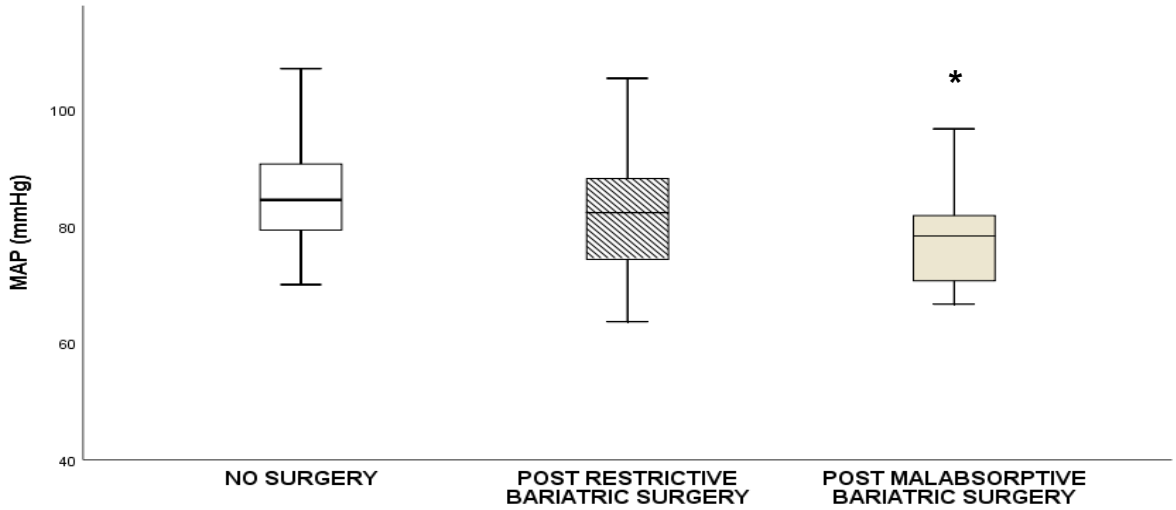


Figure 3.2 Maternal mean arterial pressure (MAP) at 28-30 weeks gestation in women with and without different types of bariatric surgery. Box plots represent interquartile range with the middle line indicating the median value of MAP. The whiskers represent minimum and maximum MAP. White box: no-surgery group; striped box restrictive bariatric surgery and grey box malabsorptive bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p < 0.05$).

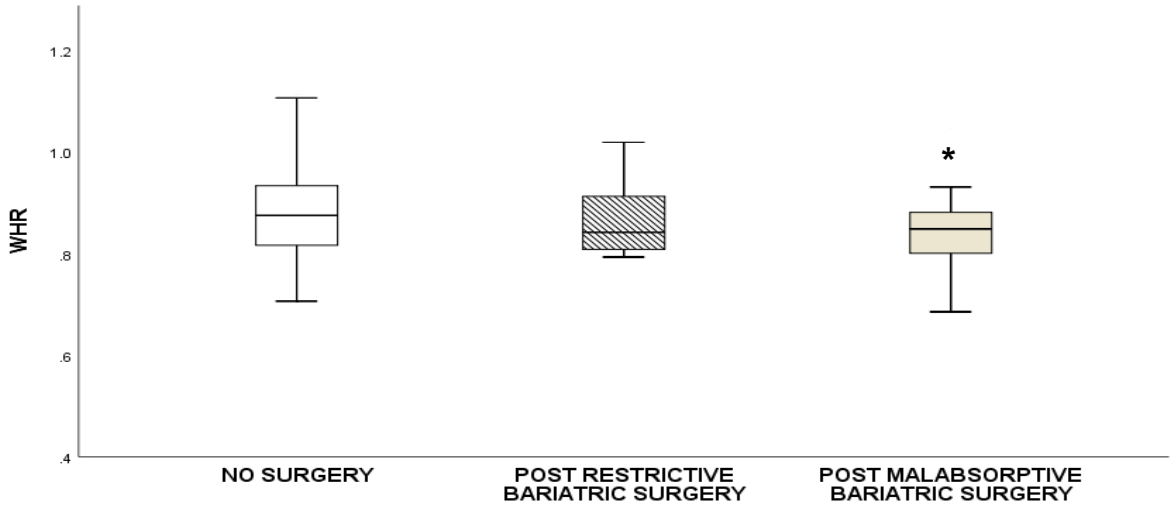


Figure 3.3 Maternal waist to hip ratio (WHR) at 28-30 weeks gestation in women with and without different types of bariatric surgery. Box plots represent interquartile range with the middle line indicating the median value of WHR. The whiskers represent minimum and maximum WHR. White box: no-surgery group; striped box restrictive bariatric surgery and grey box malabsorptive bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p < 0.05$).

Maternal Insulin Resistance

The maternal levels of fasting glucose, fasting insulin, IR, HbA1c and Matsuda index, at the time of the OGTT are given in Table 3.2 and Figures 3.4 to 3.9.

The post-bariatric surgery women, and especially the malabsorptive group had lower fasting glucose, insulin and IR, as assessed by HOMA-IR, compared to the no surgery group. However, there was no significant difference in the HbA1c and Matsuda index between groups. In the univariate analyses, maternal age (p=0.03), BMI at booking (p<0.001) and development of GDM (p=0.003) were found to be significant predictors of maternal HOMA-IR. Using these significant predictors, a multiple regression was performed and maternal BMI at booking (p<0.001) and development of GDM (p=0.01) remained significant predictors of HOMA-IR. These were then used to calculate an adjusted maternal HOMA-IR, which was still significantly lower in the post-bariatric compared to the no surgery group (Table 3.2).

Table 3.2: Maternal glucose, insulin, insulin resistance, as assessed by HOMA-IR, HbA1c and Matsuda index at 28-30 weeks of gestation.

	No surgery (N=82)	**Post-bariatric surgery (N=41)**	**Post-Restrictive (N=19)**	**Post-Malabsorptive (N=22)**
Fasting glucose (mmol/L)	4.58 (4.22-4.92)	4.25 (4.12-4.61)*	4.55 (4.23-5.25)	4.14 (4.02- 4.30)*
Fasting insulin (mu/mL)	11.10 (8.34-15.92)	6.30 (5.57-9.24)*	8.40 (6.10-16.25)	5.90 (5.35-6.30)*
HOMA-IR	2.20 (1.53 -3.38)	1.15 (1.04 – 2.07)*	1.65 (1.11-3.42)	1.08 (0.99-1.23) *
Adjusted HOMA-IR†	2.10 (1.71-2.83)	1.40 (1.14-1.93)*	1.83 (1.43-2.08)	1.29 (0.89-1.43)*
HbA1c (%)	31.54 ± 4.08	32.44 ± 3.90	33.17 ± 5.04	31.85± 2.65
Matsuda Index	6.43 (3.28-13.62) (*n*=57)	9.30 (4.29-21.45) (*n*=34)	9.30 (3.95-38.62) (*n*=15)	9.40 (4.38-20.80) (*n*=19)
Gestational diabetes, n (%)				
No	74 (90.2)	37 (90.2)	15 (78.9)	22 (100)
Yes	8 (9.8)	4 (9.8)	4 (21.1)	0 (0)

Data are given as mean ± SD or as median (interquartile range) for normally and not normally distributed values respectively. All comparisons were made with the no surgery group. *p<0.05; HOMA-IR: homeostatic model assessment of insulin resistance, HbA1c: glycosylated haemoglobin. †Values are adjusted for maternal booking body mass index and development of gestational diabetes.

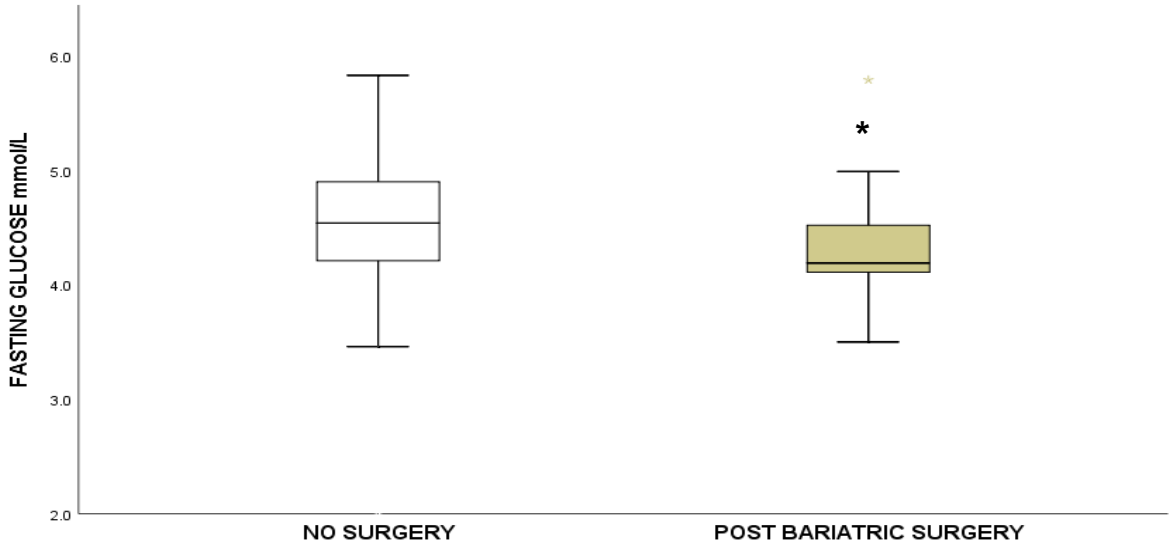


Figure 3.4 Maternal fasting glucose at 28-30 weeks gestation in women with and without previous bariatric surgery. Box plots represent interquartile range with the middle line indicating the median value of fasting glucose. The whiskers represent minimum and maximum fasting glucose levels. White box: no surgery group; Light brown box: bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p < 0.05$).

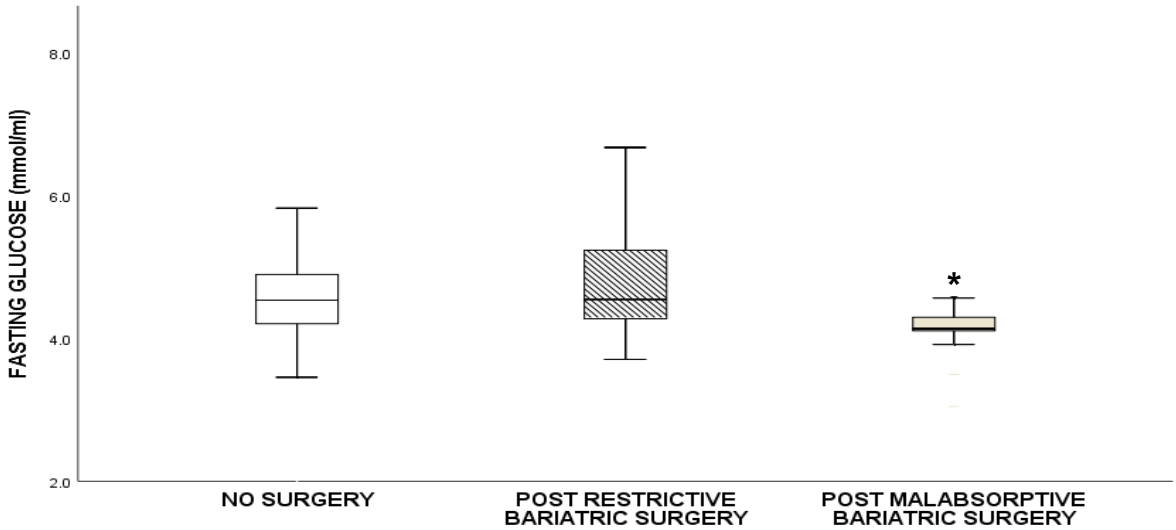


Figure 3.5 Maternal fasting glucose at 28-30 weeks gestation in women without and with previous different types of bariatric surgery. Box plots represent interquartile range with the middle line indicating the median value of fasting glucose. The whiskers represent minimum and maximum fasting glucose levels. White box: no-surgery group; striped box: restrictive bariatric surgery and grey box: malabsorptive bariatric surgery group. Asterix (*) indicates a statistical significant

difference between groups ($p < 0.05$).

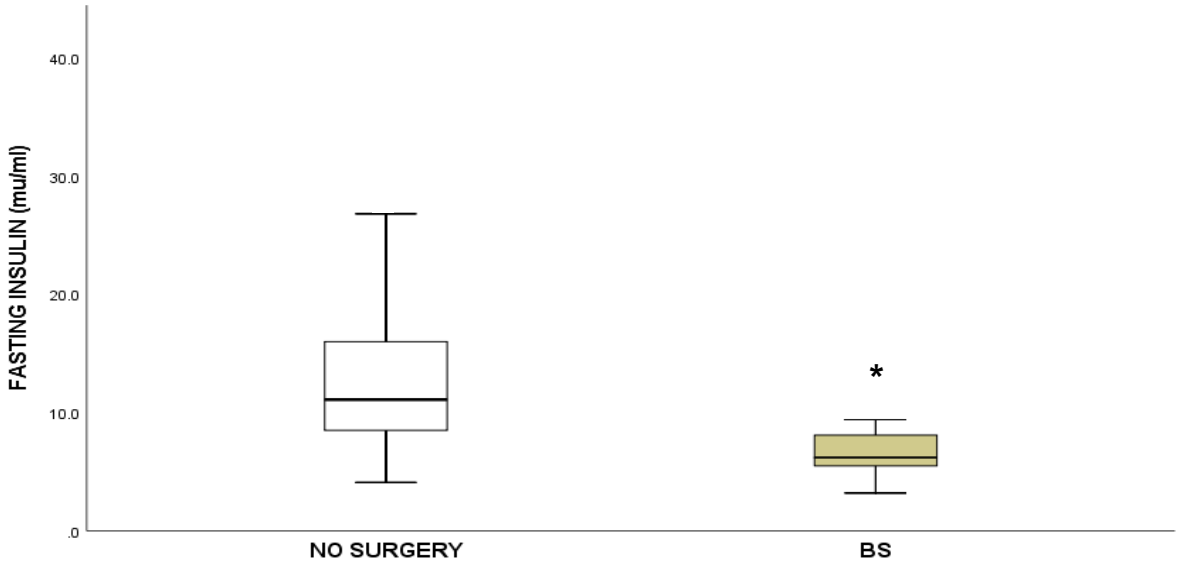


Figure 3.6 Maternal fasting insulin at 28-30 weeks gestation in women with and without previous bariatric surgery Box plots represent interquartile range with the middle line indicating the median value of fasting insulin. The whiskers represent minimum and maximum fasting insulin levels. White box: no surgery group; Light brown box: bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p < 0.05$).

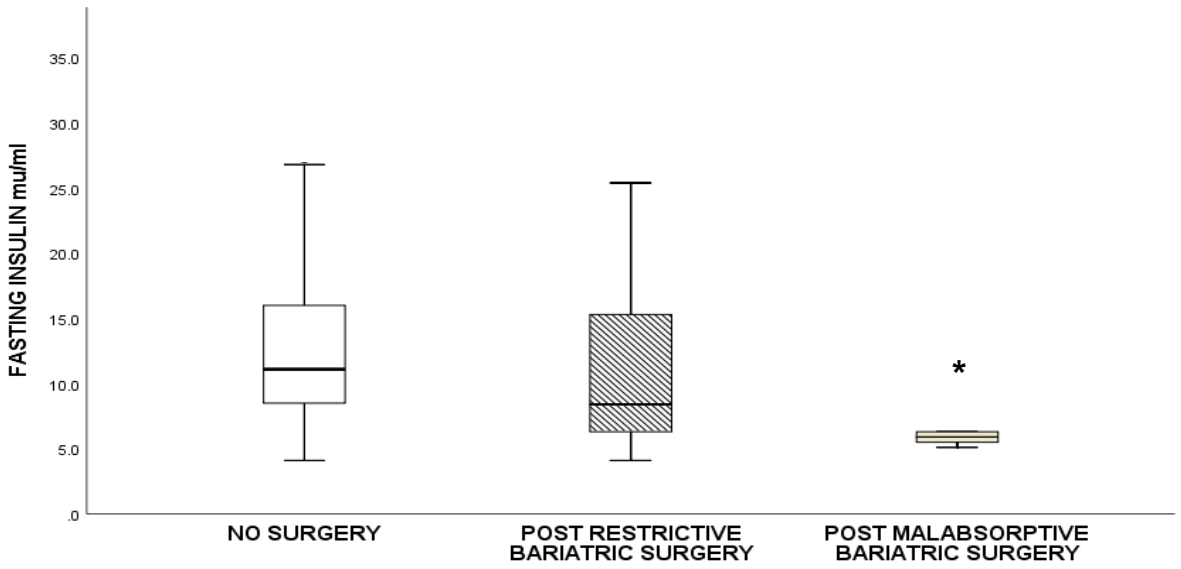


Figure 3.7 Maternal fasting insulin at 28-30 weeks of gestation in women without and with previous different types of bariatric surgery. Box plots represent interquartile range with the middle line indicating the median value of fasting insulin. The whiskers represent minimum and maximum fasting insulin levels. White box: no-surgery group; striped box restrictive bariatric surgery and grey box malabsorptive bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p < 0.05$).

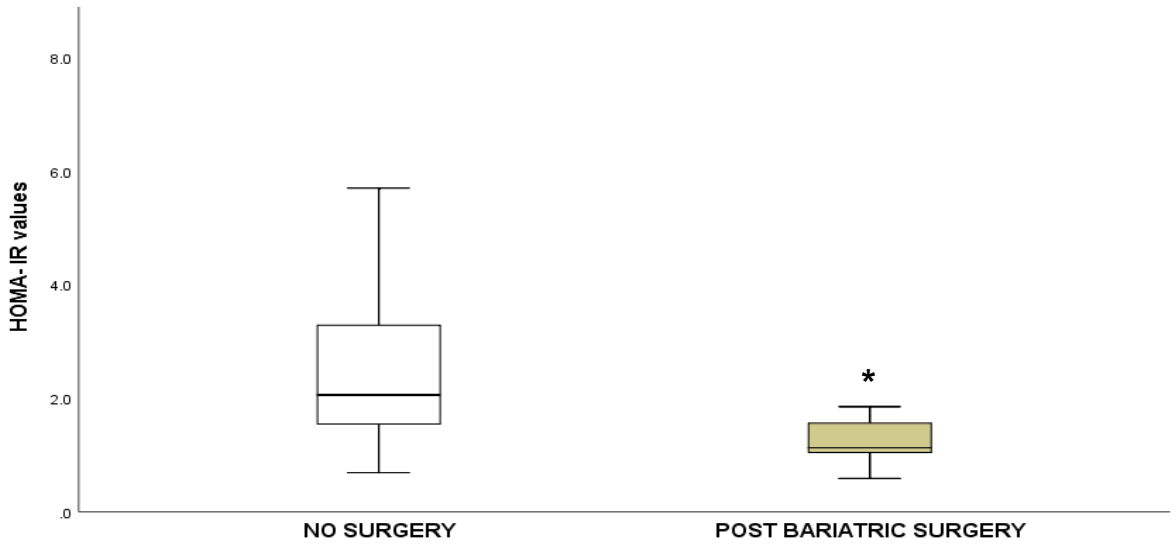


Figure 3.8: Maternal insulin resistance measured by HOMA-IR at 28-30 weeks gestation in women with and without previous bariatric surgery. Box plots represent interquartile range with the middle line indicating the median HOMA-IR value. The whiskers represent minimum and maximum HOMA-IR values. White box: no surgery group; Light brown box: Post bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p < 0.05$).

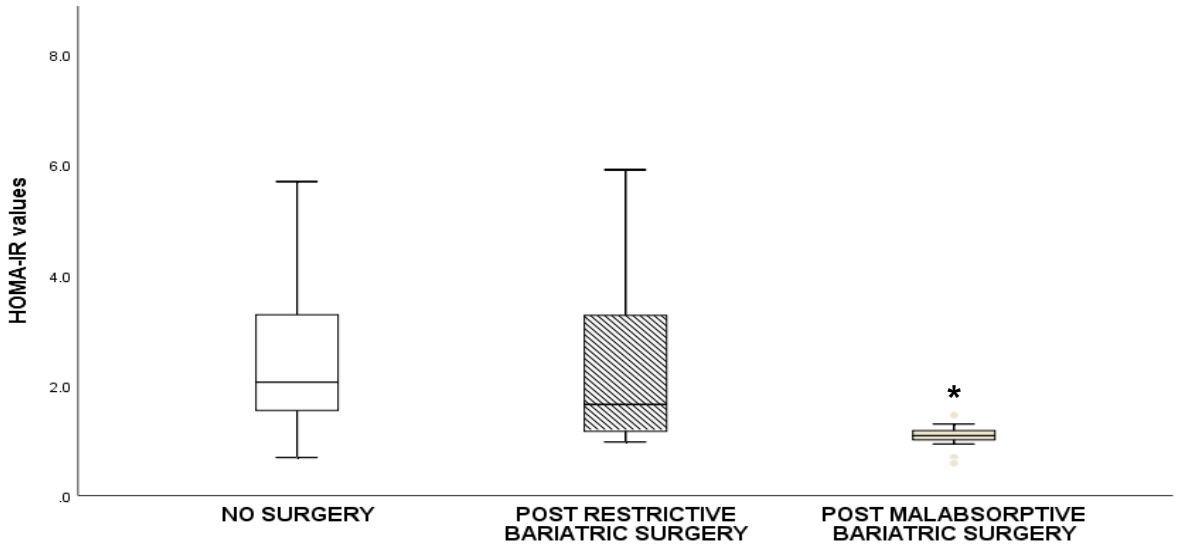


Figure 3.9: Maternal insulin resistance measured by HOMA-IR at 28-30 weeks of gestation in women with and without previous different types of bariatric surgery.

Box plots represent interquartile range with the middle line indicating the median value of HOMA-IR. Minimum and maximum fasting insulin levels represented by the whiskers. White box: no surgery group; striped box restrictive bariatric surgery and grey box malabsorptive bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p < 0.05$).

3.4 DISCUSSION

In this study, we have shown that pregnant women with previous bariatric surgery have better insulin sensitivity compared to BMI-matched pregnant woman without bariatric surgery. This is reflected by the lower maternal fasting insulin, glucose and IR seen in the post bariatric surgery group.

Studies of non-pregnant post bariatric adults, particularly following malabsorptive bariatric surgery, have linked the improved insulin resistance to calorie restriction and weight loss. (316) A recent meta-analysis of RCTs comparing RYGB and sleeve gastrectomy support this finding of a greater effectiveness of RYGB on improving IR in the short and long term (52 months) in non-pregnant participants. (317)

Our study also found malabsorptive bariatric surgery (RYGB) was superior to restrictive bariatric surgery (gastric band and sleeve gastrectomy) in improving IR in a pregnant cohort.

The reasons for this difference between malabsorptive and restrictive surgery are yet to be defined. It is plausible that there is a greater physiological and metabolic impact following bypass surgery compared to restrictive where the anatomy of the small intestine is unaltered. In gastric band surgery, for example, the stomach is structurally intact and so the ability to produce gastric hormones such as ghrelin preserved.

There are reports of improvement in IR within a week following bariatric surgery, long before significant weight loss occurs. This supports the notion that other mechanisms play an important role in reducing IR most likely as a result of the altered gastrointestinal tract anatomy. It is reported in literature that there are post bariatric changes in endocrine and adipose secretion patterns, for example an increased postprandial secretion of GLP-1 receptor agonist, signalling increased insulin secretion from beta pancreatic cells. (318)

Normal pregnancy is a state of insulin resistance which is further exacerbated by obesity. This physiological adaptation is thought to enable the mother to meet the needs of the growing fetus, delivering enough quantity of nutrients. (319) Insulin resistance in normal pregnancy is related to a decrease in the post-receptor insulin signalling cascade, specifically decreased insulin receptor substrate 1 tyrosine phosphorylation. (320) The placenta contributes inflammatory agents via

macrophages and also produces human placental lactogen that also plays a key role in inducing maternal insulin resistance. (321) These processes are known to be even more pronounced in pregnancies associated with maternal obesity where maternal insulin resistance is expected to rise even more. (322)

There are no previous studies assessing the maternal glucose haemostasis or IR in pregnancies following bariatric surgery apart from one study reporting that women who had resolution of T2DM following bariatric surgery, did not develop GDM in subsequent pregnancy despite risk factors and high BMI. (323) Certainly, our findings of lower maternal IR are likely to contribute to the reduction in the prevalence of GDM seen in pregnancies following bariatric surgery. (324) It is of interest that the maternal values of IR, reported in our study, are similar to the values reported in post bariatric surgery individuals outside the setting of pregnancy questioning the ability of these women to respond “normally” to the metabolic challenges posed by the pregnancy itself. (325) Lower maternal glucose levels may lead to reduced glucose availability for the developing feto-placental unit, which is turn could be linked to other pregnancy complications seen in pregnancies following bariatric surgery such as reduced fetal growth. (326)In the current study we also found the maternal WHR to be significantly reduced in the post-bariatric group, in particular the malabsorptive subgroup, compared to the no surgery one. Maternal fat distribution in pregnancy is related to metabolic adaptations, including IR which leads to higher levels of triglycerides, lipids and elevated leptin. As normal pregnancy advances, the result is preferential deposition of adipose tissue in the visceral compartment. (327) Pre-pregnancy central obesity that is carried into pregnancy exaggerates this maternal pattern of adipose distribution. The observed reduction in WHR, a measure of central obesity, post bariatric surgery corroborates with other studies outside of pregnancy. These studies report that the lower WHR is associated with reduced risk of cardiovascular disease and improved lung function reducing hypoventilation and sleep apnoea. (328) Although mechanisms for this finding remains unknown, it may be the result of improved IR caused by alterations of other GI hormone secretion seen after bariatric surgery.(329)

The systolic BP in the post-bariatric surgery group was also noted to be significantly reduced compared to the no surgery one. Lower systolic BP is a recognised finding in non-pregnant obese adults following bariatric surgery, reducing the risk of coronary heart disease in this high risk population. (330) The exact mechanism for this is still

unknown. Outside pregnancy, it is proposed that the early improvement in post bariatric surgery BP is related to an increased parasympathetic influence resulting in a reduced ventricular contractility and cardiac output. (331) Insulin and leptin have also been shown to cause vasodilation which could explain the early reduction in cardiac output since their levels rise after bariatric surgery. (332)

The maternal cardiovascular system progressively adapts during pregnancy. Maternal cardiac output rises by 40% throughout pregnancy. This is mediated by an increase in stroke volume and maternal heart rate by 10-20bpm. There is a 25–30% fall in systemic vascular resistance as a result of peripheral vasodilation caused by prostaglandins and progesterone. (333) Blood pressure (both diastolic and systolic) is proportional to systemic vascular resistance and cardiac output. It decreases in early pregnancy until 22-24 weeks gestation and by the third trimester, the increased cardiac output compensates for the fall in peripheral vascular resistance leading to a rise in the BP to pre-pregnant levels. (333) It appears that the post bariatric surgery group maintain a lower systolic BP by 28-30 weeks when the effect of raised cardiac output would normally compensate for the reduced peripheral vascular resistance. The mechanism for this is not clear but it is likely that weight loss and other metabolic and physiological alterations may affect the cardiac output or vasodilatory process. These findings would be consistent with the reported lower incidence of hypertensive disorders in pregnancy following bariatric surgery. (334)

The main strength of the study is its novelty. This is the first time that insulin resistance in pregnancy following bariatric surgery has been investigated. Limitations include the small sample size and the use of a non-invasive method, HOMA-IR, to estimate IR instead of the euglycaemic clamp which is regularly used in the literature. It is likely that this invasive method would not have been very acceptable in this population and there is evidence that results are comparable. (335)

3.5 CONCLUSION

This study has demonstrated that pregnancy following bariatric surgery, especially after a malabsorptive procedure, is characterised by a reduction in maternal IR, compared to pregnant women without surgery but similar BMI.

Our findings concur with findings outside the setting of pregnancy and may explain the lower incidence of GDM in this population. We have also shown a reduction in maternal waist to hip ratio and systolic BP in the post-bariatric pregnant women which may explain the lower incidence of hypertensive disorders in pregnancy in this population.

Further studies are warranted to confirm and investigate the clinical importance of our findings.

CHAPTER 4

Maternal Metabolic Profile at 28 weeks gestation and Post-delivery in pregnancy following Bariatric Surgery

ABSTRACT

Objective: To investigate the differences in the metabolic profile of pregnant women with and without previous bariatric surgery.

Methods: The study included 10 pregnant women post bariatric surgery who were matched by their early pregnancy body mass index to 10 women without surgery. Maternal bloods were taken on two occasions at 28-30 weeks of gestation (fasting bloods) and within 72 hours of delivery. Using multiplex Bio-Plex Pro Human Diabetes Panel 10-Plex, IL6 and Adiponectin Assay and Bio Plex Pro Cytokine Assay (BIO-RAD USA) peptides (C-peptide, glucagon. ghrelin), adipokines (resistin, visfatin, leptin, adiponectin), pro-inflammatory hormones (interleukin 6, Monocyte Chemotactic Protein -1 or chemokine (C-C motif) ligand 2, plasminogen activator inhibitor-1 , C-C motif Ligand 5 or RANTES and incretins (glucose-dependent insulinotropic polypeptide (GIP), glucagon-like Peptide-1) were measured. Pregnancy outcomes were obtained from the hospital database and birthweight (BW) was recorded at birth.

Results: Pregnant women with previous bariatric surgery had significantly lower leptin levels at 28-30 weeks [13.3ng/ml (IQR 9.71-15.36)] compared to women with no surgery [20.84ng/ml (IQR 18.12-24.1); $p<0.05$]. Post bariatric women also had lower leptin levels at delivery, [11.4ng/ml (IQR 8.5-24.8)] compared to women with no surgery [27.4ng/ml (IQR 19.9-30.6); $p<0.05$]. Conversely, maternal adiponectin levels at 28-30 weeks of gestation were higher in the post bariatric women [4.9µg/ml (IQR 2.9-6.7)] compared to no surgery women [2.43 µg/ml (IQR 1.8-3.2); $p <0.05$]. However, adiponectin levels after delivery were not different between post-bariatric and no surgery women [2.6 µg/ml (IQR 2.2-3) versus 1.6 µg/ml (IQR 1.0-2.8); $p = 0.2$]. There was a positive correlation between serum GIP and adiponectin with BW in women without surgery ($p = 0.01$ for both). There was no correlation between the biomarkers investigated and BW in women post bariatric surgery.

Conclusion: This study has shown that in the early third trimester, pregnant women with previous bariatric surgery have lower leptin and higher adiponectin levels compared to pregnant women without surgery. In women without bariatric surgery,

GIP and adiponectin have a positive correlation with BW which is not seen in post bariatric women. Larger studies are warranted to confirm these findings and their clinical relevance.

4.1 INTRODUCTION

According to the recent MBRRACE report, obese and overweight women account for over half of maternal deaths in the UK**.** (336) Bariatric surgery, prior to pregnancy, reduces the incidence of serious complications such as PE, GDM, and LGA babies compared to women without surgery. (337) The exact mechanism for this benefit is unknown. Our work has shown an improvement in maternal insulin sensitivity in pregnancy after bariatric surgery, however the full mechanism is likely to be multifactorial.

There are other classes of metabolic biomarkers, besides peptide hormones like insulin, which are essential in normal metabolism in pregnancy. These include adipokines, pro-inflammatory factors and incretins. These biomarkers are also implicated in obesity-related pregnancy complications and therefore warrant further investigation to identify alterations in their levels during pregnancy following bariatric surgery.

In normal pregnancy, peptide hormones such as insulin, C-peptide, glucagon and ghrelin rise indicating reduced insulin sensitivity. (338) This is driven by progesterone, oestrogen and human placental lactogen, promoting the supply of glucose to the developing fetus. (339) Adipokines produced by the placenta and adipocytes enhance insulin sensitivity, except resistin which increases insulin resistance (IR). (340) Resistin, visfatin and leptin levels rise in normal pregnancy while adiponectin decreases. (341) As far as pro-inflammatory markers is concerned, interleukin 6 (IL 6) has pro- and anti-inflammatory function, rises in pregnancy and is involved in implantation and parturition. (342) Monocyte Chemotactic Protein -1 or chemokine (C-C motif) ligand 2 (MCP-1/CCL2) has a role in normal trophoblastic invasion**.** (343) Plasminogen activator inhibitor-1 (PAI-1) is an important inhibitor of fibrinolysis, plays a role in trophoblast invasion and an increasing amount is expressed in maternal plasma**.** (344) C-C motif Ligand 5 (CCL5) or RANTES (regulated on activation, normal T cell expressed and secreted) is considered to play a significant role in implantation. (345) Finally, incretins such as Glucose-dependent insulinotropic polypeptide (GIP) and Glucagon-like Peptide-1 (GLP-1) can both stimulate insulin secretion in response to food ingestion and in normal pregnancy, GLP1 plays an important role in the reversible pregnancy-induced increase in pancreatic beta-cell mass. (346)

This study aims to identify alterations in these biomarkers (peptides, adipokines, pro-inflammatory and incretins) in women who embark on pregnancy following bariatric surgery. This may provide further insight to the mechanism behind the improvement in pregnancy outcomes seen in this group.

4.2 MATERIALS AND METHODS

This was a pilot study investigating the effect of bariatric surgery on the maternal metabolic profile. The participants were a subgroup of pregnant women already recruited to the prospective study described in Chapter 3.

Written informed consent was obtained from all women. Blood samples were obtained from pregnant women with previous bariatric surgery and no surgery at 28-30 weeks gestation (fasting bloods at the time of the OGTT) and within 72 hours of delivery. The samples were centrifuged at 4600RPM for 10 mins and the serum or plasma removed and stored immediately at -80°C. The samples were subsequently thawed and analysed for measurements of peptides (C-peptide, glucagon and ghrelin), adipokines (resistin, visfatin, leptin and adiponectin), pro-inflammatory hormones (IL 6, MCP-1/CCL2, PAI-1 and CCL5 or RANTES) and incretins (GIP and GLP-1) using multiplex Bio-Plex Pro Human Diabetes Panel 10-Plex, IL6 and Adiponectin Assay and Bio Plex Pro Cytokine Assay (BIO-RAD USA). The exact methodology is described in Chapter 2.

Pregnancy outcomes were obtained prospectively, birthweight was recorded at birth and used to calculate BW percentiles. All data was recorded on an electronic database using Microsoft Excel. Women who developed pre-eclampsia, defined as new onset hypertension after 20 weeks with or without proteinuria or evidence of end organ damage were excluded. Similarly, we excluded women who developed GDM defined as fasting plasma glucose level ≥ 5.6 mmol/litre or 2-hour post prandial plasma glucose level ≥ 7.8 mmol/litre after 75g OGTT.

Statistical Analysis

The data was assessed for normal distribution using the Kolmogoroff–Smirnoff test. Quantitative variables were reported as mean +/- standard deviation or median

(interquartile ranges). Qualitative variables were expressed as frequencies and percentages. Unpaired t-test, Mann Whitney and chi square χ^2 tests were used to analyze the differences between values obtained from the no surgery and bariatric surgery groups. Pearson correlation coefficient was used for the bivariate analysis comparing the biomarkers with birthweight in the two groups.

Since this was a pilot study with no preliminary data for sample size calculations, power calculation was not performed.

The statistical software package SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA) was used for data analyses. Statistically significant differences had a p value < 0.05.

4.3 RESULTS

The study included 20 pregnant women; 10 women with previous bariatric surgery that were matched by their early pregnancy BMI to 10 women without surgery. The maternal demographics and pregnancy outcomes are given in Table 4.1.

Within the post-bariatric group, 6 women had a previous gastric bypass, 2 women had a previous gastric band and 2 women had previous sleeve gastrectomy. Both groups of women were of similar age and were all obese, BMI >30kg/m^2. There was no significant difference between the groups with regard their BMI, ethnicity and parity. Babies born to women with previous bariatric surgery were smaller compared to women without surgery, as expected. (347) None of the women developed GDM or PE.

Table 4.1: Maternal demographics and pregnancy outcomes of the study participants.
Data are given as mean ± SD or n. Comparisons were made with the no surgery group.

Characteristics	**Post-Bariatric surgery (N=10)**	**No Surgery (N=10)**	**P values**
Maternal age (years)	32.60 ± 4.65	32.00 ± 3.65	0.75
Parity, n			
Nulliparous	4	6	1.0
Parous	6	4	1.0
Ethnic group, n			
White	8	10	1.0
Other	2	0	-
Smoking, n	1	1	-
Conception, n			
Spontaneous	9	9	-
Assisted conception	1	1	-
BMI at booking (kg/m^2)	32.02 ± 3.07	31.84 ± 4.66	0.92
BMI at 28 weeks (kg/m^2)	34.39 ± 3.45	34.03 ± 3.50	0.82
Gestational age at delivery (wks)	39.70 ± 0.95	39.40 ± 0.70	0.43
Mode of delivery, n			
Vaginal	5	8	1.0
Emergency Caesarean section	6	1	1.0
Elective Caesarean section	0	1	-
Birth weight (gr)	3237.0 ± 376.2	3570.0 ± 511.9	0.12
Birth weight percentile	32.86 ± 24.2	59.47± 32.1	0.05

Maternal blood samples were obtained at 28-30 weeks of gestation and within 72 hours post-delivery. Maternal plasma levels of peptides, adipokines, pro-inflammatory hormones and incretins were measured and summarised in Tables 4.2-4.3 and Figures 4.1-4.4.

Table 4.2: Maternal plasma levels of peptides, adipokines, pro-inflammatory hormones and incretins at 28-30 weeks. Values are given as median (IQR). Asterix (*) indicates a statistically significant difference between groups ($p<0.05$).

	28-30 weeks Gestation				
Biomarker	**Post-Bariatric Surgery (N=10)**	**No Surgery (N=10)**	**P value**	**Correlation with Birthweight Post-Bariatric Surgery** **P value**	**Correlation with Birthweight No Surgery** **P value**
C-Peptide (ng/ml)	0.66 (0.48-1.00)	0.76 (0.63-1.41)	0.15	0.06	0.28
Ghrelin (ng/ml)	0.37 (0.23-0.52)	0.32 (0.30-0.35)	0.55	0.30	0.54
GIP (pg/ml)	170.40 (151.23-207.51)	205.43 (174.22-257.97)	0.05	0.10	0.38
GLP-1 (pg/ml)	438.01 (420.63-489.10)	465.18 (448.13-477.27)	0.26	0.27	0.45
Glucagon (pg/ml)	161.27 (149.30-172.73)	163.53 (133.71-172.40)	0.91	0.28	0.74
Leptin (ng/ml)	13.50 (8.74-16.01)	20.84 (16.34-24.69)	**0.03***	0.72	0.09
PAI-1 (ng/ml)	7.95 (6.91-11.13)	7.99 (6.56-10.72)	0.71	0.18	0.13
Resistin (ng/ml)	5.63 (4.68-7.79)	6.81 (5.73-7.82)	0.29	0.76	0.69
Visfatin (ng/ml)	1.96 (1.72-2.68)	2.25 (2.14-2.59)	0.29	0.11	0.57
IL6 (pg/ml/)	4.66 (3.46-12.16)	3.18 (1.64-9.50)	0.20	0.08	0.58
Adiponectin (µg/ml)	4.55 (2.63-7.12)	2.43 (1.65-3.33)	**0.03***	0.17	0.56
RANTES (ng/ml)	11.78 (7.02 – 13.38)	5.88 (4.058-10.45)	0.11	0.30	0.42
MCP-1 (pg/ml)	19.97 (12.71 – 22.74)	22.39 (15.71 – 29.78)	0.23	0.70	0.34

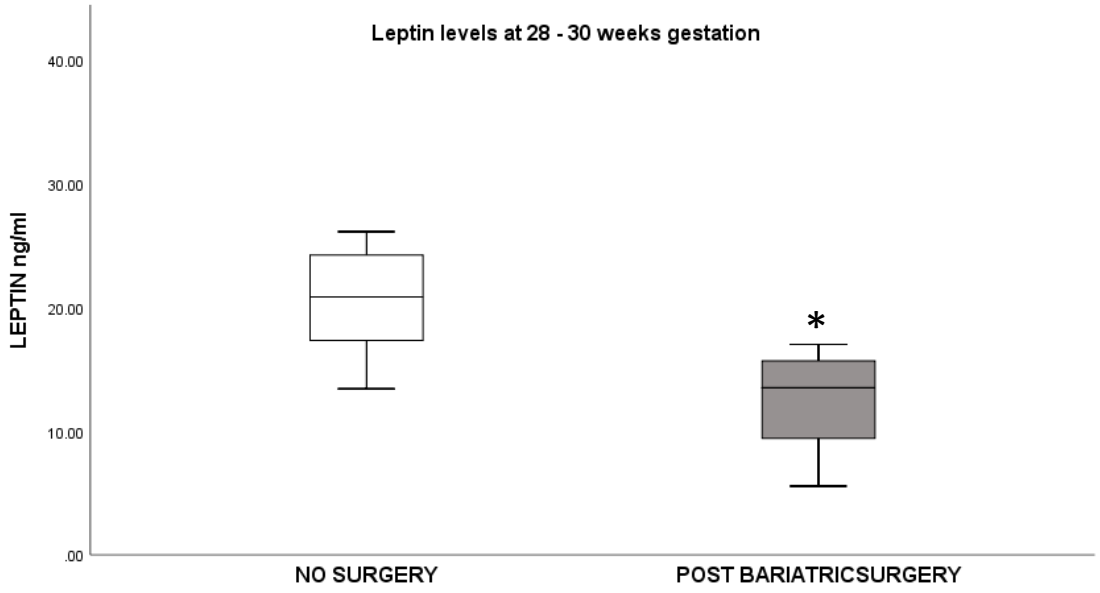
GIP: Glucose-dependent insulinotropic polypeptide, GLP-1: Glucagon-like Peptide-1, IL6: Interleukin 6, MCP-1: Monocyte Chemotactic Protein -1, PAI-1: Plasminogen activator inhibitor-1, RANTES: Regulated on activation, normal T cell expressed and secreted.

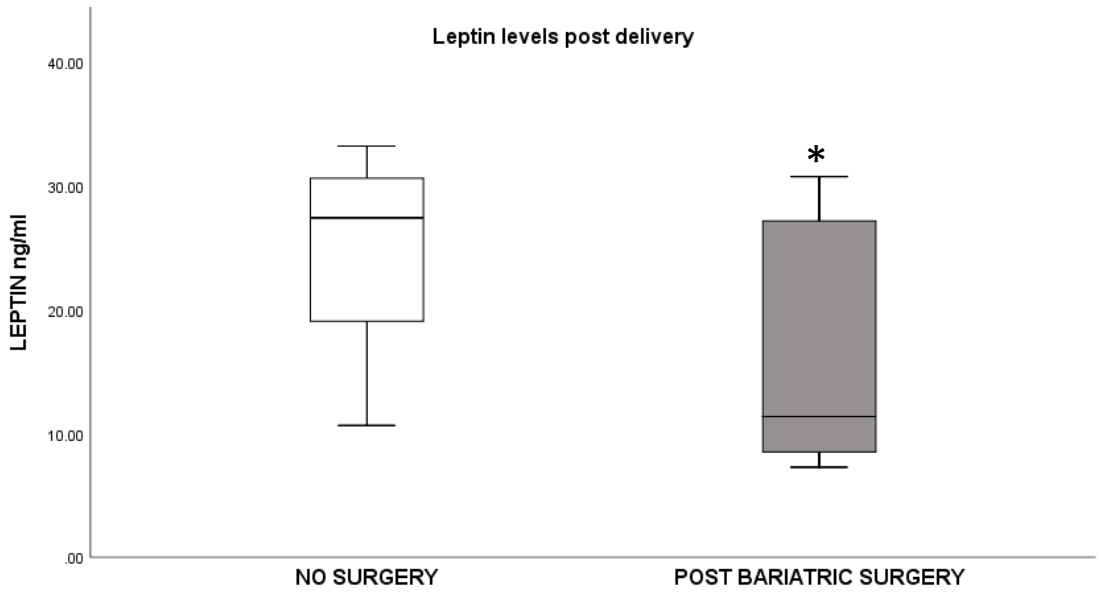
Table 4.3 Maternal plasma levels of peptides, adipokines, pro-inflammatory hormones and incretins post-delivery. Values are given as median (IQR). Asterix (*) indicates a statistical significant difference between groups ($p<0.05$).

	Post Delivery				
Biomarker	**Post-Bariatric Surgery (N=10)**	**No Surgery (N=10)**	**P value**	**Correlation with Birthweight Post-Bariatric Surgery** **P value**	**Correlation with Birthweight No Surgery** **P value**
C-Peptide (ng/ml)	1.25 (0.97-1.93)	1.46 (1.31-2.67)	0.17	0.62	0.15
Ghrelin (ng/ml)	0.25 (0.19-0.35)	0.36 (0.21-0.52)	0.17	0.50	0.18
GIP (pg/ml)	257.93 (177.35-529.10)	298.35 (157.33-703.322)	0.82	0.84	**0.01***
GLP-1 (pg/ml)	485.48 (436.81-546.26)	496.83 (462.09-521.36)	0.91	0.95	0.43
Glucagon (pg/ml)	172.53 (157.76-186.27)	164.72 (148.68-188.25)	0.68	0.99	0.90
Leptin (ng/ml)	11.36 (8.39-27.49)	27.41 (18.08-31.00)	**0.03***	0.57	0.70
PAI-1 (ng/ml)	7.39 (5.97-8.51)	5.17 (4.02-7.11)	0.10	0.82	0.64
Resistin (ng/ml)	9.26 (5.75-14.59)	9.01 (6.67 - 14.25)	0.68	0.63	0.44
Visfatin (ng/ml)	2.17 (1.83-3.04)	2.96 (2.32-3.33)	0.13	0.38	0.87
IL6 (pg/ml/)	23.19 (20.17-43.46)	14.43 (11.77-40.29)	0.26	0.41	0.68
Adiponectin (µg/ml)	2.64 (1.93-3.27)	1.64 (0.94-3.48)	0.20	0.51	**0.01***

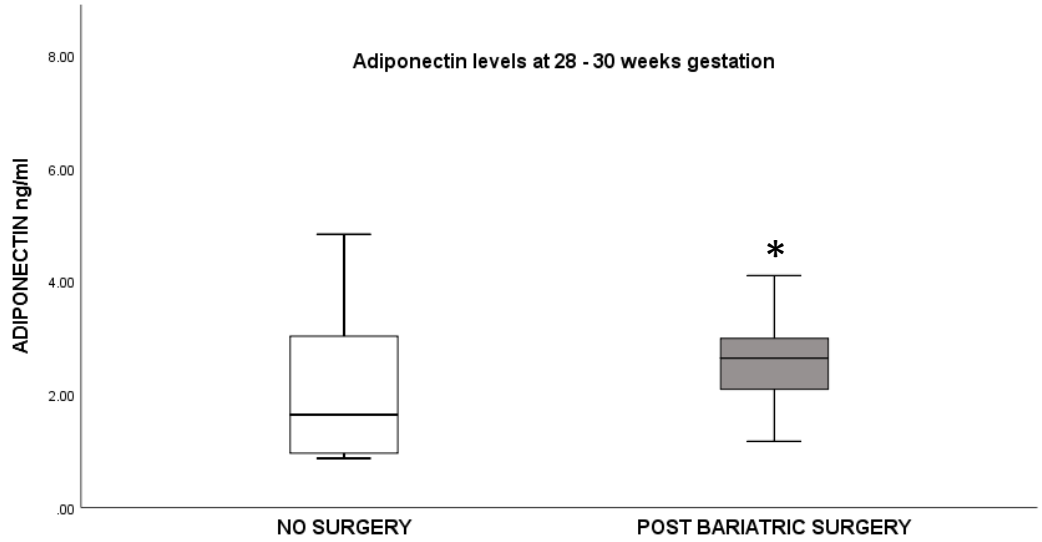
GIP: Glucose-dependent insulinotropic polypeptide, GLP-1: Glucagon-like Peptide-1, IL6: Interleukin 6; PAI-1: Plasminogen activator inhibitor-1.

Leptin, adiponectin and GIP showed a significant difference between the post bariatric surgery and no surgery women. In particular, post-bariatric pregnant women had significantly lower leptin levels at 28-30 weeks and post-delivery compared to women with no surgery ($p<0.05$) (Figures 4.1 and 4.2). Conversely, adiponectin levels were higher in the post bariatric group compared to no surgery one ($p<0.05$). This was also the case post-delivery, however this upward trend did not reach statistical significance ($p=0.2$), (Figures 4.3 and 4.4).





Figures 4.1 and 4.2: Maternal leptin levels at 28-30 weeks of gestation and post-delivery women without and with previous bariatric surgery (different types). Box plots represent interquartile range with the middle line indicating the median value of leptin. Minimum and maximum leptin levels are represented by the whiskers. White box: no surgery group and dark grey box bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p<0.05$).



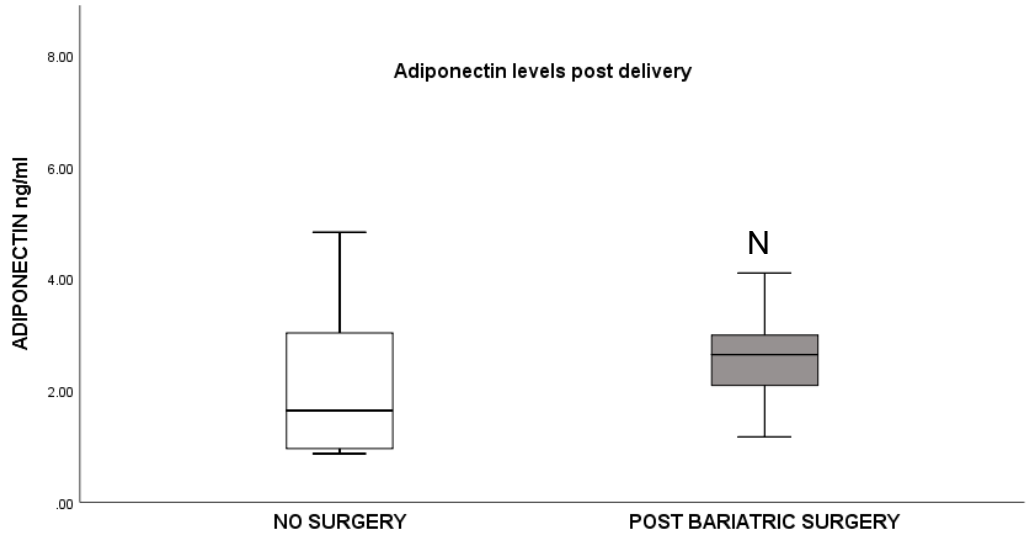


Figure 4.3 and 4.4: Maternal adiponectin levels at 28-30 weeks of gestation and post-delivery in women without and with previous bariatric surgery (different types). Box plots represent interquartile range with the middle line indicating the median value of adiponectin. Minimum and maximum adiponectin levels are represented by the whiskers. White box: no surgery group; dark grey box bariatric surgery group. Asterix (*) indicates a statistical significant difference between groups ($p < 0.05$). NS, indicates, a non-significant difference.

There was a positive correlation between serum GIP and infant BW from women without surgery ($p = 0.01$), however adiponectin had a negative correlation with BW from no surgery women compared women to those with previous bariatric surgery ($p=0.01$), Table 4.3 and Figure 4.5.

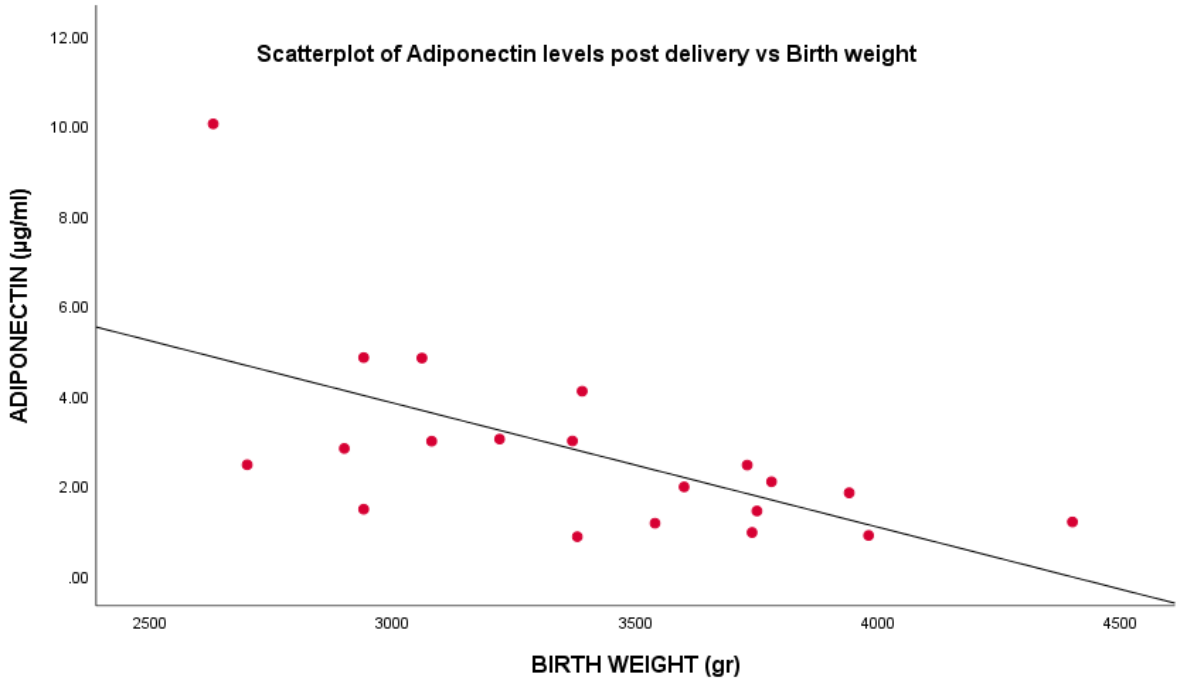


Figure 4.5: Scatter plot of post delivery maternal adiponectin levels versus birth weight in women with no surgery (p=0.01).

4.4 DISCUSSION

Our study has shown that pregnant women with previous bariatric surgery have lower fasting levels of leptin in the third trimester and at delivery, compared to women without bariatric surgery. We have also shown a higher level of adiponectin in the third trimester in women with previous bariatric surgery compared to women without surgery. There was a negative correlation between adiponectin levels at delivery and BW in women without previous bariatric surgery. We also identified a positive correlation between GIP levels at delivery and BW in women without surgery.

The data from this pilot study could go some way to explaining the known pregnancy outcomes observed post bariatric surgery such as a reduced incidence of GDM, PE and LGA infants and an increased incidence of SGA infants. (348)

Our findings of reduced maternal leptin and elevated adiponectin following bariatric surgery are in line with current literature outside the context of pregnancy. Lower leptin levels in post bariatric individuals is a reflection of reduced fat mass, a finding that has been reported following different types of bariatric surgery. (349) Reduced leptin sensitivity is a hypothesis offered to explain high levels of leptin seen in obese individuals. (350) Reduced fat mass is unlikely to completely explain the lower leptin in this cohort as the post bariatric women had the similar BMI as those without surgery. It is more plausible that post bariatric pregnant women also have an improved sensitivity to leptin, resulting in the lower levels observed.

Changes in adiponectin levels are inversely proportional to fat mass. (351) Higher adiponectin levels post bariatric surgery enhance insulin sensitivity similar to leptin. It also has additional benefits of anti-inflammatory and antioxidant properties. (352) High leptin and low adiponectin levels are associated with the onset of GDM and have been proposed as potential biomarkers to predict GDM development from early pregnancy. (353) The opposite findings were seen in our study of post bariatric pregnant women which could explain why the incidence of GDM is reported to be lower in this group.

The role of leptin and adiponectin in the pathogenesis of PE in the general population is not clear, with several conflicting reports. Some studies have found a rise in adiponectin and others no difference in the level of leptin or adiponectin in pregnancies complicated by PE compared to healthy controls. (354) Some studies have shown higher leptin levels in pregnancies complicated by PE that predate its clinical manifestation making it a good predictive biomarker. (355) Conversely, there are reports of the opposite result, a lower level of leptin associated with PE. (356) There is convincing evidence that the incidence of PE is lower in post bariatric pregnancies. (357) Our data of lower leptin and higher adiponectin levels in the third trimester of post-bariatric women, when PE typically occurs, could be indicative of a mechanism that counters the onset of PE in this group of women. A larger study would be required to confirm this.

With regard to BW, our findings of a negative correlation between adiponectin and BW from women without surgery, is in line with published research. Studies of women without GDM have reported a lower level of adiponectin in the mothers of macrosomic babies but, similar to our work, they found no change in the leptin levels. Interestingly, they also report lower levels of adiponectin in mothers of growth restricted babies.

(357, 358) In contrast to this, Büke et al conducted a study comparing FGR babies with and without PE and found that only isolated FGR pregnancies had higher maternal levels of adiponectin. (360) The negative correlation between adiponectin and BW in women without surgery and the absence of a correlation post bariatric surgery, needs further investigation to identify its clinical relevance.

We also identified a positive correlation between maternal GIP levels at delivery and BW in women without surgery. GIP and GLP-1 are incretins associated with glucose homeostasis and have been linked with the pathogenesis of T2DM and GDM, although the mechanism is unclear. (361) Most studies investigating an association of both GIP and GLP-1 with BW have found that only GLP-1 has a significant correlation. This is partly explained by evidence of an inverse correlation between fasting GLP-1 and the risk of GDM. (362) Valsamakis et al. proposed that maternal GLP-1 may be responsible for mitigating maternal hyperglycemia and IR, and they inferred that it has a role in governing maternal weight and fetal growth. (363) Our finding of a positive correlation between GIP and the higher BW observed in obese women without bariatric surgery, is novel. A larger cohort would confirm this and may ascribe a role of GIP in fetal growth, providing further insight for the mechanism behind the lower incidence of LGA babies post bariatric surgery.

Strengths and Limitations

This is the first time these biomarkers have been investigated in pregnant women with previous bariatric surgery; a major strength of the study. We were careful to exclude confounding factors such as GDM and used standardised, reliable laboratory procedures which adds to the strength of this work.

This was a pilot study that obtained significant preliminary data. The small sample size is a limitation with insufficient data to draw definitive conclusions. Future work would use our results to design a study with a large cohort such that data could be stratified into different types of bariatric surgery and the biomarkers could be quantified in relation to the woman's fat mass.

4.5 CONCLUSION

This pilot study has shown reduced maternal fasting leptin and increased adiponectin levels in pregnant women with previous bariatric surgery, compared to pregnant women without surgery, in the third trimester. It has also shown a novel positive association of maternal GIP and a negative association of maternal adiponectin at delivery and BW in women without surgery. Most of our findings are in line with current research in post bariatric individuals in the general population and in pregnant women without previous bariatric surgery. Our data offer an explanation for post bariatric pregnancy outcomes, including reduced incidence of GDM, PE and LGA infants. Larger, conclusive studies are warranted to confirm these findings and their clinical relevance.

CHAPTER 5

Effect of Bariatric Surgery on the Metabolomic Profile of Maternal and Cord blood serum.

ABSTRACT

Objective: The aim of the current study was to investigate the effect of bariatric surgery on the maternal and fetal (at birth) metabolome.

Methods: Maternal blood serum was obtained from pregnant women with previous bariatric surgery (n=47; 21 with a restrictive and 26 with a malabsorptive procedure) and without surgery (n=118) at six time-points during pregnancy: 11^{+0}-14^{+0} (T1), 20^{+0}-24^{+0} (T2), 28^{+0}-30^{+0} (T3), 30^{+0}-33^{+0} (T4), 35^{+0}-37^{+6} (T5) weeks' gestation and within 72 hours of delivery (T6). Cord blood serum was also obtained at delivery from the umbilical vein. Untargeted H^1 NMR metabolomics profiling was performed.

Results: Pregnant women with previous malabsorptive bariatric surgery had changes in the following serum metabolites: lower maternal serum levels of unsaturated lipids, isobutyrate, leucine, isoleucine and N-acetyl glycoprotein and higher levels of glutamine and D-ß-hydroxybutyrate, by T4 (30-33 weeks) and T5 (35-37 weeks) compared to those without bariatric surgery (p=0.027 and p=0.006, respectively). There were no significant changes in cord blood metabolites between the groups.

Conclusion: Our study has demonstrated significant changes in serum metabolites of pregnant women with a previous malabsorptive bariatric surgery compared to women with no surgery. These changes may have a positive influence on maternal health following malabsorptive bariatric surgery.

5.1 INTRODUCTION

Metabolomics is a study of the metabolome in any biological specimen with the aim of detecting low molecular weight compounds (<1500 Daltons) during a genetic alteration or physiological stimulus. (364) These metabolites are molecules that result from metabolic processes within an organism and the majority of them are substrates and products of enzymes.(365) In most metabolomics studies, blood and urine are the biofluids of choice, as the sample collection is minimally invasive, and these contain a multitude of detectable metabolites.

Metabolomics is a powerful tool for the study of alterations in physiological processes and it has been widely used in the medical field for disease pathogenesis and biomarker discovery. Pharmaco-metabonomics, a term interchangeable with pharmaco-metabolomics, is the evaluation of therapeutic outcomes of clinical drugs by correlating the baseline metabolic profiles of patients with their responses. (366) This evaluation of metabolic response to treatment could enable personalised therapeutic management. For example, aspirin is used in the primary prevention of cardiovascular disease in high risk patients due to its antiplatelet aggregation action. In the Heredity and Phenotype Intervention heart study, metabolomics analysis was applied to investigate the mechanisms underlying aspirin resistance; a phenomenon encountered in 25% of high risk cardiac patients. (367) In the study, 76 healthy volunteers were recruited (40 good and 36 poor responders) and put on aspirin therapy for 2 weeks. The pre-dose metabolic signatures was correlated with inter-individual variations after aspirin therapy and as a result, inosine and serotonin were identified as key metabolites as they were increased in the plasma of poor-responders.

Metabolomics can identify metabolites involved in disease mechanisms and susceptibility. For instance, the Framingam Offspring Study analysed baseline metabolic profiles in 189 new-onset diabetics during a 12-year follow-up period and propensity-matched controls. (368) Metabolites that were significantly different between cases and controls were leucine, isoleucine, valine (branch-chained amino acids), phenylalanine and tyrosine (aromatic amino acids). Participants with the highest plasma amino acids levels had a 2-fold higher chance of developing diabetes during the following 12 years, compared to those with the lowest levels of plasma amino acids. In oncology, a Nuclear Magnetic Resonance (NMR) based metabolomics study has examined the serum metabolomic profiles of patients with early stage,

untreated chronic lymphocytic leukaemia patients and found higher concentrations of pyruvate and glutamate and decreased concentrations of isoleucine compared with controls. (369)

In obstetrics, studies have shown potential clinical application of metabolomics to predict, diagnose and monitor pregnancy-related disease. (370) To identify metabolic changes underlying fetal malformations and early biomarkers, a metabolomics study of second trimester maternal plasma and urine was conducted and showed that in cases of fetal malformation, the maternal plasma had lower betaine and trimethylamine-N-oxide concentrations and elevated levels of urinary levels of amino acids involved in gluconeogenesis; Cis-aconitate, acetone, 3-hydroxybutyrric and hypoxanthine. (371) These findings suggested enhanced gluconeogenesis and tricarboxylic acid cycle in malformed fetuses, possibly due to hypoxic metabolism. (372) Similarly, metabolomic studies have been used in the prediction and diagnosis of pre-eclampsia, small for gestational age neonates, preterm birth, gestational diabetes and maternal and fetal infections. (373)

The aim of this study was to address the lack of data on metabolic changes in pregnancy following bariatric surgery, particularly in light of the known association between bariatric surgery and altered pregnancy outcomes notably a reduction in the prevalence of pre-eclampsia and large for gestational age neonates (LGA) but an increased prevalence of SGA. (374) Variation in any metabolites between women with and without surgery may highlight the metabolic pathways responsible for altered pregnancy outcomes that have been observed.

5.2 MATERIALS AND METHODS

This was a prospective, observational study investigating the effect of bariatric surgery on the maternal and fetal serum metabolome.

Pregnant women with previous bariatric surgery (n=47) and without surgery (n = 118) were recruited from the Chelsea & Westminster Hospital from May 2015 until April 2017. Written informed consent was obtained from all women.

Maternal demographic, biophysical characteristics were recorded in our research database and samples obtained as described in Chapter 2. Maternal blood serum was

obtained at six time-points during the pregnancy: 11^{+0}-14^{+0} (T1), 20^{+0}-24^{+0} (T2), 28^{+0}-30^{+0} (T3), 30^{+0}-33^{+0} (T4), 35^{+0}-37^{+6} (T5) weeks' gestation and within 72 hours of delivery (T6). Cord blood serum was also obtained at delivery from the umbilical vein. All the samples were centrifuged and stored at -80°C within 30 minutes of collection.

Samples were subsequently randomised, thawed at room temperature and put into groups of 92 and 93 then placed into ten 96-well plates. The metabolites were not known a-priori therefore untargeted NMR metabolomics was used. In the literature, NMR spectroscopy has been used for multivariate metabolic profiling of biological fluids and tissue for almost 50 years. (375) In light of this and previously discussed advantages over MS in Chapter 2, H^1 NMR spectroscopy was the chosen profiling method in this study. The NMR sample preparation and analysis is described in material and methods Chapter 2.

Statistical Analysis

The data was assessed for normal distribution using the Kolmogoroff–Smirnoff test. Quantitative variables were reported as mean +/- standard deviation (SD) or median (interquartile ranges). Qualitative variables were expressed as frequencies and percentages. Unpaired t-test, Mann Whitney and chi square χ^2 tests were used to analyze the differences between values obtained from the no surgery and bariatric surgery groups.

Multivariate statistical analysis was used to aid pattern recognition and decipher metabolic signatures from the large data sets obtained. This was performed in Soft Independent Modelling of Class Analogy (SIMCA) software, version 14.1 (MKS Umetrics, Umeå Sweden).

Multivariate modelling

Initially, unsupervised principal component analysis (PCA) models were created for each time point. This meant that no information on group identity was used to construct the models.

The PCA models gave an unbiased overview of the variability in the study dataset, analysed as one block (X data block), reducing the data dimensionality. (301) Each subject is represented as a single point in the scores plot. PCA inspects data homogeneity and highlights extreme outliers based on the Hotelling's T^2 statistic, a

multivariate generalization of the 95% confidence interval. (376) Statistical tests are not performed on this model.

Next, a supervised model: Orthogonal projections to latent structures discriminant analysis (OPLS-DA) was built for each time point. In this model, group identity was defined e.g. no bariatric surgery (NBS), malabsorptive bariatric surgery (MAL) and restrictive bariatric surgery (RES). The OPLS-DA models maximize covariance between the spectral data (predictors, X block) and the group labels (outcomes, Y block). These models determine which spectral features (metabolites) are important for group discrimination (e.g. what is driving the separation between the datasets represented as individual points in the model). Extreme outliers were excluded from this supervised model. (376) OPLS-DA separates the systematic variation in X into two parts: one part that is correlated or predictive to Y and one part that is uncorrelated (orthogonal) to Y.

The quality of the OPLS-DA models created had to be evaluated to rule out overfitting. This was achieved by determining the following parameters(377)

1. R^2 value, the measure of fit. The variance of the original dataset explained by the model. Expressed as a fraction.
2. Q^2 value, the predicted variation**.** Calculated using cross-validation. A measure of the predictive power of the model**.**
3. Cross-validated analysis of variance (CV-ANOVA) provides a p-value indicating the level of significance of group separation in supervised analysis, Orthogonal projections to latent structures discriminant analysis, OPLS-DA.
4. Outliers were identified using the following functions in SIMCA: (i) Hotellings T2 Multivariate generalization (95% confidence interval) and (ii) Distance to model X (DModX); the distance of a given observation to the model plane.
5. NMR Data was represented as p(corr) Loadings scaled as a correlation coefficient (ranging from 1.0 to 1.0) between the model and original data.

5.3 RESULTS

Between May 2015 and April 2017, 165 pregnant women were recruited; 47 women had previous bariatric surgery (21 with a restrictive and 26 with a malabsorptive procedure) and 118 women with no previous weight loss surgery. Women with diabetes (Type 2 or gestational diabetes mellitus) and maternal body mass index (BMI)

<25 or >50 were identified as potential confounders and therefore excluded. In the post-bariatric surgery group, at total of 6 diabetic women were excluded. In the no surgery group, 11 diabetic women and 35 women with BMI <25 or >50 were also excluded. One woman had a miscarriage and one withdrew from the study (Figure 5.1 and Table 5.1).

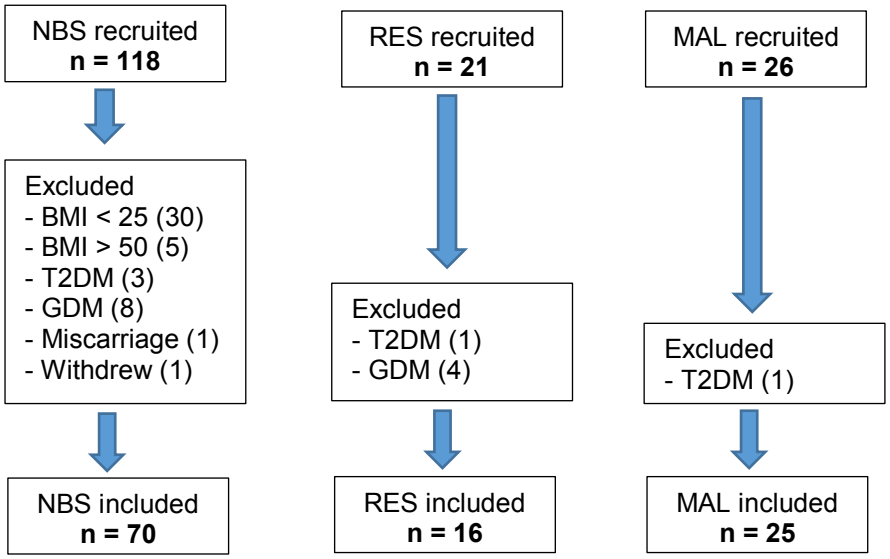


Figure 5.1: Total number of patients that were initially recruited, subsequent exclusions and final number included in the study. NBS: No previous Bariatric Surgery; RES: Restrictive Bariatric Surgery; MAL: Malabsorptive Bariatric Surgery.

Table 5.1: Number of study participants at each time point.

Participants, n	No bariatric surgery (n=70)	Post-bariatric surgery (n=41)	Restrictive (n=16)	Malabsorptive (n=25)
T1	55	73	6	12
T2	47	73	10	16
T3	47	74	12	15
T4	45	68	8	15
T5	39	66	10	17
T6	27	45	7	11

Demographic, biophysical characteristics of the participants and their pregnancy outcomes are summarised in Table 5.2. There were 70 participants without bariatric surgery with a mean age of 29.72 years (SD 5.26), mean booking weight of 91.22kg

and BMI 34.12. There were 41 participants with post bariatric surgery (16 with a restrictive and 25 with a malabsorptive surgery) with a mean age of 33.46 years, mean booking weight of 87.55kg and BMI of 32.94.

Table 5.2: Maternal demographics and pregnancy outcomes of the study participants

Variable	No bariatric surgery (n=70)	Post-bariatric surgery (n=41)	*P* value	Restrictive (n=16)	Malabsorptive (n=25)
Maternal age (years)	29.72 (5.26)	33.46 (4.58)	<0.01	32.56 (4.32)*	34.15 (4.73)*
Parity, n (%)					
Nulliparous	43 (61.4)	20 (48.8)	0.19	9 (56.3)	11 (44.0)
Multiparous	27 (38.6)	21 (51.2)		7 (43.8)	14 (56.0)
Racial group, n (%)					
White	54 (77.1)	31 (75.6)	0.85	12 (75)	19 (76.0)
Other	16 (22.9)	10 (24.4)		4 (25)	6 (24.0)
Conception, n (%)					
Spontaneous	67 (95.7)	38 (92.7)	0.49	16 (100.0)	22 (88.0)
Assisted reproductive techniques	3 (4.3)	3 (7.3)		0 (0)	3 (12.0)
Smoking, n (%)					
No	67 (95.7)	36 (87.8)	0.12	15 (93.8)	21 (84.0)
Yes	3 (4.3)	5 (12.2)		1 (6.3)	4 (16.0)
Body mass index prior to surgery (kg/m^2)	-	47.19 (7.95)	-	41.95 (6.47)	50.54 (7.01)ǂ
Booking weight (kg)	91.22 (16.30)	87.55 (17.20)	0.33	87.37 (24.56)	87.63 (13.88)
Booking body mass index (kg/m^2)	34.12 (5.68)	32.94 (5.10)	0.35	32.96 (7.29)	32.93 (4.10)
Booking waist to hip ratio	0.88 (0.08)	0.85 (0.07)	0.14	0.85 (0.05)	0.86 (0.08)
Gestational age at delivery (weeks)	39.44 (1.34)	38.67 (2.266)	0.03	38.95 (2.31)	38.49 (2.26)*
Birth weight (grams)	3520.92 (603.86)	3062.53 (588.32)	<0.01	3096.75 (519.07)*	3040.64 (638.12)*
Birth weight percentile	59.48 (33.30)	39.01 (27.35)	<0.01	39.29 (26.25)*	38.83 (28.57)*

Comparisons were made with the no bariatric surgery group. Asterix (*) refers to statistical significant difference (p<0.05) between the groups. Data are expressed as mean (standard deviation) or number (%).T1: 11^{+0}-14^{+0}, T2: 20^{+0}-24^{+0}, T3: 28^{+0}-30^{+0}, T4: 30^{+0}-33^{+0}, T5: 35^{+0}-37^{+6} and T6: Delivery. NS: No significance, p=1.

Principal component analysis (PCA) was performed on maternal serum samples at all time-points (T1-T6), Figures 5.1 – 5.7. The Hotelling T2 test did not identify any strong outliers.

Time point 1 (12-14 weeks)

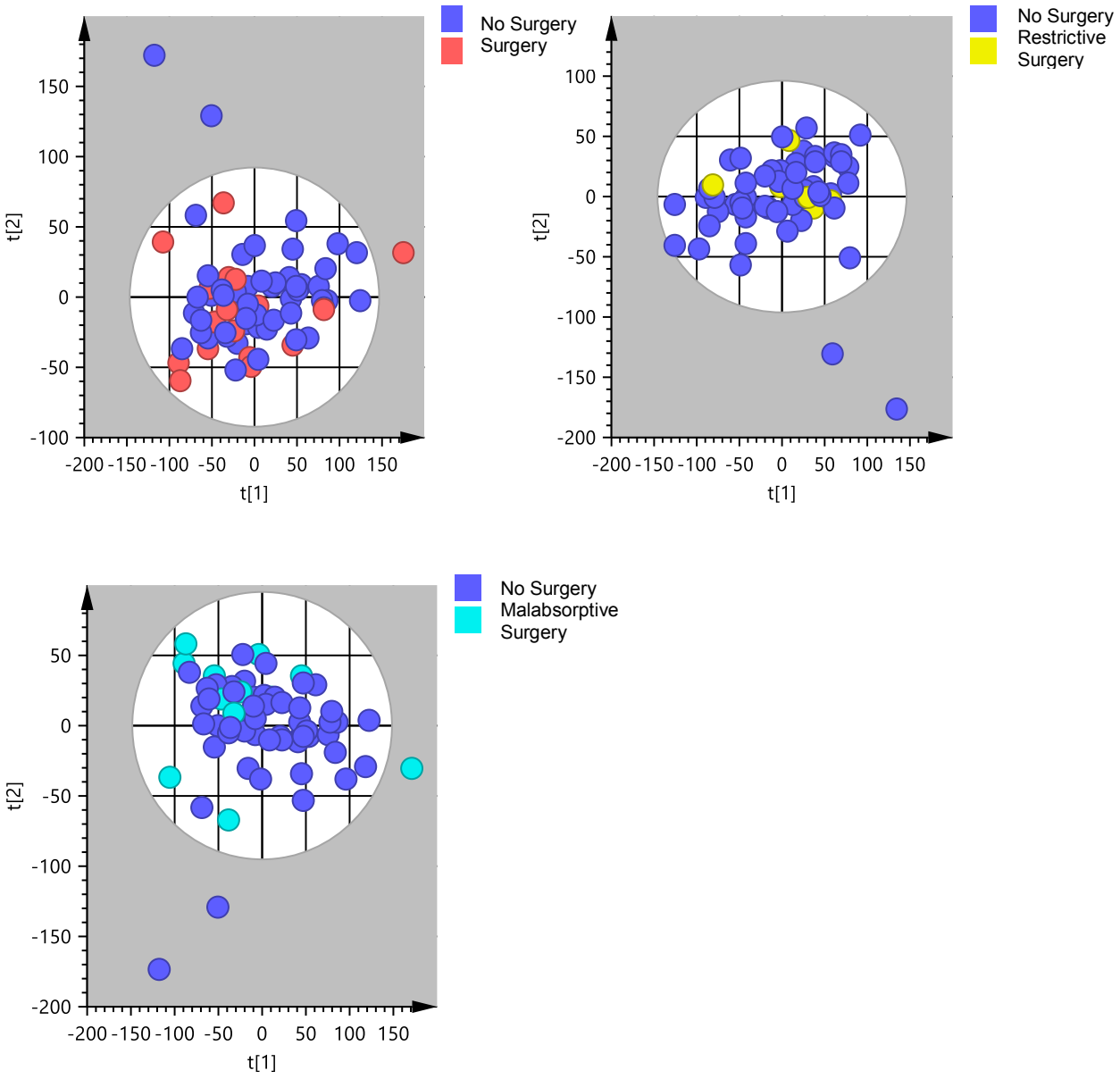


Figure 5.2: PCA models of maternal serum ^{1}H NMR spectral data from women at T1 (12-14wks) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. A summary of fit for all models (not shown) had similar, positive R2 and Q2 values for all components of the models.

Time point 2 (20-24 weeks)

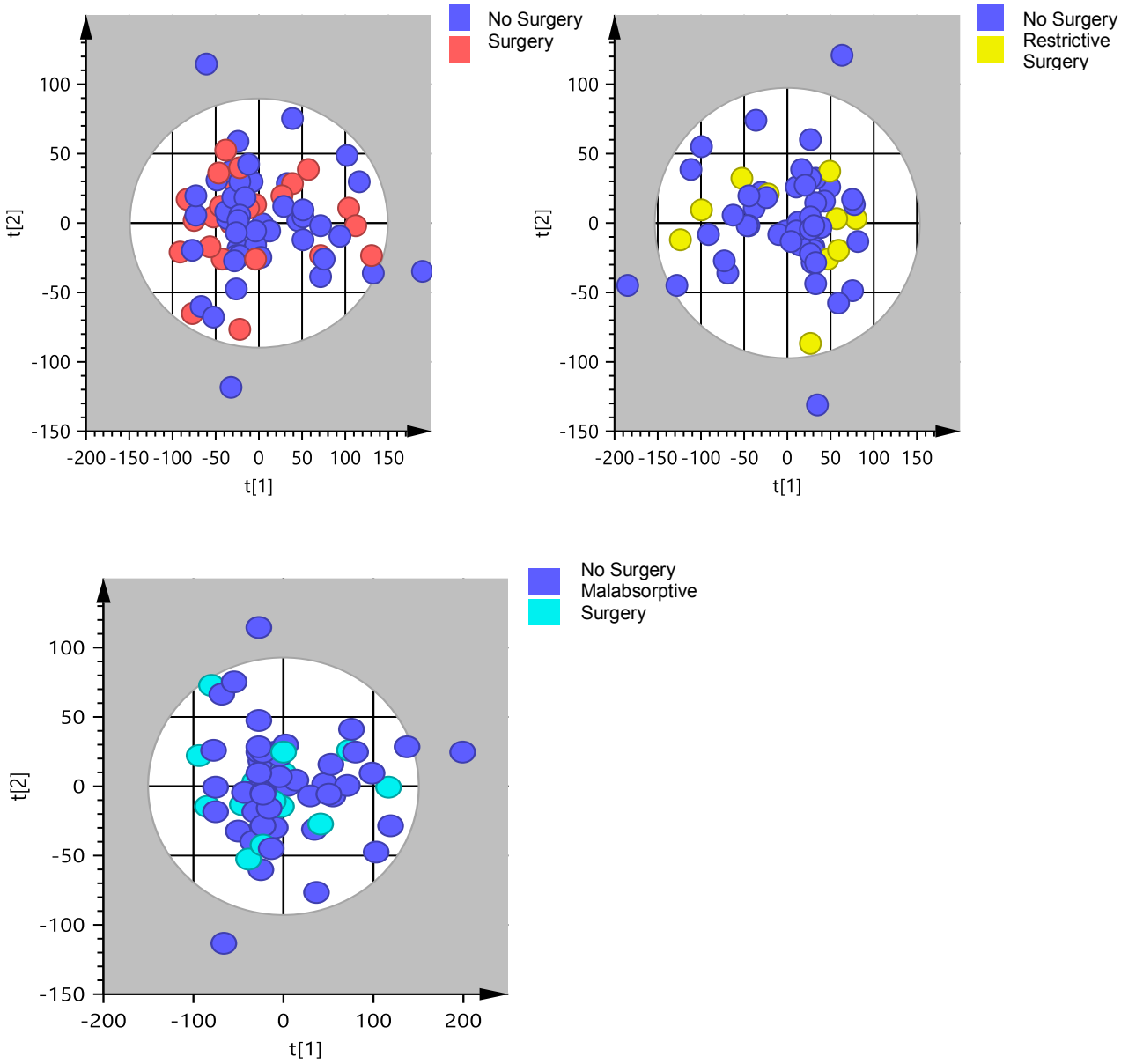


Figure 5.3: PCA models of maternal serum ^{1}H NMR spectral data from women at T2 (20^{+0}-24^{+0}). (a) No surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. A summary of fit for all models (not shown) had similar, positive R2 and Q2 values for all components of the models.

Time point 3 (28-30 weeks)

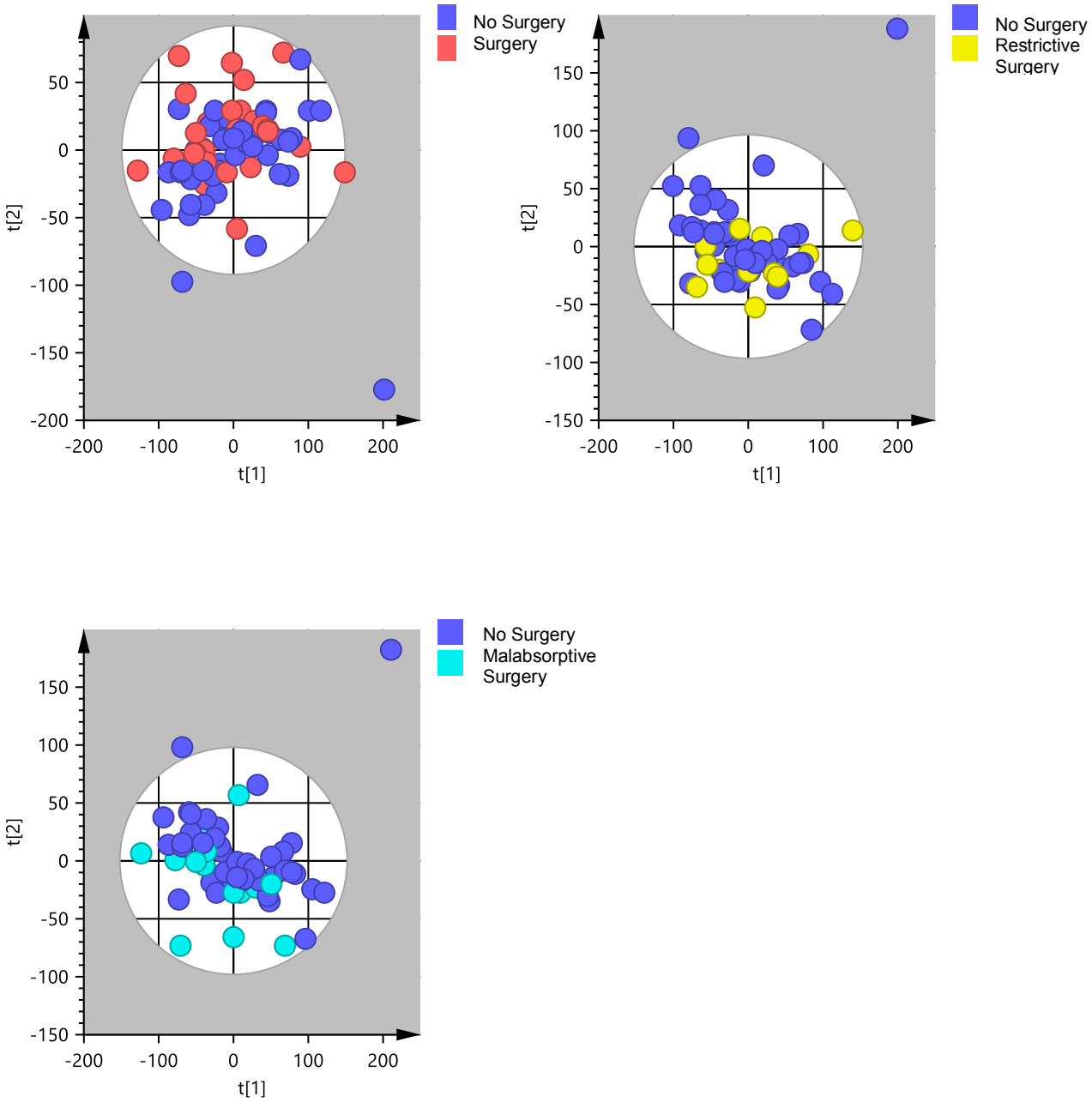


Figure 5.4: PCA models of maternal serum ^{1}H NMR spectral data from women at T3 (28^{+0}-30^{+0}) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. A summary of fit for all models (not shown) had similar, positive R2 and Q2 values for all components of the models.

Time point 4 (30-33 weeks)

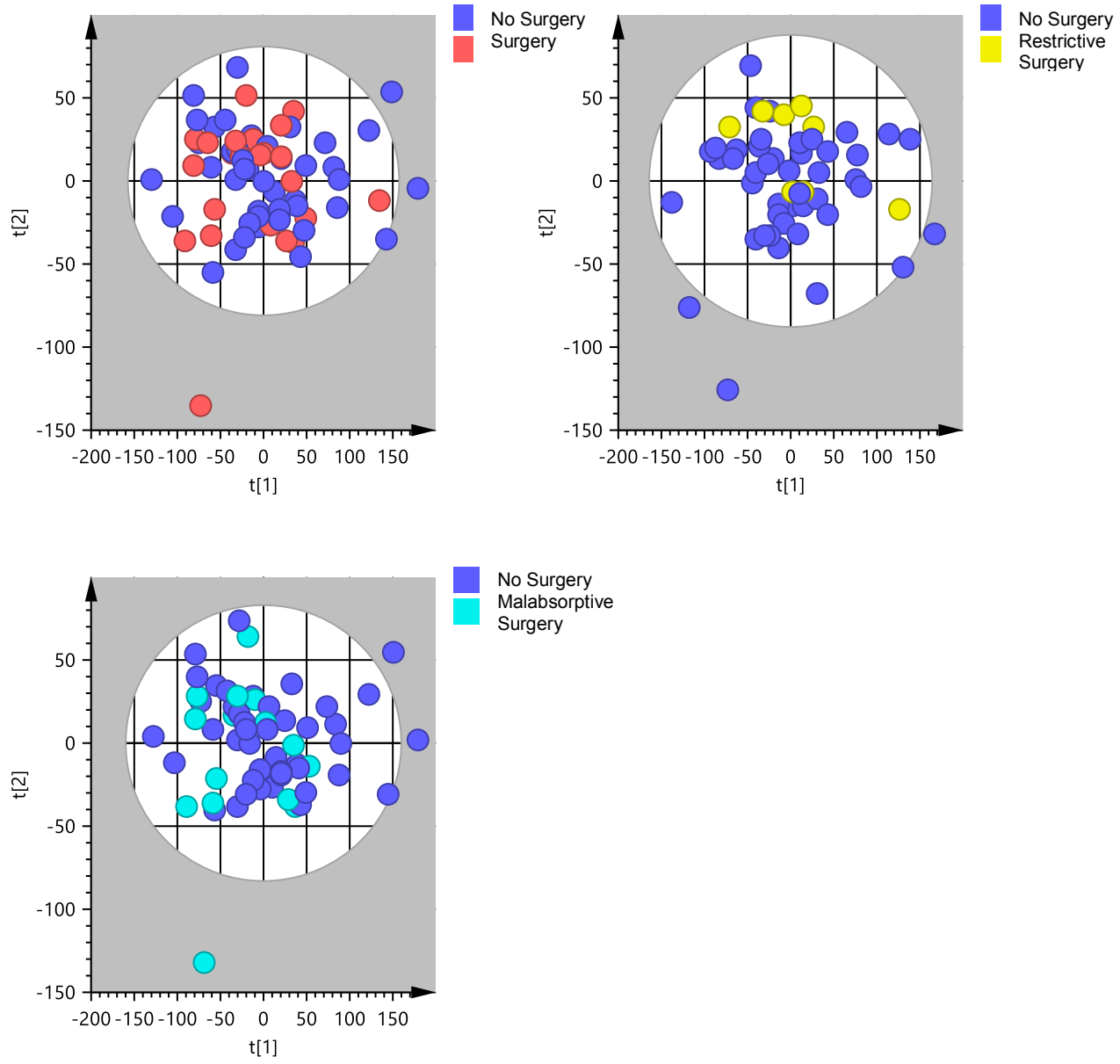


Figure 5.5: PCA models of maternal serum ^{1}H NMR spectral data from women at T4 (30^{+0}-33^{+0}) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. A summary of fit for all models (not shown) had similar, positive R2 and Q2 values for all components of the models.

Time point 5 (35-37 weeks)

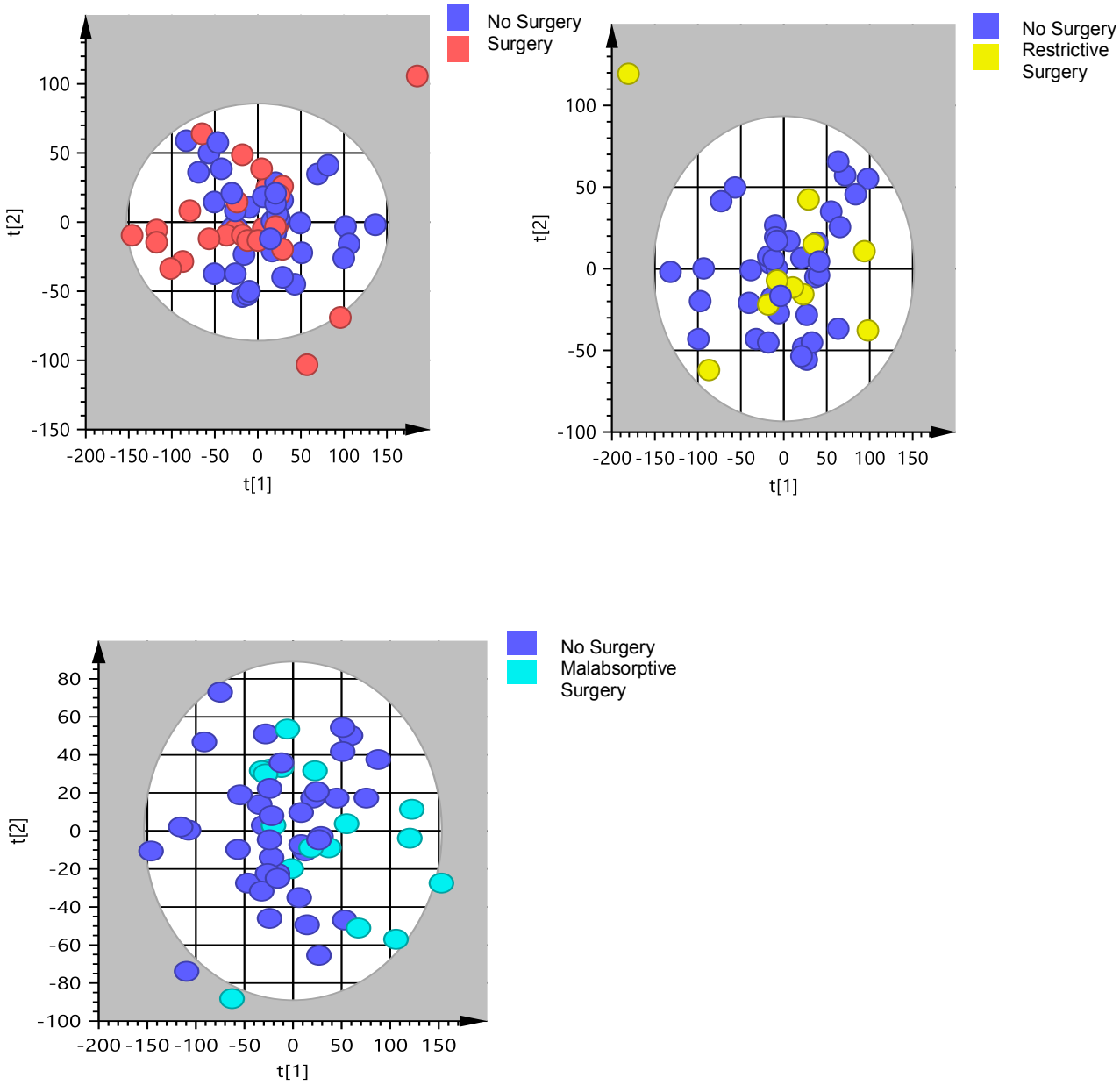


Figure 5.6: PCA models of maternal serum ^{1}H NMR spectral data from women at T5 (35^{+0}-37^{+6}) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. A summary of fit for all models (not shown) had similar, positive R2 and Q2 values for all components of the models.

Time point 6 (Delivery)

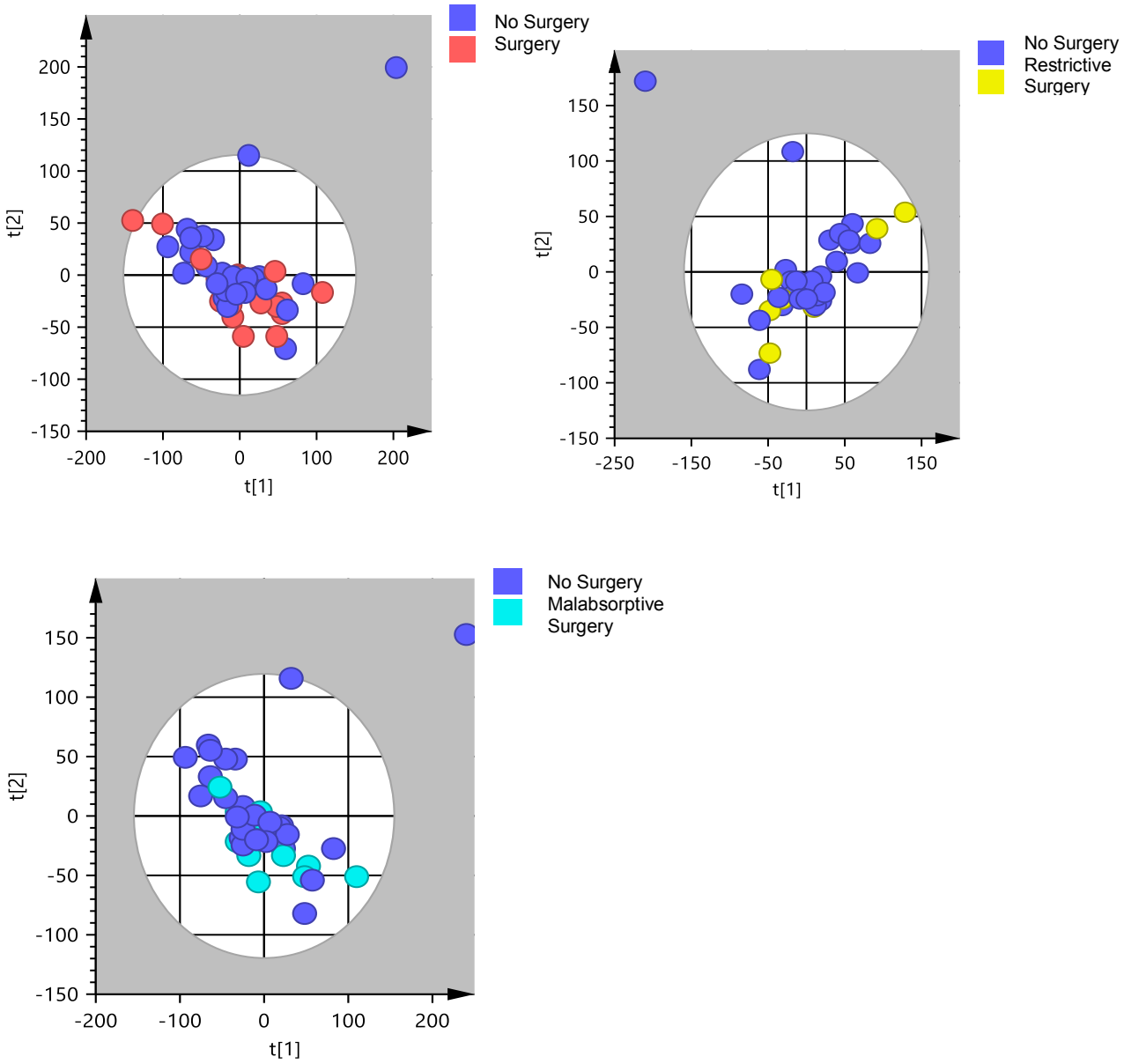


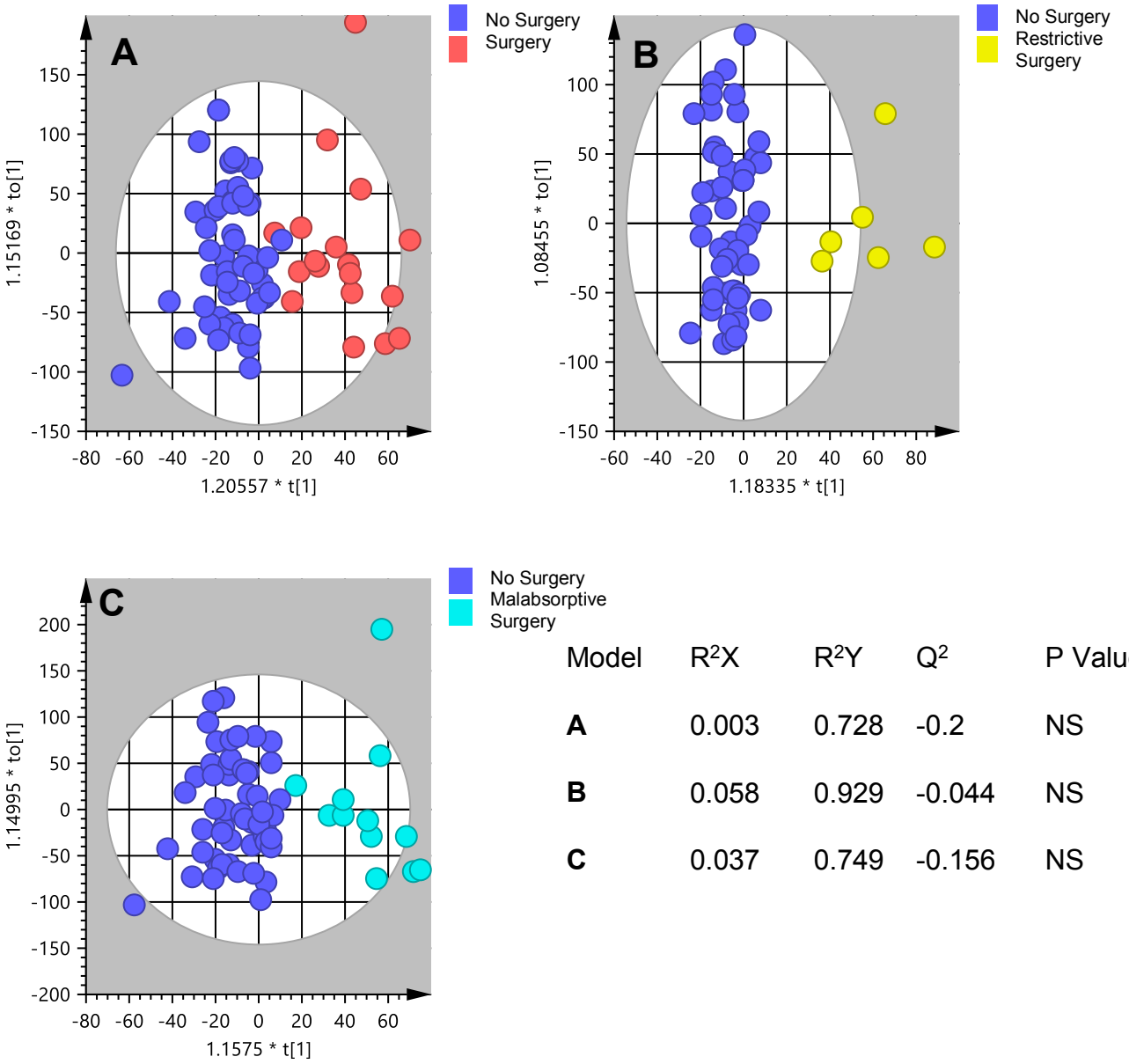
Figure 5.7: PCA models of maternal serum ^{1}H NMR spectral data from women at delivery (T6) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. A summary of fit for all models (not shown) had similar, positive R2 and Q2 values for all components of the models.

Following PCA, a supervised analysis, Orthogonal projections to latent structures discriminant analysis (OPLS-DA) was performed on maternal serum samples at all time-points (T1-T6) to identify discriminating metabolites (Figures 5.8- 5.13) and the model statistics computed from cross-validation (R^2X, R^2Y, Q^2) are summarised in the

Figures. The scores from each OPLS-DA model were subjected to a cross-validated analysis of variance (ANOVA) to test for significance ($p<0.05$).

There were no significant differences between the groups from the first to the second trimester (T1-T3) and at delivery (T6). In the third trimester (T4 and T5), an obvious distinction between the metabolic profiles of serum from women who had malabsorptive surgery versus no surgery was identified which reached statistical significance ($p=0.027$ and $p=0.006$ respectively).

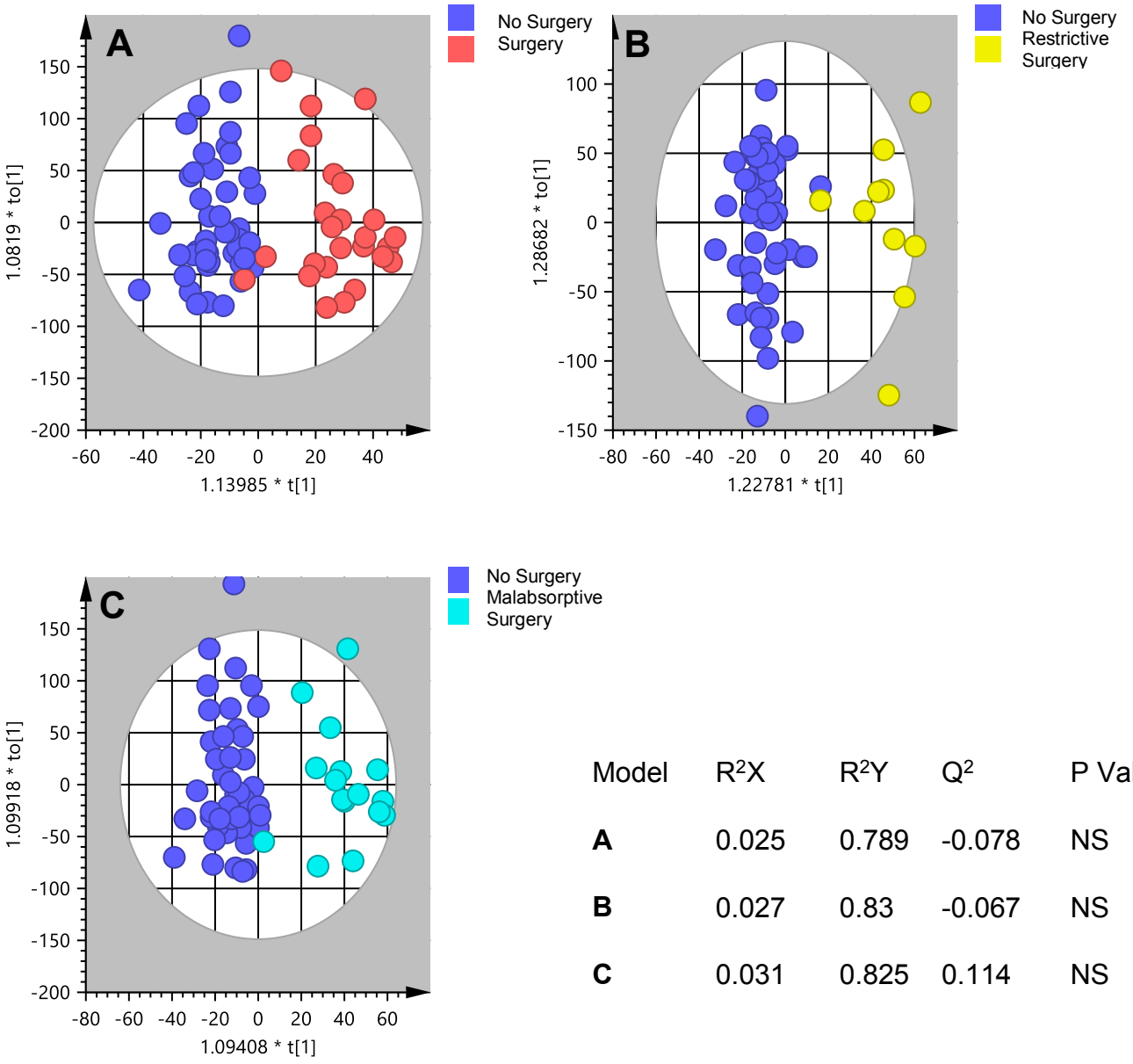
Time point 1 (12-14 weeks)



Model	R^2X	R^2Y	Q^2	P Value
A	0.003	0.728	-0.2	NS
B	0.058	0.929	-0.044	NS
C	0.037	0.749	-0.156	NS

Figure 5.8: OPLS-DA models of maternal serum ^{1}H NMR spectral data from women at T1 (12-14wks) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. P value NS: not statistically significant.

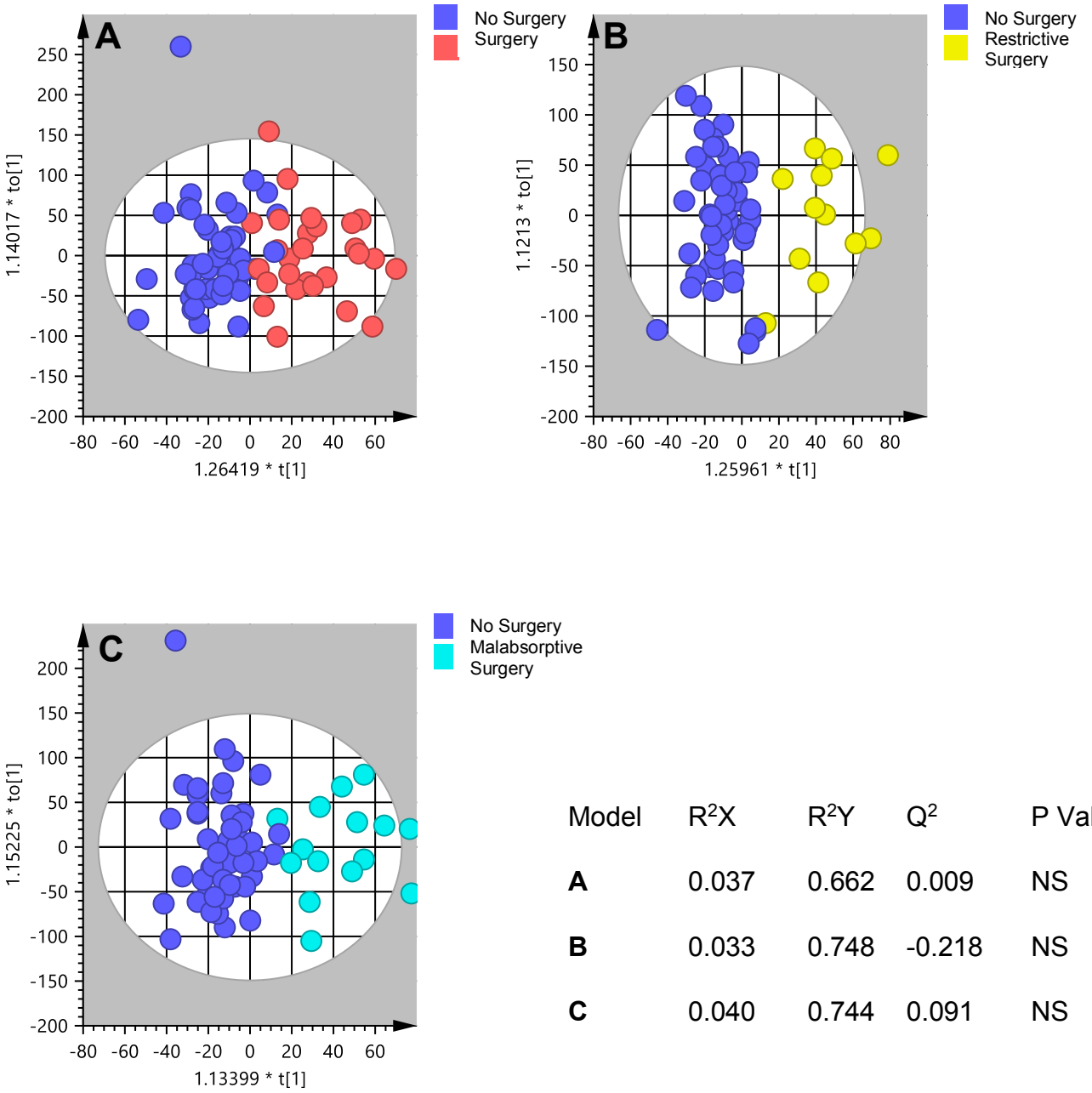
Time point 2 (20-24 weeks)



Model	R^2X	R^2Y	Q^2	P Value
A	0.025	0.789	-0.078	NS
B	0.027	0.83	-0.067	NS
C	0.031	0.825	0.114	NS

Figure 5.9: OPLS-DA models of maternal serum ^{1}H NMR spectral data from women at T2 (20^{+0}-24^{+0}) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. P value NS: not statistically significant.

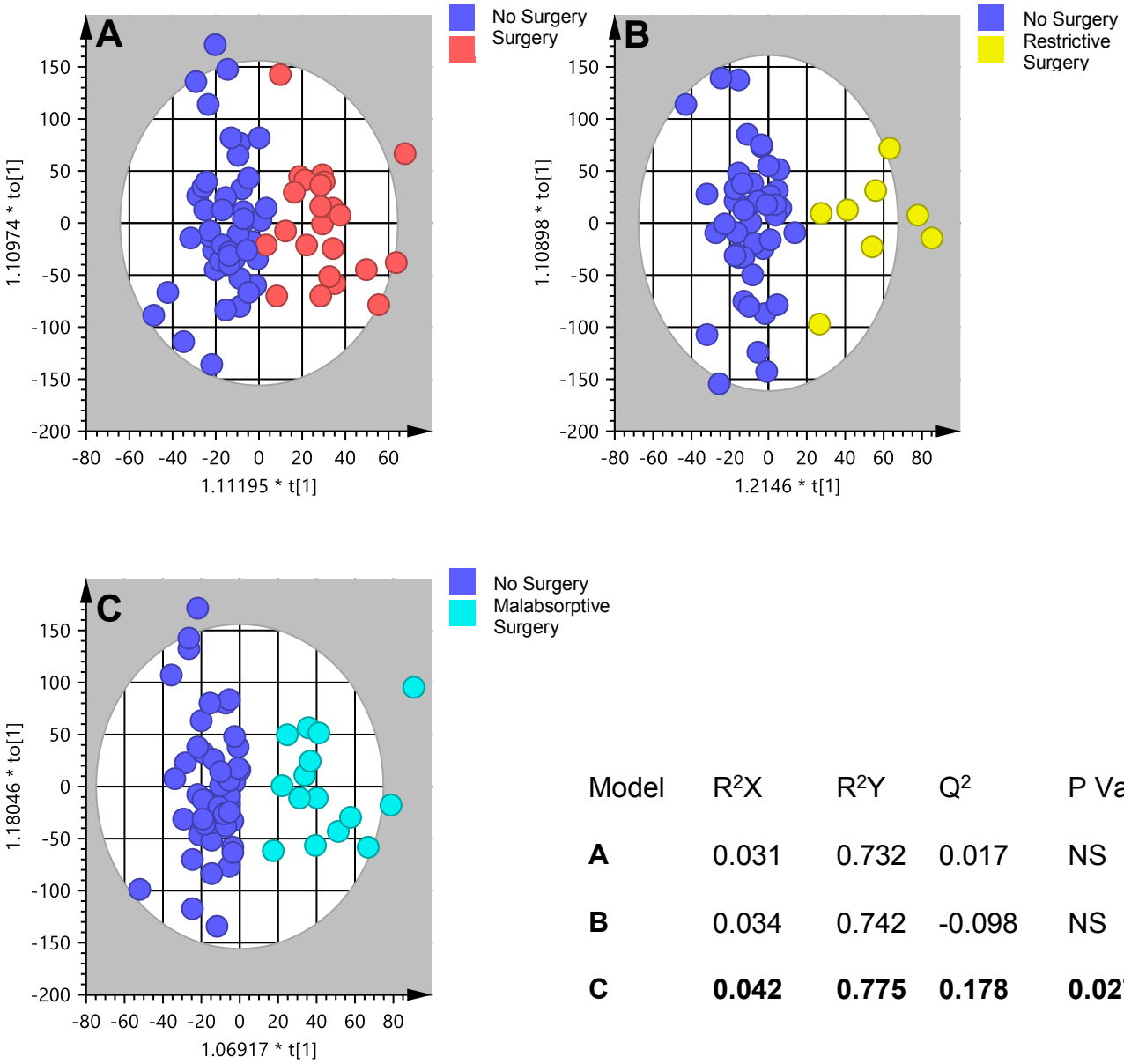
Time point 3 (28-30 weeks)



Model	R^2X	R^2Y	Q^2	P Value
A	0.037	0.662	0.009	NS
B	0.033	0.748	-0.218	NS
C	0.040	0.744	0.091	NS

Figure 5.10: OPLS-DA models of maternal serum ^{1}H NMR spectral data from women at T3 (28^{+0}-30^{+0}) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. P value NS: not statistically significant.

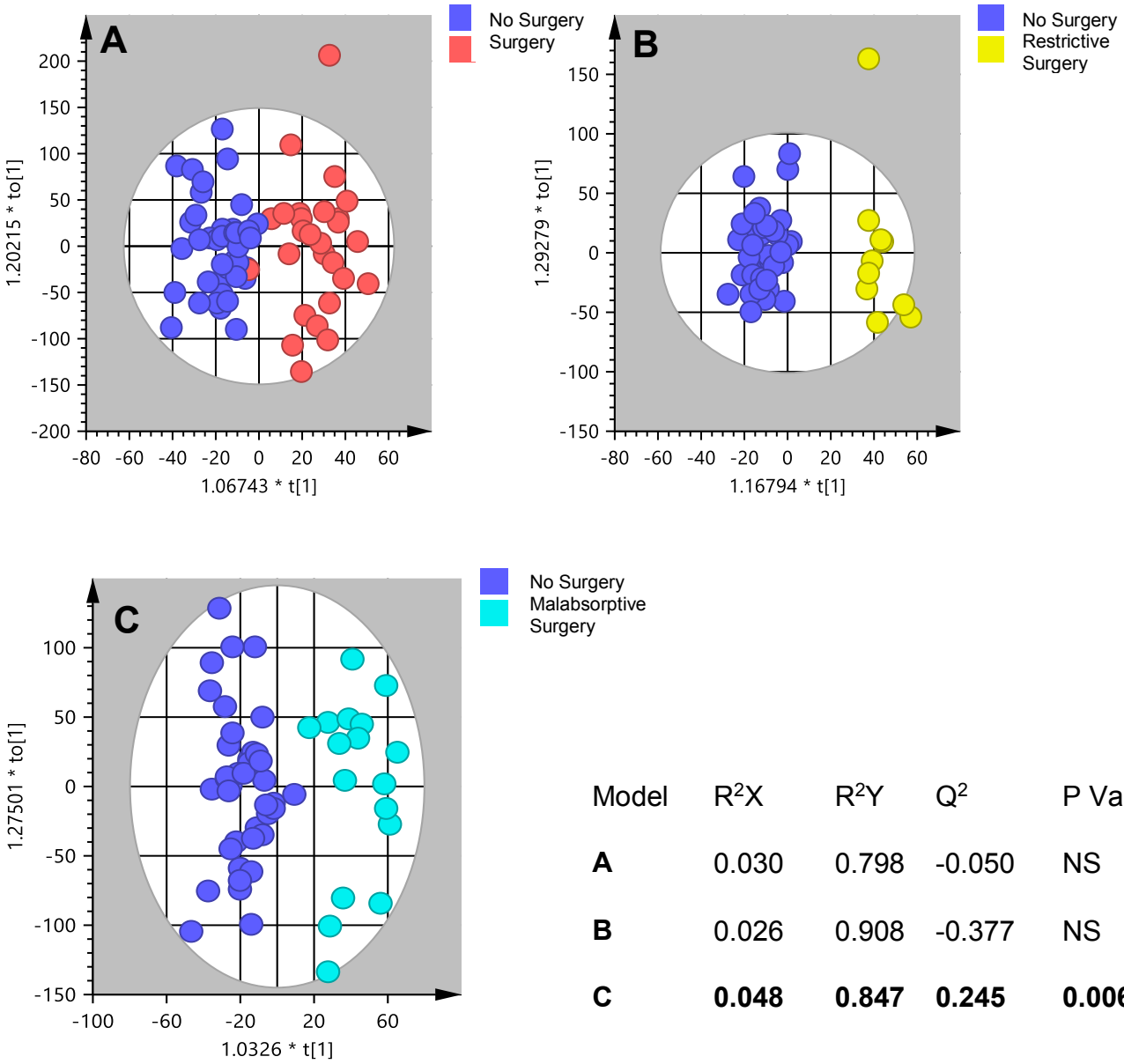
Time point 4 (30-33 weeks)



Model	R^2X	R^2Y	Q^2	P Value
A	0.031	0.732	0.017	NS
B	0.034	0.742	-0.098	NS
C	**0.042**	**0.775**	**0.178**	**0.027**

Figure 5.11: OPLS-DA models of maternal serum ^{1}H NMR spectral data from women at T4 (30^{+0}-33^{+0}) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. P value NS: not statistically significant.

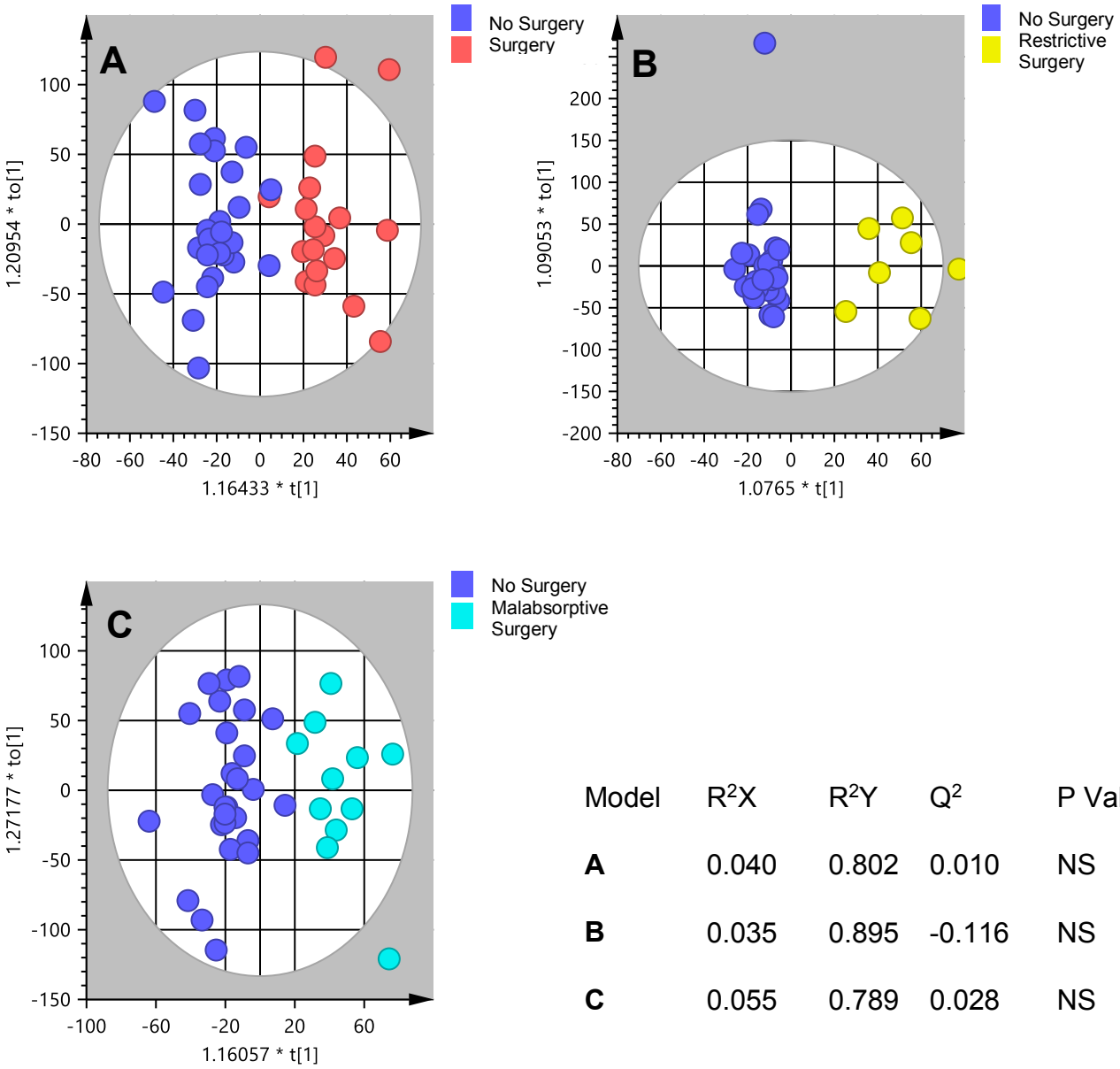
Time point 5 (35 – 37 weeks)



Model	R^2X	R^2Y	Q^2	P Value
A	0.030	0.798	-0.050	NS
B	0.026	0.908	-0.377	NS
C	**0.048**	**0.847**	**0.245**	**0.006**

Figure 5.12: OPLS-DA models of maternal serum ^{1}H NMR spectral data from women at T5 (35^{+0}-37^{+6}) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. P value NS: not statistically significant.

Time point 6 (Delivery)



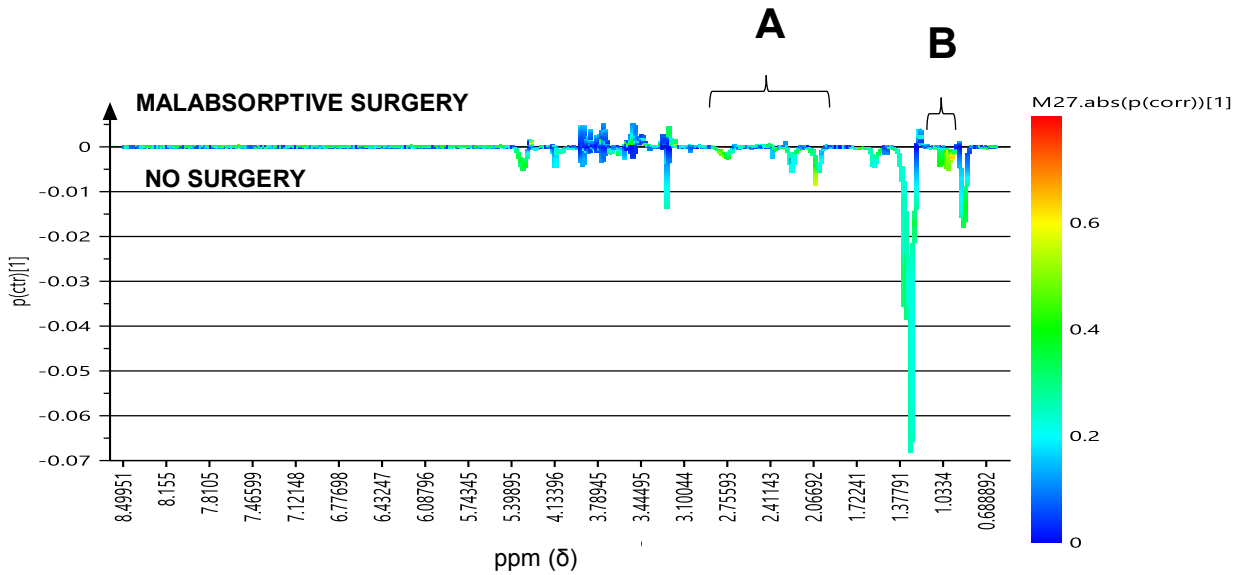
Model	R^2X	R^2Y	Q^2	P Value
A	0.040	0.802	0.010	NS
B	0.035	0.895	-0.116	NS
C	0.055	0.789	0.028	NS

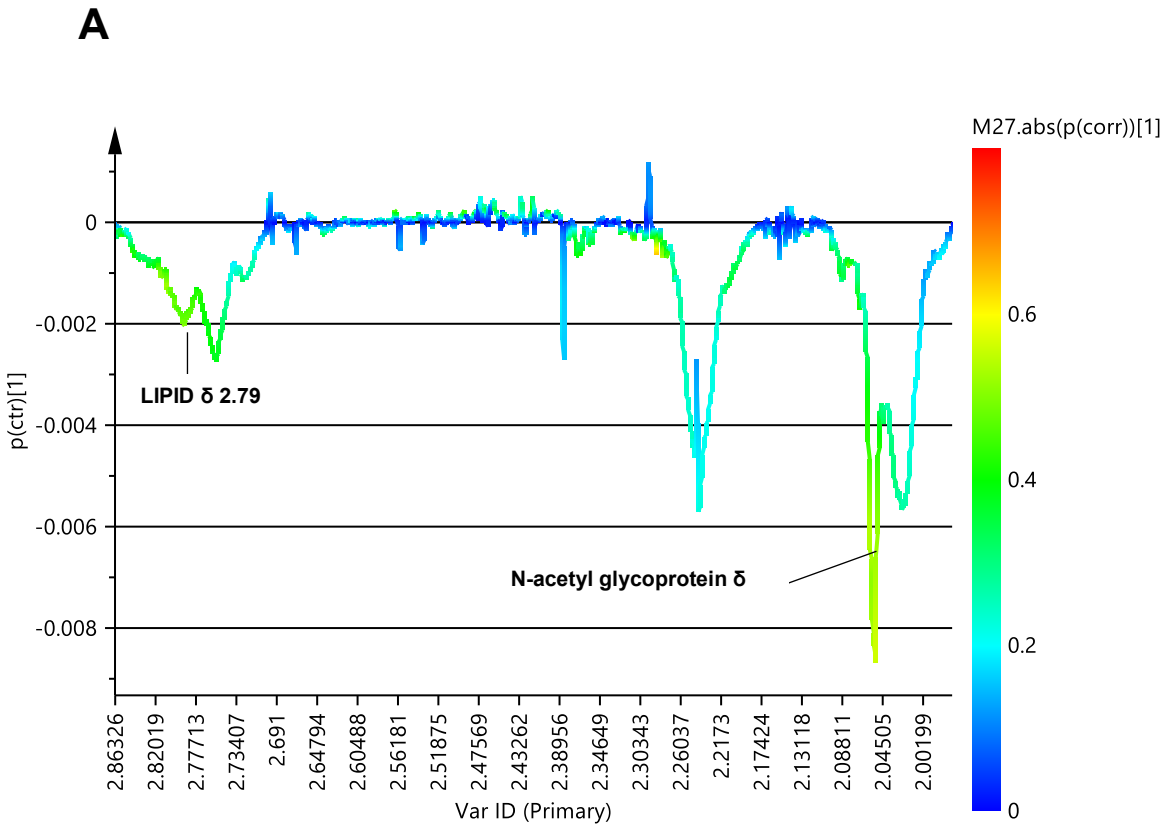
Figure 5.13: OPLS-DA models of maternal serum ^{1}H NMR spectral data from women at delivery (T6) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. P value NS: not statistically significant.

S-line plots were generated from the OPLS-DA models at T4 and T5, comparing maternal serum from malabsorptive and no surgery groups shown in Figures 5.14 and 5.15. The s-line plots identified the areas of NMR spectra representing class-separating metabolites. This data was subsequently used in STOCSY® plots and database cross-referencing to confirm metabolite identities.

Compared with the no surgery, the malabsorptive surgery group had lower serum levels of lipids including unsaturated lipids, saturated fatty acid isobutyrate, amino acids leucine and Isoleucine and N-acetyl glycoprotein. The malabsorptive group had higher serum levels of amino acid glutamine and ketone body D-ß-hydroxybutyrate compared with no surgery. The time series analysis of these discriminatory metabolites across all the time points is shown in Figure 5.16.

Time point 4


A
B
MALABSORPTIVE SURGERY
NO SURGERY
M27.abs(p(corr))[1]
p(ctr)[1]
0
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
-0.07
0.6
0.4
0.2
0
8.49951
8.155
7.8105
7.46599
7.12148
6.77698
6.43247
6.08796
5.74345
5.39895
4.13396
3.78945
3.44495
3.10044
2.75593
2.41143
2.06692
1.72241
1.37791
1.0334
0.688892
ppm (δ)


A
M27.abs(p(corr))[1]
p(ctr)[1]
0
-0.002
-0.004
-0.006
-0.008
LIPID δ 2.79
N-acetyl glycoprotein δ
0.6
0.4
0.2
0
2.86326
2.82019
2.77713
2.73407
2.691
2.64794
2.60488
2.56181
2.51875
2.47569
2.43262
2.38956
2.34649
2.30343
2.26037
2.2173
2.17424
2.13118
2.08811
2.04505
2.00199
Var ID (Primary)

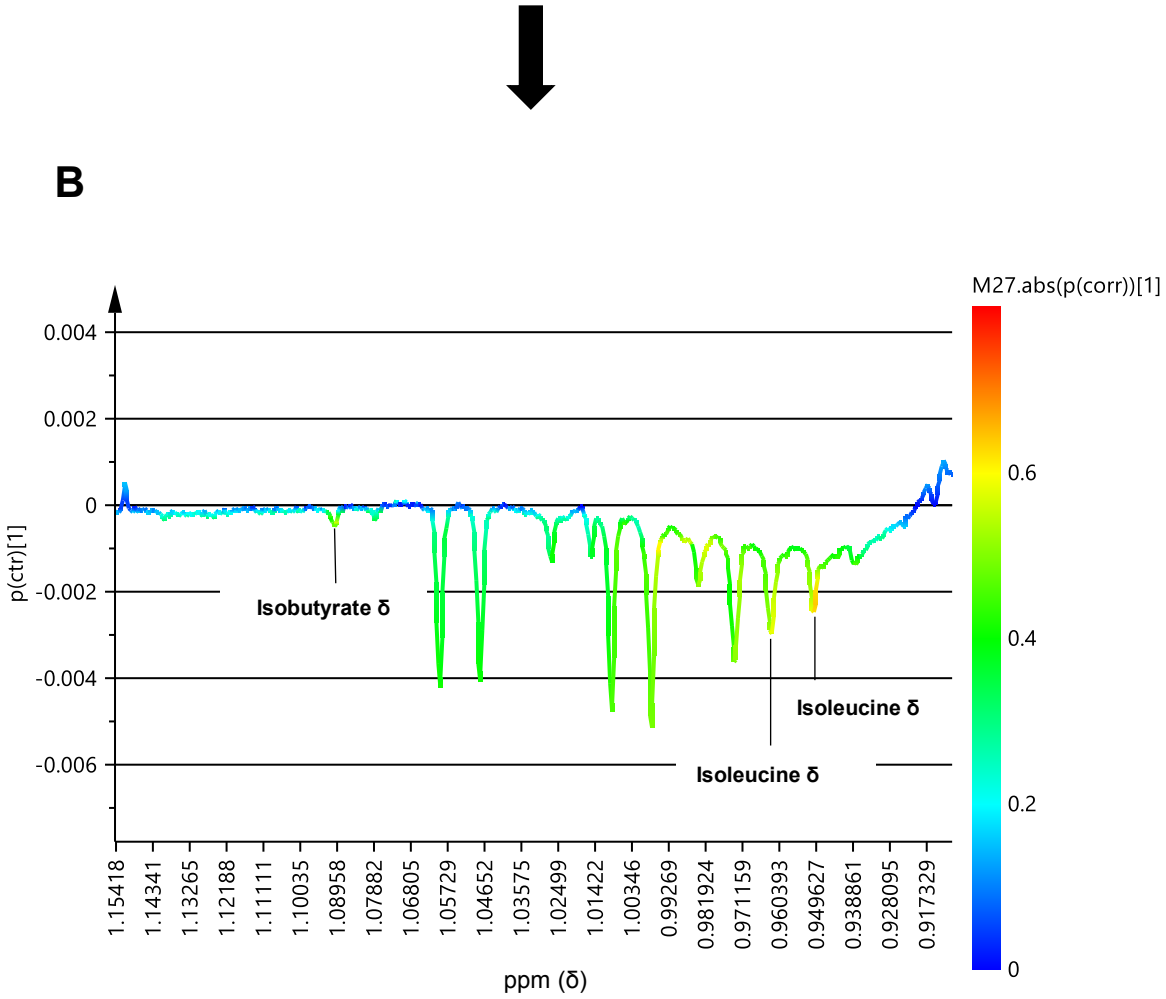
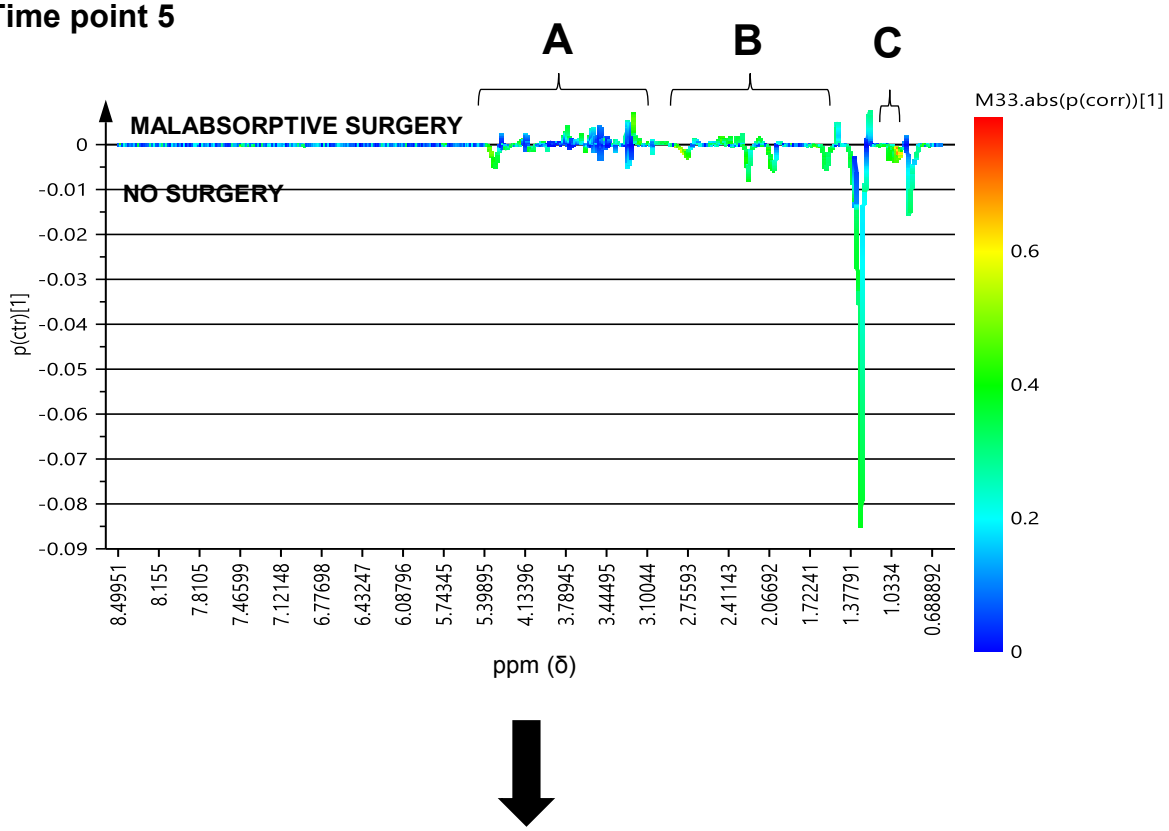
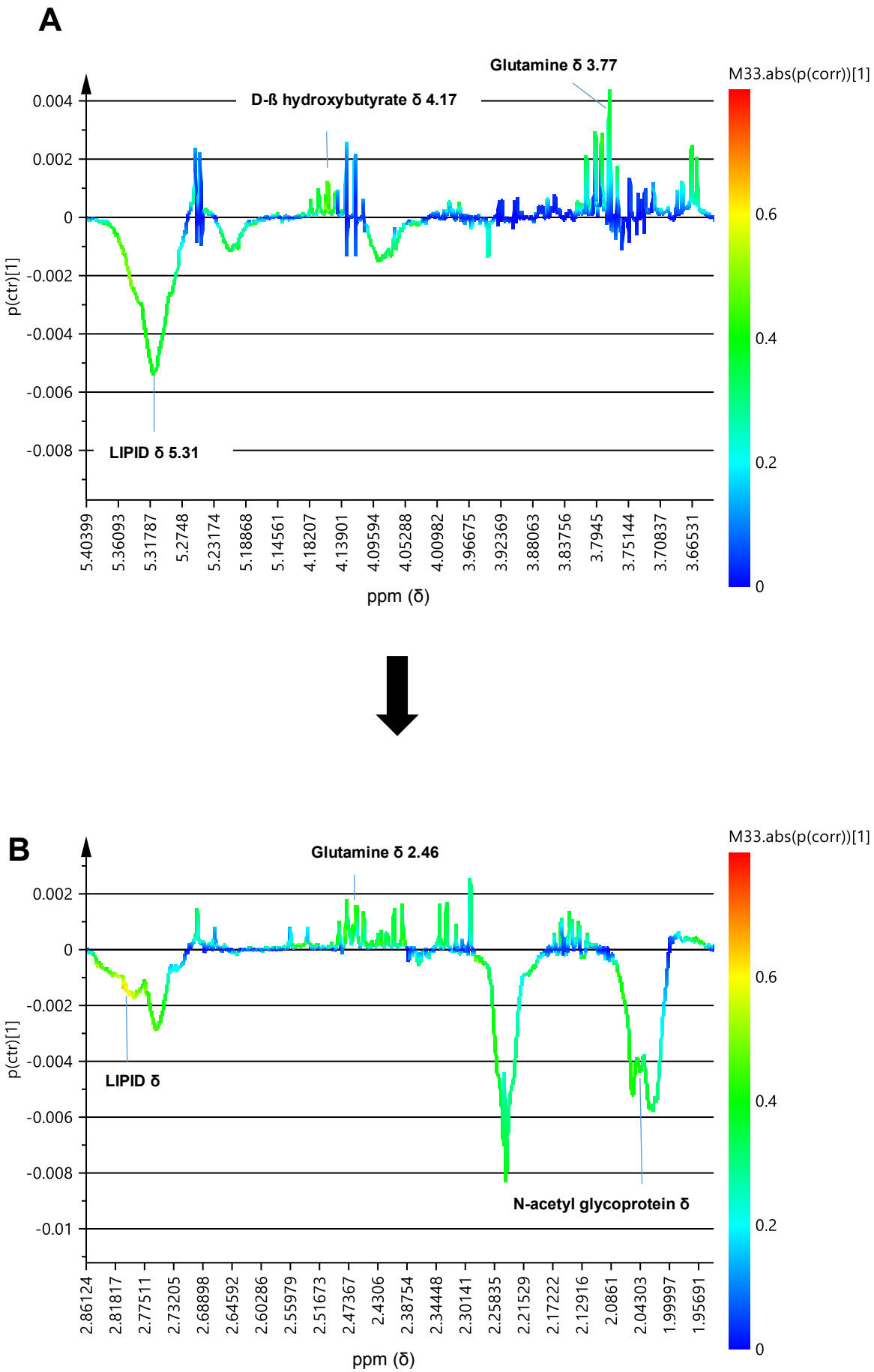


Figure 5.14: S-line plot for OPLS-DA models to separate the metabolic profiling of malabsorptive surgery and no surgery at T4 (30-33 weeks). A positive peak indicates higher metabolite levels in the malabsorptive group. A negative peak indicates higher levels in the no surgery group. Class-separating metabolites are indicated on the magnified sections of the plots, sections A & B.

Time point 5


A
B
C
MALABSORPTIVE SURGERY
NO SURGERY
M33.abs(p(corr))[1]
p(ctr)[1]
0
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
-0.07
-0.08
-0.09
0.6
0.4
0.2
0
8.49951
8.155
7.8105
7.46599
7.12148
6.77698
6.43247
6.08796
5.74345
5.39895
4.13396
3.78945
3.44495
3.10044
2.75593
2.41143
2.06692
1.72241
1.37791
1.0334
0.688892
ppm (δ)


A
Glutamine δ 3.77
D-ß hydroxybutyrate δ 4.17
LIPID δ 5.31
p(ctr)[1]
0.004
0.002
0
-0.002
-0.004
-0.006
-0.008
5.40399
5.36093
5.31787
5.2748
5.23174
5.18868
5.14561
4.18207
4.13901
4.09594
4.05288
4.00982
3.96675
3.92369
3.88063
3.83756
3.7945
3.75144
3.70837
3.66531
ppm (δ)
M33.abs(p(corr))[1]
0.6
0.4
0.2
0
B
Glutamine δ 2.46
LIPID δ
N-acetyl glycoprotein δ
p(ctr)[1]
0.002
0
-0.002
-0.004
-0.006
-0.008
-0.01
2.86124
2.81817
2.77511
2.73205
2.68898
2.64592
2.60286
2.55979
2.51673
2.47367
2.4306
2.38754
2.34448
2.30141
2.25835
2.21529
2.17222
2.12916
2.0861
2.04303
1.99997
1.95691
ppm (δ)
M33.abs(p(corr))[1]
0.6
0.4
0.2
0

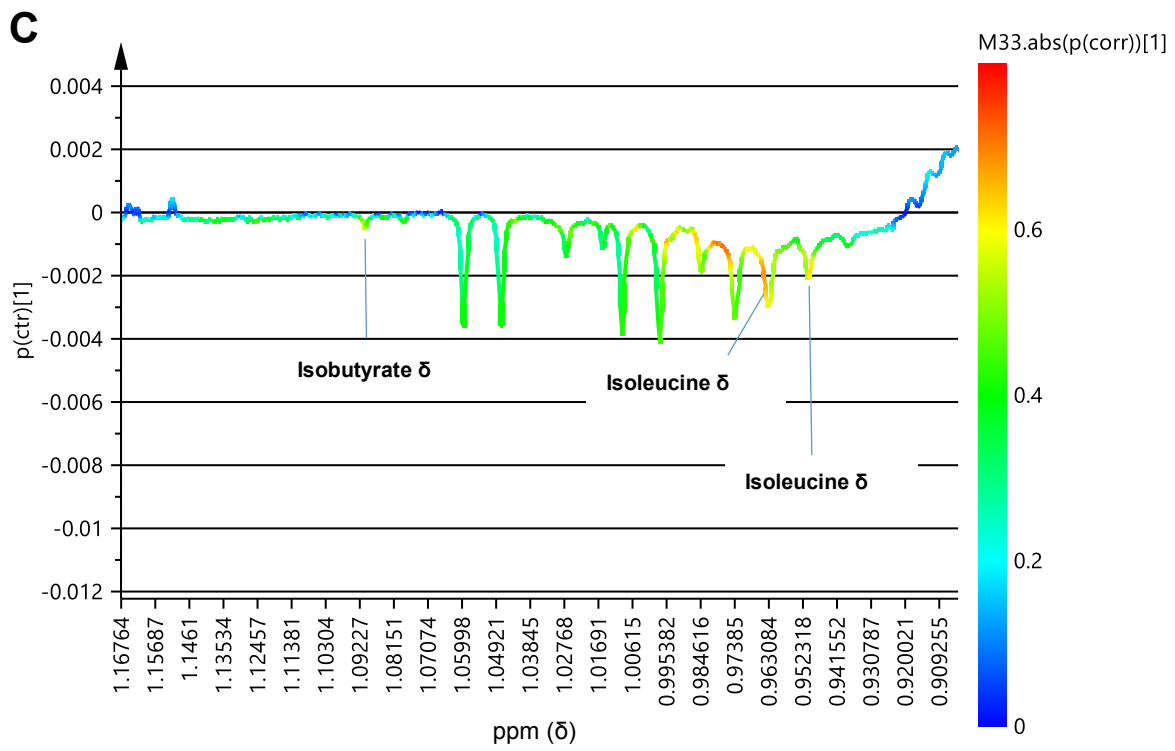


Figure 5.15: S-line plot for OPLS-DA models to separate the metabolic profiling of malabsorptive surgery and no surgery at T5 (35-37 weeks). A positive peak indicates higher metabolite levels in the malabsorptive group. A negative peak indicates higher levels in the no surgery group. Class-separating metabolites are indicated on the magnified sections of the plots, section A, B & C.

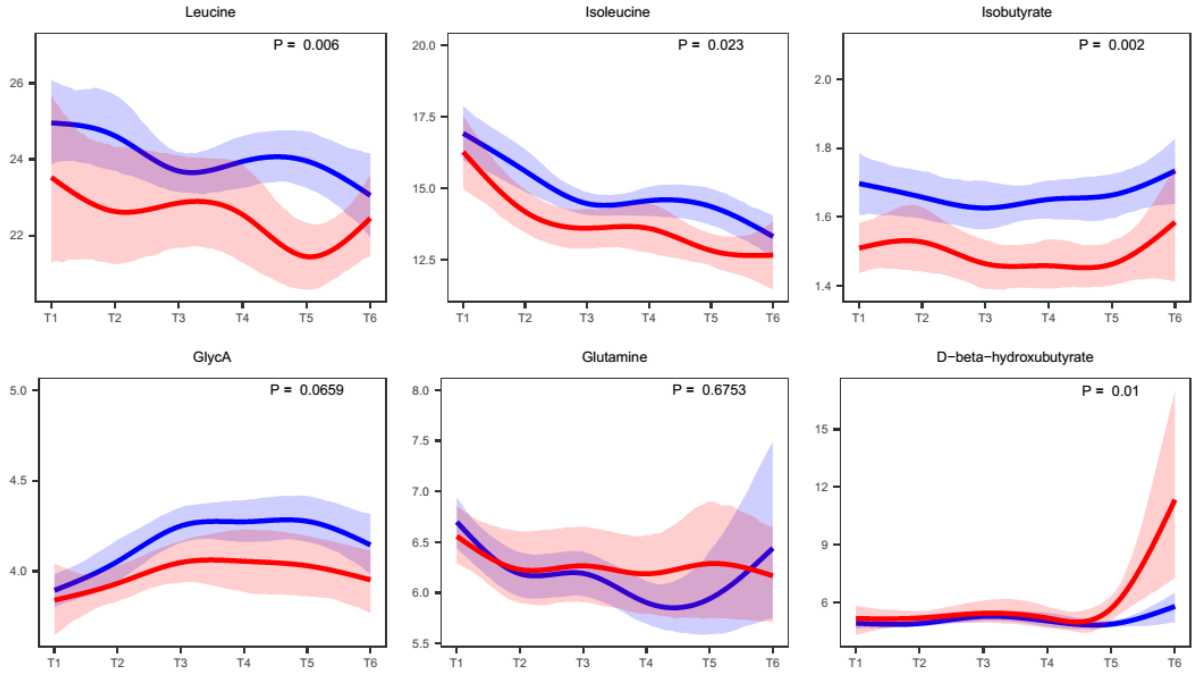


Figure 5.16: Time series analysis of discriminatory metabolites. The lines represent the mean curves of the metabolite concentrations. Metabolite levels in malabsorptive post-bariatric surgery patients is represented by the red lines and no surgery patients represented by the blue lines. The shaded areas represent the 95% confidence intervals. $p<0.05$ Mann Whitney U test.

Principal component analysis (PCA) and OPLS-DA was performed on cord blood samples (T7), summarized in Figures 5.17 and 5.18. There were no differences between metabolic profiles of samples from infants of women with previous bariatric surgery and those with no surgery.

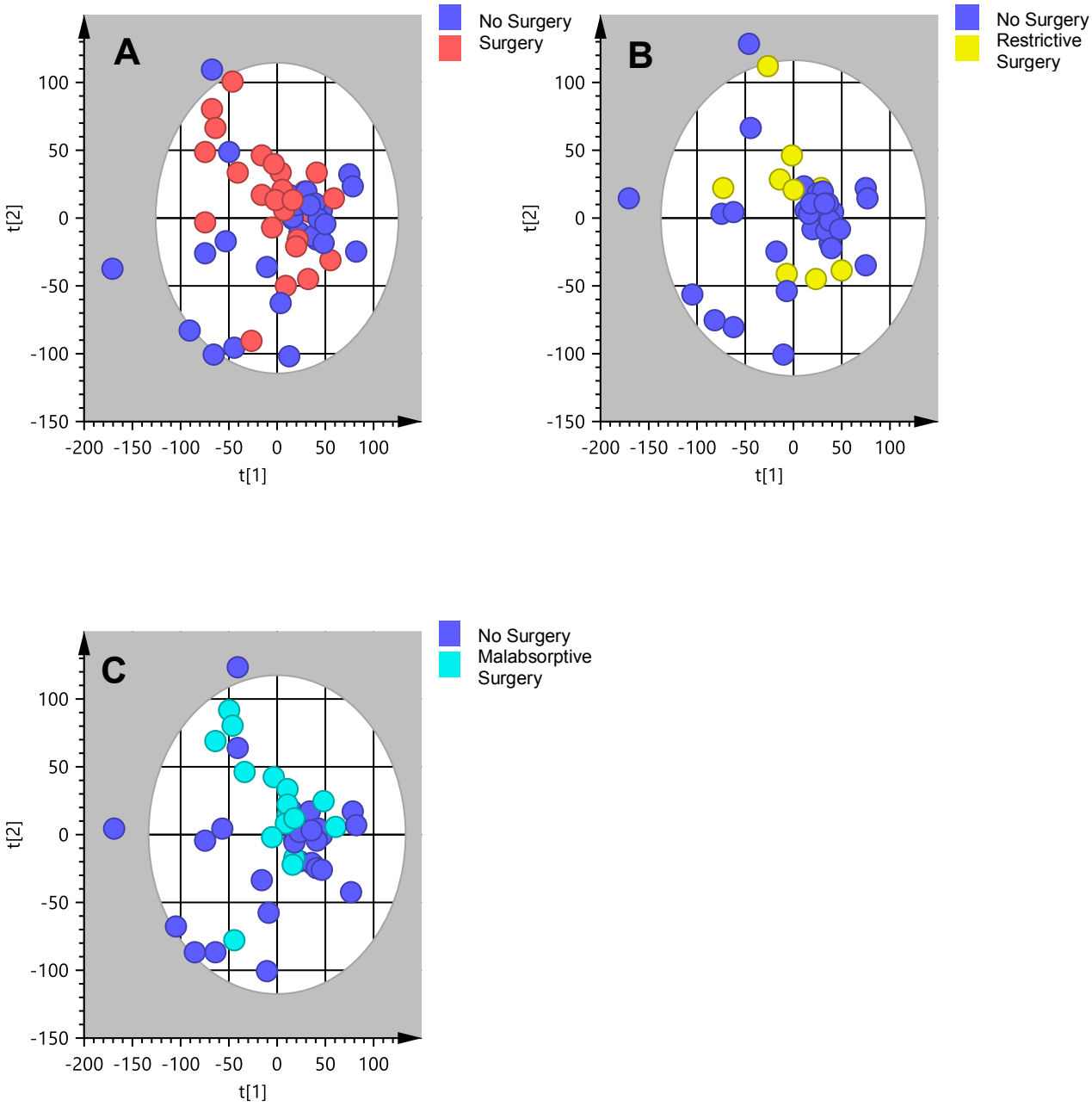
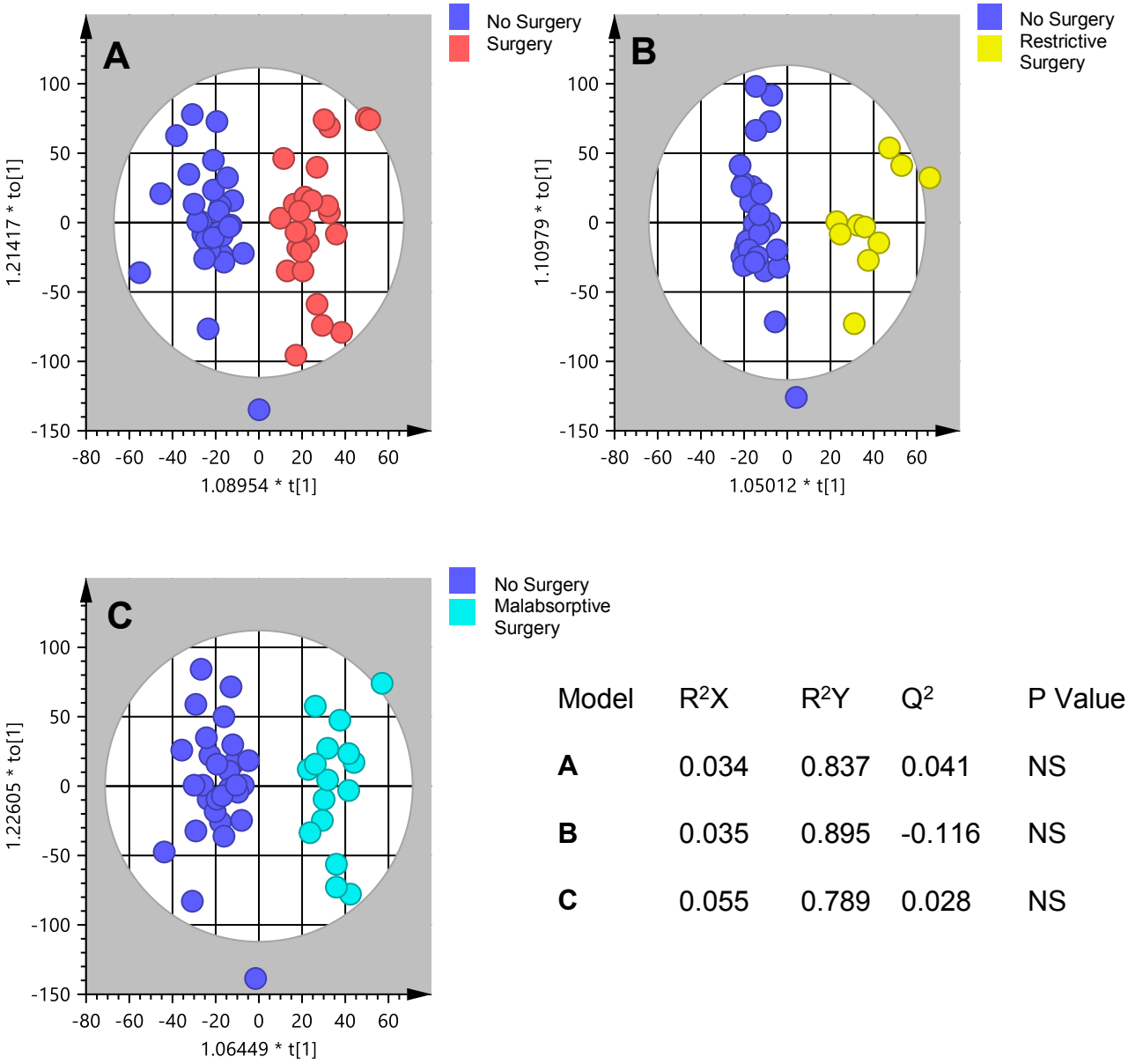


Figure 5.17: PCA models ^{1}H NMR spectral data from cord blood at delivery (T7) (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. A summary of fit for all models (not shown) had negative R2 and Q2 values for all components of the models, demonstrating over-fitting.



Model	R^2X	R^2Y	Q^2	P Value
A	0.034	0.837	0.041	NS
B	0.035	0.895	-0.116	NS
C	0.055	0.789	0.028	NS

Figure 5.18: OPLS-DA models of ^{1}H NMR spectral data from cord blood at T7 (a) no surgery and bariatric surgery; (b) no surgery and restrictive bariatric surgery and (c) no surgery and malabsorptive bariatric surgery. P value NS: not statistically significant.

Table 5.3: Summary of findings

Time Points	OPLS-DA Models	P value	Metabolite Alterations identified.
T1, T2, T3	NBS vs RES	NS	-
	RES vs MAL	NS	-
	NBS vs MAL	NS	-
T4	NBS vs RES	NS	-
	RES vs MAL	NS	-
	NBS vs MAL	**0.027**	**MAL compared to NBS group: ↓Unsaturated lipids, ↓Isobutyrate, ↓isoleucine ↓N-acetyl glycoprotein**
T5	NBS vs RES	NS	-
	RES vs MAL	NS	-
	NBS vs MAL	**0.006**	**MAL compared to NBS group: ↓Unsaturated lipids, ↓Isobutyrate, ↓leucine and isoleucine ↓N-acetyl glycoprotein ↑Glutamine ↑ D-ß-hydroxybutyrate,**
T6	NBS vs RES	NS	-
	RES vs MAL	NS	-
	NBS vs MAL	NS	-

T1:11-14 weeks; **T2:** 20-24 weeks; **T3:** 28-30 weeks; **T4:** 30-33 weeks; **T5:** 35-37weeks; **T6:** Delivery
NBS: No bariatric surgery; **RES:** Restrictive Bariatric surgery; **MAL:** Malabsorptive bariatric surgery

5.4 DISCUSSION

Our study has shown that pregnant women with previous malabsorptive bariatric surgery have an altered maternal serum metabolome in the third trimester, notably at 30-33 weeks and 35-37 weeks, compared to those without bariatric surgery. In particular, post-bariatric pregnant women had lower serum level of unsaturated lipids, isobutyrate, leucine, isoleucine and N-acetyl glycoprotein and higher level of glutamine and D-ß-hydroxybutyrate. There was no significant difference in cord blood metabolite levels between the groups.

Lipids

With respect to the observed lower serum level of unsaturated lipids, similar serum metabolite alterations are seen in studies on non-pregnant adults following malabsorptive bariatric surgery. Mika et al conducted a study of morbidly obese patients post bariatric surgery (sleeve gastrectomy and gastric bypass) and found a significant decrease in various serum lipids, in particular an 82.5% decrease in the level of 7-lathosterol, a precursor of cholesterol, 6 months post-operative. They concluded that the reduced serum lipid profile may be due to a reduction in dietary intake, hepatic cholesterol production and desaturation of fatty acids. (378)F Lopes et al also showed decreased levels of very low density lipoproteins (VLDL) and unsaturated lipids, following Roux-en-Y gastric bypass (RYGB), which could indicate excessive lipid peroxidation and/or oxidative stress with this type of surgery.(379)

During normal pregnancy, regardless of BMI, researchers have found that lipids (including total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides-TG), insulin, leptin and interleukin 1b (IL-1b) all increase significantly ($p<0.05$). (380)

N-acetyl-glycoprotein

Reduction in N-acetyl-glycoprotein levels has also been documented in a previous study following RYGB. (381) Chronic inflammation, a known feature of obesity, is associated with pathogenesis of several diseases including atherosclerosis, metabolic syndrome and insulin resistance. A reduction in N acetyl glycoprotein, an inflammatory marker, may reflect reduced inflammation post RYGB.

The reduction in maternal lipid and inflammatory marker(s) following malabsorptive bariatric surgery, seen in our study, may be positively associated with a lower incidence obesity-related pregnancy complications such as pre-eclampsia and gestational diabetes and their adverse perinatal consequences.(382)

Amino acids

Reduction in serum levels of branched chain amino acids (BCAA) leucine and isoleucine post malabsorptive bariatric surgery is a finding corroborated by several other studies outside pregnancy. Following RYGB, Wijayatunga et al demonstrated a

reduction of 2- oxoisocaproate, which is a surrogate for leucine since it is an intermediate of leucine metabolism, and this could also be due to excessive lipid peroxidation and/or oxidative stress associated with this type of operation with RYGB. (383) Gralka et al had similar findings following three types of bariatric surgery: sleeve gastrectomy, proximal RYGB and distal RYGB. (384) The significant reduction of isoleucine and leucine ($p<0.001$) was not dependent on the type of bariatric surgery, although all three have a malabsorptive component. Both leucine and isoleucine stimulate increased insulin secretion and reduced levels improve insulin resistance. (385) This is important in the context of pregnancy after bariatric surgery and may go some way to explain the reduced incidence of GDM seen in post bariatric pregnant women. Future work including participants with and without GDM would be required to confirm this.

Our findings of a higher serum glutamine level post bariatric surgery are in line with published studies outside pregnancy. Glutamine is an essential amino acid with an important regulatory role in glucose metabolism. An animal study of obese rats by Wolff et al demonstrated that RYGB increased intestinal glutamine transport and absorption. (386) The clinical implication of this is uncertain as the same researchers found that the post RYGB rats had a down-regulation of the enzymes responsible for glutamine metabolism and subsequent gluconeogenesis. More work is needed to understand the clinical effect of its raised levels in pregnancy following bariatric surgery.

D-ß-hydroxybutyrate

Researchers Herzog et al, also observed a transient post-operative rise in ketone bodies acetoacetate and 3-hydroxybutyrate (also known as beta hydroxybutyrate) peaking the first few months after surgery, reflecting both a post-operative catabolic state and perioperative dietary intervention. (387) It is possible that malabsorptive bariatric surgery causing reduced availability of glucose substrate, may render pregnant women unable to meet the higher metabolic demands of pregnancy, resulting in greater lipid metabolism and ketogenesis. The clinical implication of this in pregnancy following bariatric surgery is yet unknown. Most studies on ketosis in pregnant women are in the context of diabetes and have found a significant link between ketosis in pregnancy and congenital malformations including cleft lip and

palate, cardiac malformations and neural tube defects such spina bifida. (388) Some animal models have also shown that pregnancies exposed to high levels of ketones have a greater incidence of fetal malformations, mainly cardiac and neural tube defects. It is important to note that the levels of ketone exposure was 20- 40 times higher than what is achieved in normal human ketosis. (389) More work is required to determine the effect of ketosis in non-diabetic pregnant women post bariatric surgery.

Isobutyrate

Contrary to the rise of hydroxybutyrate, the branched chain amino acid, Wijayatunga et al also showed that Butyrate, produced from dietary fibre by gut microbia, had lower serum levels 6 months post RYGB compared with pre-surgery levels. (390) A reduction of butyrate-producing gut microbes and/or lower consumption of dietary fibre are thought to be responsible for this. Our study of pregnant women found a reduction of butyrate's isomer, isobutyrate, following malabsorptive bariatric surgery. Since stool specimens were not analysed in our study, conclusions cannot be confidently drawn without further research.

Strengths and Limitations

There are several strengths of our study. This is a novel study of serum metabolomic profiles in pregnant women following bariatric surgery. The longitudinal design, different gestational time points and inclusion of different types of bariatric surgery are important strengths. The use of NMR allows comparison with other published work in metabolomics work. We were also able to obtain fasting samples at 28 weeks gestation, allowing accurate measurements of metabolites.

A limitation of this study is the relatively small sample size of the cord samples, which may explain the lack of cord blood metabolite alterations seen between the groups. Future work would include higher number of participants within each group. Further work could also compare metabolite alterations with BMI and the interval between bariatric surgery and pregnancy.

5.5 Conclusion

This study has shown that, in the late third trimester, pregnant women with previous malabsoptive bariatric surgery have reduced levels of unsaturated lipids, isobutyrate, leucine, isoleucine and N-acetyl glycoprotein but higher levels of glutamine and D-ß-hydroxybutyrate, compared to pregnant women without surgery. Further research will be required to investigate the role of these alterations in the pregnancy outcomes of women with previous malabsorptive bariatric surgery.

CHAPTER 6

Lipoprotein Profile of Women with different BMI at 28 weeks of gestation

ABSTRACT

Objective: To investigate how maternal lipid profiles, at 28 weeks of gestation, vary according to body mass index (BMI).

Methods: Eighty five pregnant women were prospectively recruited from the antenatal clinics at Chelsea & Westminster Hospital, London, from May 2015 until April 2017. Maternal blood serum (fasting blood) was obtained at 28^{+0}-30^{+0} weeks' gestation. H^1 NMR lipoprotein profiling was performed on the samples. The lipoprotein classes including very low density lipoproteins (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density Lipoproteins (HDL) were compared between the BMI groups.

Results: In obese women, with BMI ≥ 30kg/m^2, compared to women with normal BMI (18.5-24.9 kg/m^2), there was a higher triglyceride content in the HDL sub-fraction 4 (HDL4) (p=0.02) and a lower cholesterol and phospholipid content in the HDL sub-fraction 1 (HDL1) (p=0.02 and p=0.03 respectively). There was also a lower cholesterol content in LDL sub-fraction 2 and 3 (LDL2, LDL3) (p=0.03 and p=0.02 respectively). Conversely, obese women had a higher phospholipid content in LDL3 (p=0.02) and higher content of cholesterol and phospholipid in VLDL sub-fraction 1 (VLDL1) (p=0.02). In overweight women (BMI 25–29.9 kg/m^2), compared to women with normal BMI, there was a higher Apolipoprotein A2 (Apo-A2) content in HDL sub-fraction 3 (HDL3), (p=0.02).

Conclusion: Our study has demonstrated significant differences in the maternal lipoprotein lipid profile between BMI groups, at 28 weeks gestation. These changes could have implications on perinatal outcome.

6.1 INTRODUCTION

Lipids are essential, dynamic components of biological membranes. As well as a cellular barrier they act as signal receptors, transmitters and membrane transporters. They are substrates for hormones, an important energy source and participate in numerous vital metabolic processes. The three main types of lipids are: triglycerides (TG), phospholipids and sterols (e.g. cholesterol). (391) Besides enabling the normal function of healthy cells, lipids are also responsible for the pathophysiology of several disease processes such as atherosclerosis, resulting in cardiovascular disease. (392)

Lipid protein complexes that transport insoluble lipids in the blood are called lipoproteins which have varying density depending on the amount of protein. Low density lipoprotein (LDL) transport cholesterol and triglycerides from the liver to tissues. Conversely, high density lipoprotein (HDL) remove cholesterol from tissues. (393) HDL has a diverse, complex composition, apart from cholesterol. This includes: ApoA-I, almost a hundred different proteins including microRNAs, hundreds of different lipids and it is encased in phosphatidylcholine. (394)

A lipidome is the entire lipid content in a cell, organ or biological system. It is not static but can be altered over time or as a result of environmental, metabolic, physiological or pathological changes. (395) It is predicted to be in the range of tens of thousands to hundreds of thousands at concentrations of nmol/mg of protein. (396) The lipidome is the subject of interest in lipidomics. Outside of pregnancy, changes in the lipid profile is associated with obesity and metabolic diseases. (397) Similarly, alterations in the maternal lipid profile have been associated with the development of pre-eclampsia and gestational diabetes (GDM); conditions typically arising in the third trimester of pregnancy. (398) The analysis of lipidomic distinction may provide some explanation of the different prevalence of pregnancy complications in the different BMI groups.

The aim of this study is to identify how maternal lipid profiles vary according to BMI in the third trimester, at 28 weeks gestation.

6.2 MATERIALS AND METHODS

This was a cross sectional study comparing serum lipid composition of women with different BMI at 28 weeks of gestation.

The study participants were part of an on-going prospective, observational study investigating pregnancy outcomes of women with previous bariatric surgery. The study included 85 pregnant women with no previous history of bariatric surgery that were recruited from the antenatal clinics at Chelsea & Westminster Hospital, London, from May 2015 until April 2017. Women were included if they were over 18 years old, had a singleton pregnancy and not diabetic. Written informed consent was obtained from all women.

At 28-30 weeks of gestation, weight and height were measured in the women standing upright, without shoes and in light clothing. The BMI was calculated as weight in Kg divided by the square of height in meters. Women were put into three BMI groups as per World Health Organisation (WHO) guidelines: (i) Normal BMI (18.5-24.9 kg/m^2), (ii) Overweight (25 – 29.9 kg/m^2) and (iii) Obese (≥ 30kg/m^2). The waist to hip ratio (WHR) was calculated; waist circumference measured just above the iliac crest and hip circumference from the widest diameter of the hips, over the great trochanters of the femur bones.

At the same time (28-30 weeks of gestation), all women underwent a 75gr oral glucose tolerance test (OGTT) after an overnight fast of at least 8 hours. Fasting maternal plasma and serum samples were collected at the time of the OGTT, centrifuged (4600RPM for 10mins) and stored at -80°C, within 30mins of collection.

Lipoprotein Profiling

^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy was used to quantify lipoproteins in serum samples (*Bruker Biospin GmbH*, *Germany*). NMR is a faster tool with a high throughput (100 samples/day) than the alternative Ultracentrifugation which can take 24h to complete analysis. (399) Lipoprotein classes were quantified by NMR spectral measurement of the terminal methyl groups. The lipoprotein classes compared between BMI groups were: Very low density lipoproteins (VLDL), Intermediate density lipoprotein (IDL), LDL and HDL. Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA ™) prediction algorithm calculated the particle number in the different lipoprotein sub fractions directly from the ^{1}H NMR spectra of serum

samples. It used regression models to predict the concentration of the lipoprotein parameters of interest. (400) The sub fraction categories are based on particle diameter i.e. the higher the sub fraction number, the smaller the particle size. (401) Other lipid parameters that were measured included: TG, Cholesterol and Apolipoprotein-A1, A2 and B100.

Statistical Analysis

The Kolmogoroff–Smirnoff test was used to assess normal distribution of the data. The mean and standard deviation were used to describe continuous (dependent) variables with normal distribution. The median and interquartile range (IQR) described continuous variables without normal distribution. Categorical (independent) variables were represented by percentage or frequency counts. Analysis of the differences between the groups required the use of the Mann-Whitney U test, one-way analysis of variance (ANOVA), chi-squared and Independent-samples T-test as appropriate.

Multivariate statistical analysis was performed in Soft Independent Modelling of Class Analogy (SIMCA) software, version 14.1 (MKS Umetrics, Umeå Sweden).
Univariate statistical analyses was performed using SPSS Software version 25.0 (IBM, Chicago, IL, USA). Differences were considered statistically significant at p value <0.05.

6.3 RESULTS

The study included 85 pregnant women divided into three groups according to their BMI at 28 weeks' gestation; at the time of the OGTT. From this cohort, 28 women had a normal BMI, 16 women were overweight and 41 were obese.

Participant Characteristics

The demographics and clinical characteristics are demonstrated in Table 6.1. Obese pregnant women were younger than overweight and normal BMI pregnant women ($p=0.03$). There was a similar spread of parity and conception between the groups. Most of the women were white and non-smokers.

The WHR was significantly higher in the obese and overweight group compared to the normal BMI group (p<0.01). Regarding birth outcomes, all women had live births and all babies were born at term (>37 weeks gestation). The obese women had their babies slightly early then the normal weight women (p = 0.02). Overweight and obese women both had babies with higher birth weights than normal BMI women (p = 0.01, 0.04) respectively.

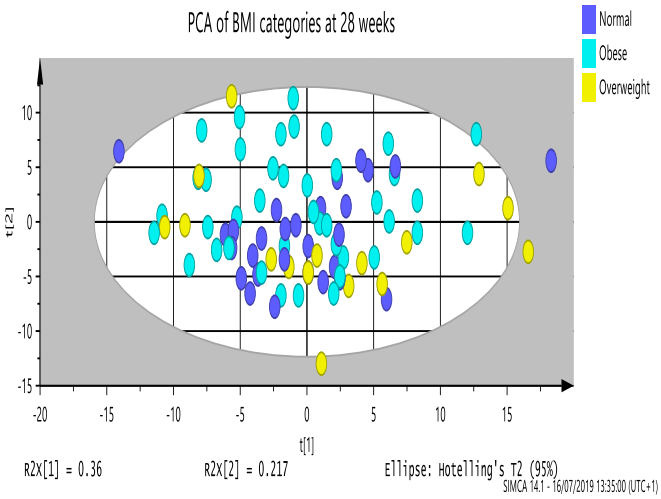
Table 6.1: Demographics & clinical characteristics of participants.

Characteristics	**Normal BMI* (n=28)**	**Overweight (n=16)**	**P value Overweight vs Normal**	**Obese (n=41)**	**P value Obese vs Normal**
Maternal age (years)	33 (30-35)	33 (31-36)	0.46	29 (26-33.5)	0.03
Parity, n (%)					
Nulliparous	19 (67.9)	9 (56.3)	0.11	24 (58.5)	0.43
Parous	9 (32.1)	7 (43.8)		17 (41.5)	
Ethnic group, n (%)					
White	25 (89.3)	13 (81.3)	0.36	29 (70.7)	0.17
Other	3 (10.7)	3 (18.8)		12 (29.3)	
Conception, n (%)					
Spontaneous	28 (100)	15 (93.8)	0.68	40 (97.6)	0.78
Assisted reproductive techniques	0	1 (6.3)		1 (2.4)	
Smoking, n (%)					
No	28 (100)	16 (100)	-	39 (95.1)	0.41
Yes	0	0		2 (4.9)	
First Trimester BMI	21.5 ± 1.7	27.1 ± 1.7	<0.01	38.1 ± 5.8	<0.01
BMI at OGTT	22.9 ± 1.4	27.0 ± 1.3	<0.01	39.8 ± 6.2	<0.01
Gestational age at OGTT	28.5±0.7	28.4±0.6	0.72	28.4±0.7	0.80
Waist to Hip ratio at OGTT	0.9±0.06	0.96±0.05	<0.01	0.98±0.8	<0.01
Gestational age at delivery (wks)	40.4 (39.1–40.9)	39.5 (39.0–40.2)	0.89	39.1 (38.1–39.8)	0.02
Birth weight (gr)	3268.6±531.7	3598.1±442.5	0.04	3361.6±565.1	0.49
Birth weight percentile	40.6±29.7	64.3±26.9	0.01	56.8±33.1	0.04

Data are expressed as mean (±SD) or median (IQR). All comparisons were done with the normal body mass index (BMI) group. *p<0.05. BMI: body mass index; OGTT: Oral glucose tolerance test. *WHO BMI classification (Kg/m^2): Normal = 18.5-24.9; Overweight 25-29.9; Obese ≥ 30.

Lipoprotein subclass NMR analysis

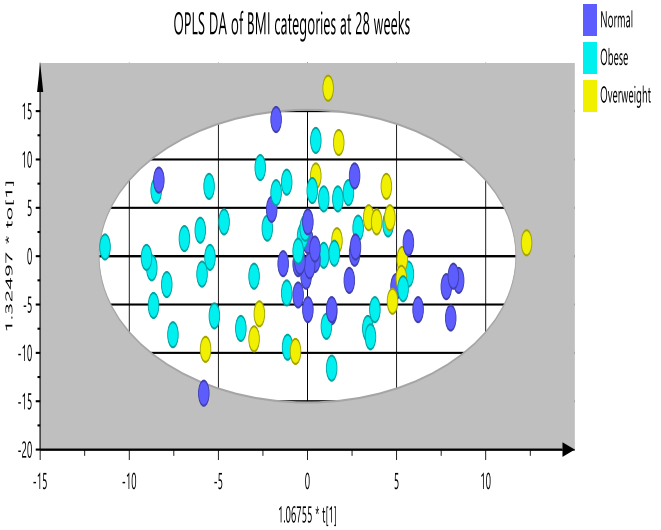
This analysis compared 111 lipoprotein measurements in maternal serum. Initially, multivariate analysis (MVA) using principal component analysis (PCA) was conducted to demonstrate variation in the dataset (Figure 6.1). There was no dominant systematic variation between the BMI groups when all lipid metabolites were compared in this unsupervised model.



Component	R^2	R^2 (cum)	Q^2	Q^2 (cum)
1	0.36	0.36	0.318	0.318
2	0.217	0.577	0.282	0.51

Figure 6.1: PCA score plot of lipoprotein NMR data from the pregnant women at 28 week gestation.
Each score plot was coloured according to BMI. The model quality parameters are summarised on the right.

A supervised model, orthogonal partial least squares discriminant analysis (OPLS-DA) also did not show discrimination between sample classes (Figure 6.2).



	R2X	R2	Q2	R2Y
P1	0.194	0.091	**- 0.037**	0.554
O1	0.325			

Figure6.2: OPLS-DA score plot of lipoprotein NMR data from the pregnant women at 28 weeks gestation. Each score plot was coloured according to BMI. The model quality parameters are summarised on the right.

On the contrary, the model had evidence of over-fitting with a negative Q^2 value. For this reason univariate analysis with the Mann Whitney U test was used instead, to compare individual lipid components between the BMI groups:

Lipoproteins

Comparing the different lipoproteins, there were higher levels of LDL in overweight compared to normal BMI pregnant women, although this did not reach statistical significance (p=0.08) (Figure 6.3).

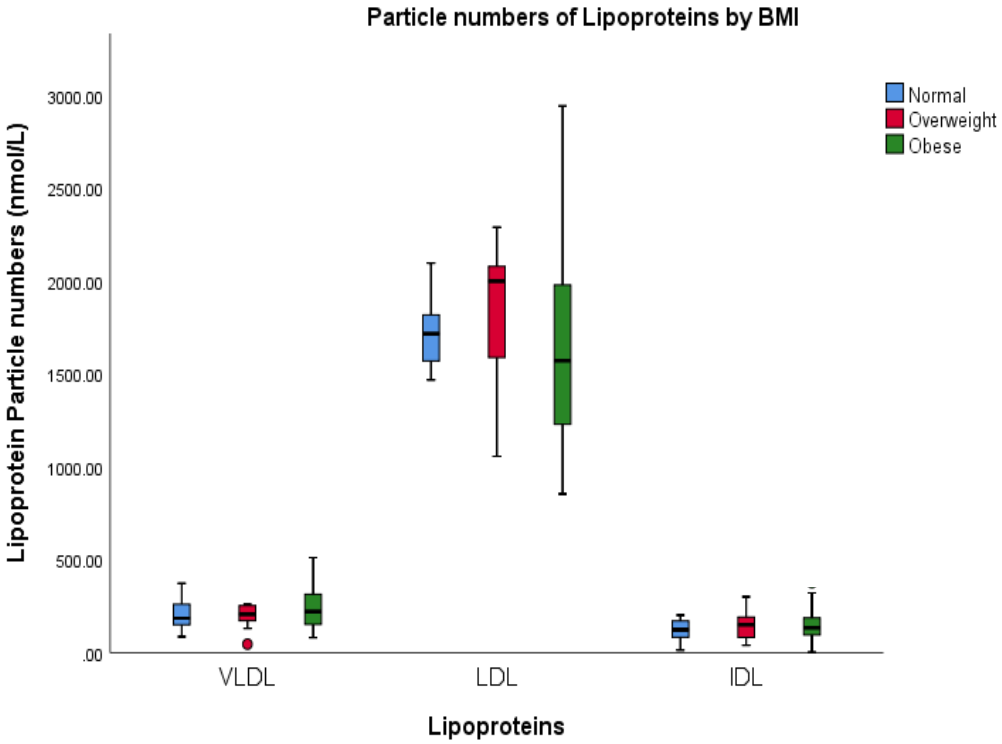


Figure 6.3: Box plot illustrating the distribution of lipoprotein particle numbers (PN) between BMI groups at 28 weeks gestation. All comparisons are made with the normal BMI group. Upward trend of LDL in overweight women did not reach statistical significance (p=0.08).
VLDL: Very Low Density Lipoprotein, IDL: Intermediate Density Lipoprotein, LDL: Low Density Lipoprotein

Interrogation of the LDL sub fractions showed no variation in LDL1 to LDL6 levels between the BMI groups (not shown). Total Triglyceride (TG) levels were of similar proportion between BMI groups at 28 weeks gestation (Figure 6.4). TG within lipoproteins as a whole also showed no variation between the BMI groups. The TG content of lipoprotein sub fractions however, showed that HDL4 had significantly higher TG content in obese compared to pregnant women with normal BMI at this gestation (p=0.02), (Figure 6.5).

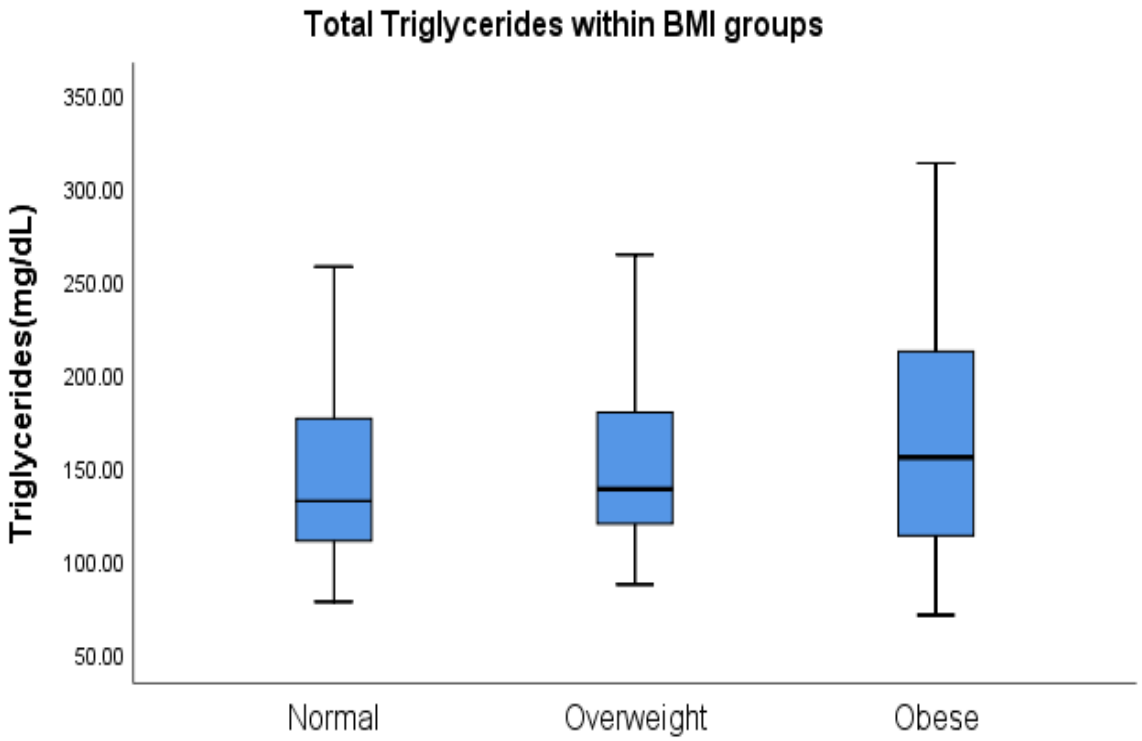


Figure 6.4: Box plot illustrating the triglyceride levels between BMI groups at 28 weeks gestation. All comparisons are made with the normal BMI group. No significant difference identified.

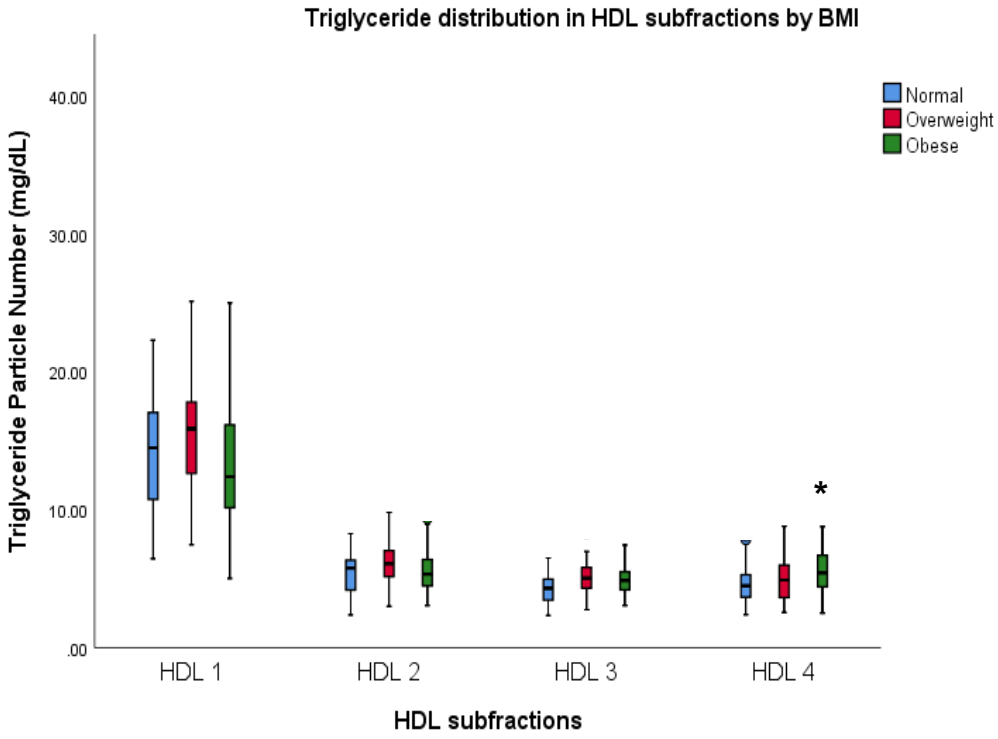


Figure 6.5: Box plot illustrating the triglyceride content of HDL between BMI groups at 28 weeks gestation. All comparisons are made with the normal BMI group. *p<0.05.

There was no significant difference between serum cholesterol levels between BMI groups at 28 weeks gestation.

In the lipoprotein sub fractions, HDL1, LDL2 and LDL3, cholesterol content was shown to be significantly lower in obese compared to those with normal BMI (p=0.02, p=0.03 and p=0.02 respectively), (Figures 6.6a-c). The same findings were shown with both total and free cholesterol. The reverse was the case for VLDL1, where the cholesterol content (free and total) was significantly higher in the obese compared to normal BMI pregnant women (p=0.009), (Figure 6d).

Serum phospholipid showed no significant variation between BMI groups at this gestation. The distribution in LDL3 and VLDL1 subgroups was significantly higher in obese compared to women with normal BMI (p=0.04 and p=0.02 respectively), (Figure 6.7a-c). In HDL1 subgroup the phospholipid content was significantly lower in obese compared to normal BMI pregnant women (Figure 6.7b).

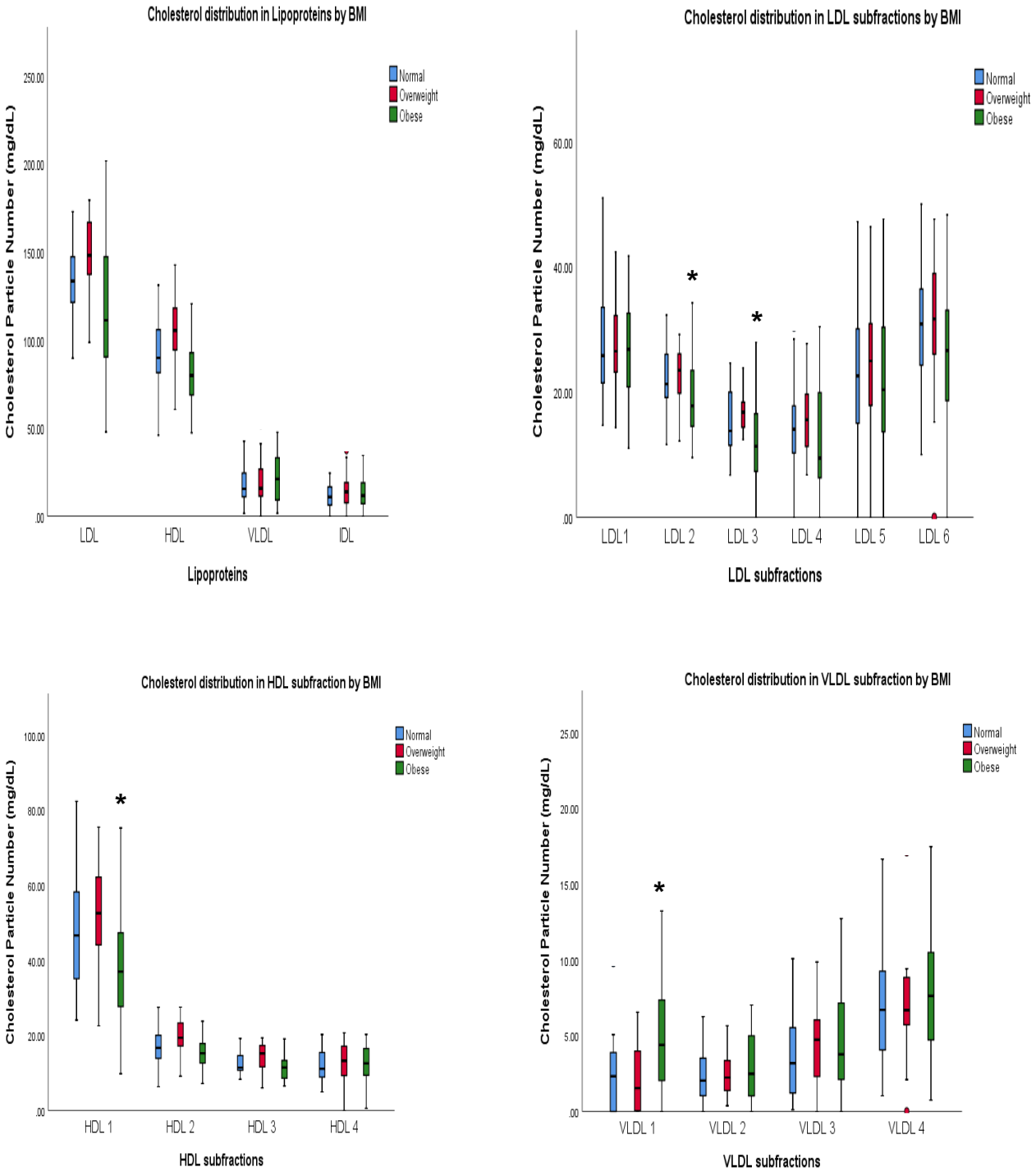
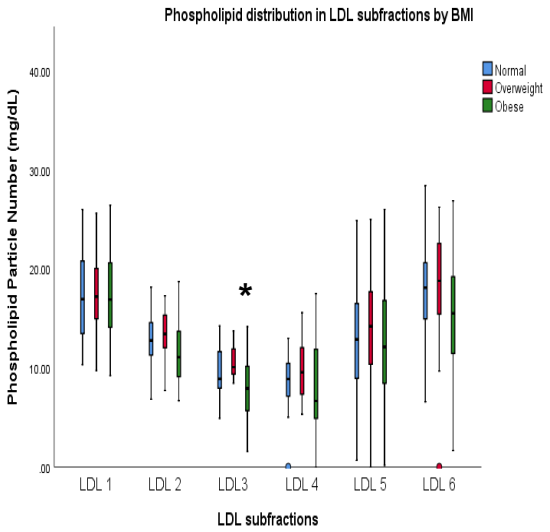
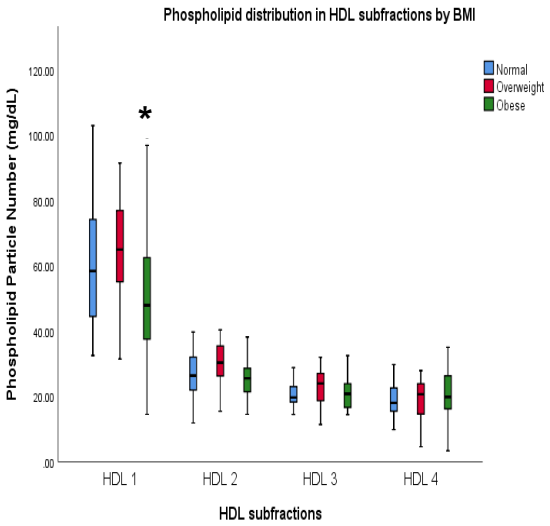


Figure 6.6: Box plots illustrating the cholesterol content of (a) Lipoproteins (b) LDL (c) HDL and (d) VLDL sub-fractions between BMI groups at 28 weeks gestation. All comparisons are made with the normal BMI group. *p<0.05.





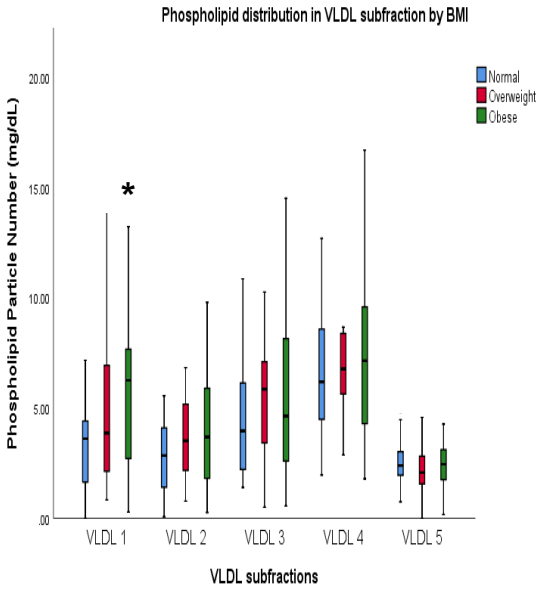


Figure 6.7a-c: Box plot illustrating the phospholipid content of LDL, HDL and VLDL sub-fractions between BMI groups at 28 weeks gestation. All comparisons are made with the normal BMI group. *p<0.05.

Apolipoproteins

The distribution and composition of apolipoproteins A1, A2 and B100 was analysed. Apolipoprotein A1 (ApoA1) levels showed no difference between BMI groups. When comparing levels within HDL and HDL sub fractions by BMI groups, there remained no difference. Overweight pregnant women had significantly higher levels of

Apolipoprotein A2 (ApoA2) compared to normal BMI pregnant women at 28 weeks gestation. HDL and its sub fraction HDL3 had the highest amount of ApoA2 in overweight women (Figure 6.8a & b). Apolipoprotein B100 (Apo B100) levels did not differ significantly between BMI groups, though there was a higher level in the overweight compared to normal BMI women which trended towards statistical significance (p=0.05). Further analysis did not find any variation in its distribution within LDL, VLDL or IDL. The ratio of Apo B100 and Apo A1 was also similar between the BMI groups.

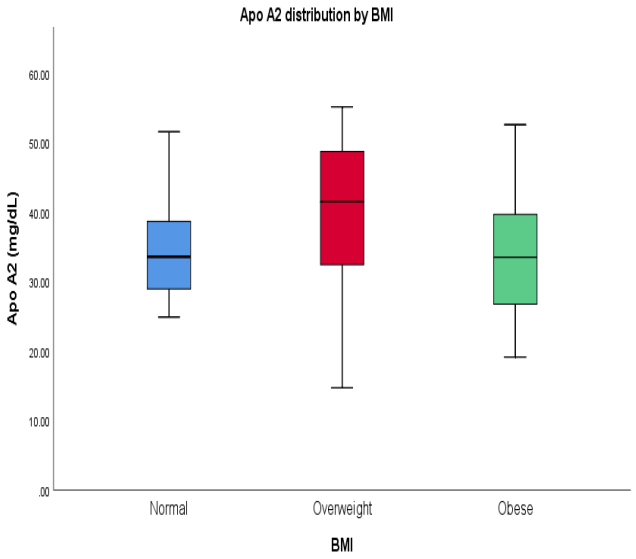


Figure 6.8a: Box plot illustrating the Apolipoprotein A2 (Apo A2) levels between BMI groups at 28 weeks gestation. All comparisons are made with the normal BMI group. *p<0.05.

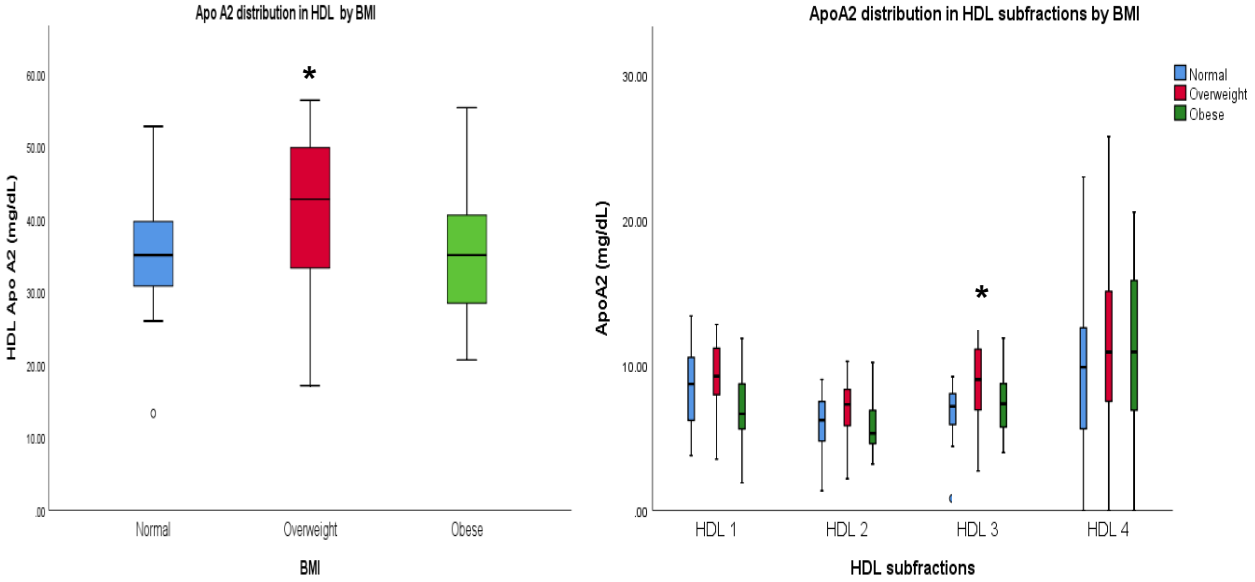


Figure 6.8b: Box plot illustrating the Apo A2 content of HDL between BMI groups at 28 weeks gestation. All comparisons are made with the normal BMI group. *p<0.05.

A summary of the above findings is given in Figure 6.9.

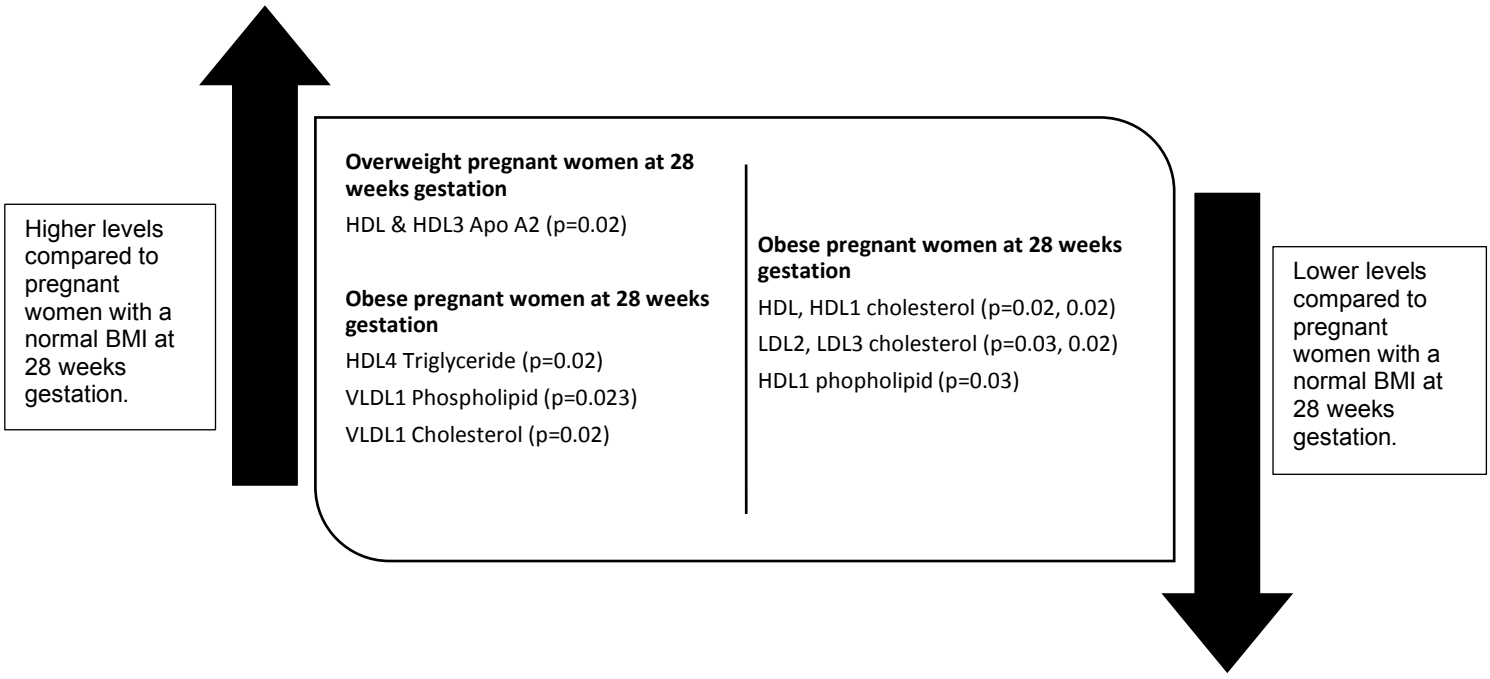


Figure 6.9: Summary of findings from analysis of serum lipid components in pregnant women at 28 weeks gestation in relation to BMI.

6.4 DISCUSSION

Our study has shown that at 28 weeks gestation, obese pregnant women have higher levels of HDL4 Triglyceride, VLDL1 Phospholipid and VLDL1 Cholesterol. Conversely, they have lower levels of HDL, HDL1 cholesterol, LDL2, LDL3 cholesterol and HDL1 phospholipid.

Most studies report a significant elevation of TG, phospholipids and cholesterol throughout the stages of pregnancy reaching a peak in the third trimester. (402) Our study showed that total serum TG, cholesterol and phospholipid levels at 28 weeks were not significantly different between BMI groups. However, there was variation in the lipid composition of lipoprotein sub fractions.

HDL sub-fractions

The traditional view of HDL as the 'good cholesterol' has been challenged over recent years. Historically, evidence from landmark studies such as the Framington study showed cardiovascular disease (CVD) risk is inversely associated with the level of HDL cholesterol (HDL-C). (403) Research has since found that this association is not reliable and very high levels of HDL-C can be detrimental and linked to high CVD risk. (404) The reasons for the paradox is still unknown.

This shift in the perception of HDL, reducing the focus on its ability to remove cholesterol from macrophages (cholesterol efflux capacity) has led to more work into the functionality of HDL and its sub-fractions. There is recognition that HDL sub-fractions are heterogeneous due to variations in their protein and lipid content. This could translate into different functionalities in HDL sub-fractions such as an antioxidant function which could be an alternative mechanism by which HDL provides cardiovascular protection.(405)

NMR analysis divides HDL into four groups with HDL1 being large, HDL2 and HDL3 intermediate and HDL4 being small. (406)

Within the HDL sub fractions in our study, obese pregnant women had significantly higher TG in HDL4 and lower cholesterol and phospholipid content in HDL1 compared to normal BMI women.

Outside the context of pregnancy, total HDL in studies of dyslipidaemia in type 2 diabetics (T2DM) has a similar composition to our results. (407) The researchers proposed that HDL becomes enriched in TG transferred from the increased amount of VLDL found in T2DM. The lower HDL cholesterol and phospholipid content is then attributed to increased HDL catabolism by insulin-enhanced liver lipase.

In pregnancy, findings are mixed; low HDL cholesterol content (HDL-C) in women with GDM has been reported, whereas another study reported that HDL-C does not change significantly during gestation even in GDM patients. (408) A meta-analysis showed that, in late pregnancy, levels of HDL-C were significantly lower in women with GDM compared to women without insulin resistance; in line with our findings of obese pregnant women that are known to have higher insulin resistance. (409)

The significance of these findings in HDL1 and HDL4 sub-fractions in particular is not clear. HDL4 is the smallest HDL sub-fraction and it has been shown in in-vitro studies that the cholesterol efflux is related to its size with the smaller sub-fractions associated with a higher cholesterol efflux than the larger sub-fractions.(410) However, findings are conflicting, as an in vivo study demonstrated the opposite finding, that smaller HDL sub-fractions were associated with lower efflux capacity and higher concentrations found in patients at high risk of CVD. (411) Overall, it can be said that HDL efflux is affected at a sub-fraction level, the exact mechanism is yet to be determined. Results so far indicate a possible disruption in the normal process of HDL development due to obesity and associated insulin resistance.

Lipidomics studies have shown that alterations in the composition of HDL can interfere with the integrity of the HDL particle. A higher TG content as seen in our study in HDL4 subclass, is associated with reduced esterified cholesterol and instability of the particle leading to increased clearance by liver lipase. (412) This would further reduce the level of HDL-C and increase CV risk.

These findings could point to a dysfunctional picture of HDL seen in pregnant obese women, putting them at greater cardiovascular risk. Future work could link these findings with the incidence of gestational hypertension, pre-eclampsia, VTE and GDM. If an association is found, pregnancy-safe methods to mitigate this low HDL1 cholesterol level such as lifestyle changes in diet and exercise, stop smoking, eliminate alcohol could be employed and assessed for effectiveness. HDL1 and HDL4 could also potentially be used as a biomarkers for CV risk in pregnancy.

In overweight women, our results showed a higher Apolipoprotein A2 content in HDL3 than women with normal BMI.

The mechanism behind higher levels of Apo A2 in overweight pregnant women is uncertain, however, it may be of some benefit as it is reported, outside pregnancy, that small HDL particles such as HDL3, enriched in Apo A2 have enhanced cardio-protective ability due to its antioxidant and anti-inflammatory properties. They are able to protect LDL against oxidation. (413) Future work could corroborate this with the clinical picture in overweight women. Outcome measures could compare the incidence of CVD, Pre-eclampsia, gestational HT and VTE with women with normal and obese BMI.

LDL Sub-fractions

LDL has a well-known causal link with atherosclerotic CVD and, similar to HDL, the cholesterol content of LDL has been used as a biomarker for cardiovascular risk. However, recent evidence has shown that this has its limitations and there is benefit to interrogating subclasses of LDL which vary in constitution, with some being more atherogenic in nature than others. (414) Thus, the content and functionality of LDL subclasses has been the focus of recent research to fully understand pathophysiology of cardiovascular disease and how to mitigate the risk.

NMR separates LDL into 6 subclasses: LDL1 to LDL6 from the most light and buoyant to the least (LDL6). (415)

With regards to LDL sub fractions in our study, there was a significantly lower cholesterol content in LDL2 and LDL3 but higher phospholipid content of LDL3 in obese women compared to normal BMI group. In the literature results are conflicting; a meta-analysis showed no differences in aggregate total cholesterol or LDL-C levels between pregnant women with GDM and those without insulin resistance. Studies conducted in the US and Italy also showed lower LDL-C levels in pregnant women with GDM compared with those with normal glucose tolerance but, studies conducted in other countries did not show this change.(416)

The clinical implication of these alteration at subclass level is unclear. It is likely that the obesity related insulin resistance and inflammation relative to normal BMI women

may have an influence. Lower cholesterol content of LDL2 and 3 implies a benefit since this would indicate a lower CV risk. However, these subclasses are light and buoyant molecules which should have a high lipid to protein ratio. These changes in composition would cause a reduced lipid to protein ratio more in keeping with the dense, atherogenic LDL subclasses. (417) Further work would have to clarify this distinction by, for example, increasing the dataset and linking the results to markers for IR and inflammation. It would also be of interest to compare results from different ethnicities as the very dense LDL subclasses are more prevalent in Asian population. (418)

VLDL sub-fractions

Regarding VLDL sub-fractions in our study, there was a significantly higher content of cholesterol and phospholipid in VLDL1 in obese women compared to women with normal BMI. This is a novel finding. It is known that obese pregnant women have a raised IR compared to normal BMI women, albeit subclinical, and the altered content of VLDL1 may be the result of the agonistic effect of IR on adipose tissue lipase resulting in increased lipolysis with a subsequent increased acquisition of cholesterol and phospholipid into the VLDL1 molecule. (419)

It has also been reported that insulin resistance with and without diabetes, outside the context of pregnancy, is a factor that increases production and decreases catabolism of VLDL1, halting the associated reduction of VLDL cholesterol. (420)

Future work would endeavour to prove these hypotheses by linking the composition of VLDL1 in obese pregnant women to markers of IR.

WHR

Women in the obese and overweight BMI group in our study had significantly higher waist to hip ratio compared to the normal BMI group. WHR is a measure of central obesity. In the non-pregnant adult population WHR is a better predictor of obesity-related outcomes than BMI. (421) A high WHR is associated with an increased risk of cardiovascular disease. Literature confirms that fat distribution in pregnancy is related to metabolic adaptations, including insulin resistance which alters the lipid profile and GI hormone secretion.(422) The suggested clinical importance of these findings was

outlined in a study that reported that BMI ≥30 kg/m^2 and WHR ≥0.85 during early pregnancy are significant risk factors for development of GDM and insulin resistance. (423) Although this is not surprising, taken together with the atherogenic changes in the lipoprotein profile of obese pregnant women, this study goes some way to demonstrate CVD risk in pregnancies complicated by obesity.

Strengths and limitations

The main strength of this study is its novelty. To our knowledge, there are no other studies comparing alterations in lipid profile between BMI groups in pregnant women at 28 weeks gestation.

The number of women included in the study was relatively small. A larger, longitudinal study in the future could investigate the relation between altered maternal lipid profiles of different BMI groups, at different gestations and include pregnancy outcome such as birthweight. It would also allow reassessment of the total serum TG, cholesterol and phospholipid levels between BMI groups on a larger scale and may uncover any differences masked by the smaller numbers.

Future work would test the proposed hypotheses from this study and explore potential for clinical use of lipoprotein sub-fraction measurements as a biomarker in pregnancies complicated by obesity, for example to quantify CVD risk and track the effectiveness of risk-reduction initiatives.

This study investigated women without prior bariatric surgery. A future study could compare analysis of the lipoprotein sub-fractions in the post bariatric group with those without previous surgery. This could provide a mechanistic explanation for the differences in pregnancy outcome seen in post bariatric group such as reduction in LGA babies and reduced gestational hypertensive disorders such as pre-eclampsia.

6.5 CONCLUSION

In conclusion, our study shows variation in the lipid composition of lipoprotein sub-fractions in obese pregnant women compared to women with normal BMI at 28 weeks. Larger studies are needed to determine the clinical implications of these findings.

CHAPTER 7

Principle Findings and Future Work

7.1 SUMMARY

The rise in obesity and its consequences including T2DM and subfertility has led to an increase in the uptake of bariatric surgery, particularly among women of child-bearing age. As a result there is a rising cohort of women entering pregnancy following previous bariatric surgery.

Pregnancy outcomes for these women have been well documented, including a reduction in GDM, PE and LGA neonates compared to women without previous surgery but similar pre-pregnancy BMI. There is also a rise in the occurrence of SGA neonates. The mechanism to explain these outcomes have not been well defined.

This thesis has examined the insulin resistance, metabolic and metabolomic profile of these women. The findings from analysis of these areas of interest could give an explanation for the mechanisms driving the reported observations in post bariatric pregnancies. It would also shed some light on the aetiology behind pathological processes that lead to pregnancy-related complications such as GDM and PE.

The effect of obesity on pregnancy at a lipoprotein level was also analysed in an effort to understand mechanistically, how obesity complicates pregnancy, rendering the woman high risk for cardiovascular disease (CVD). Such an understanding would aid development of risk-reduction strategies and potentially develop clinical tools such as biomarkers for risk assessment.

7.2 PRINCIPLE FINDINGS

The findings from the work presented in this thesis are summarised:

In pregnancy following bariatric surgery, compared to women without surgery:

- There is a reduction in maternal insulin resistance, fasting insulin and glucose levels, especially after a malabsorptive procedure at 28 weeks gestation.
- There is a lower fasting leptin level in the third trimester and at delivery.
- There is a higher level of adiponectin in the third trimester.
- Post malabsorptive bariatric surgery, there is a lower serum level of unsaturated lipids, isobutyrate, leucine, isoleucine and N-acetyl glycoprotein and higher level of glutamine and D-ß-hydroxybutyrate at 30-33 weeks and 35-37 weeks gestation.

In pregnancy without previous bariatric surgery:

- There is negative correlation between adiponectin levels at delivery and BW.
- There is a positive correlation between GIP levels at delivery and BW.
- At 28 weeks gestation, obese women have higher levels of HDL4 Triglyceride, VLDL1 Phospholipid and VLDL1 Cholesterol and lower levels of HDL, HDL1 cholesterol, LDL2, LDL3 cholesterol and HDL1 phospholipid, compared to women with normal BMI.

7.3 DISCUSSION

The analysis from this thesis has shown that, in post bariatric women, there is a reduction in maternal insulin resistance, fasting insulin and glucose levels, especially after a malabsorptive procedure at 28 weeks gestation. This is a novel finding that corroborates with research outside the context of pregnancy. It tells us that the metabolic benefits gained from bariatric surgery are carried into pregnancy. The results offer an explanation for the reduced incidence of GDM in this group. At the time of the research, it was acceptable practice for women with previous bariatric surgery

to have an OGTT at 28 weeks. However, as work progressed, published research highlighted the effect of dumping syndrome and altered GI transit such that the accuracy of the OGTT post malabsorptive bariatric surgery was in question. Since then, guidelines have been updated and any future work examining IR in post bariatric women should endeavour to measure glucose levels either with HBGM or continuous glucose monitoring (CGM) as a more accurate means of gaining clearer insight into glucose homeostasis in these women. We also found a positive association between maternal glucose levels (at the time of OGTT) and BW. The lower maternal glucose level seen in post-bariatric pregnant women may go some way to explain the increased incidence of SGA neonates in this population.

Data from a pilot study conducted of post bariatric women showed a lower fasting leptin and higher adiponectin level in the third trimester. These biomarkers have been associated with the development of PE and may be linked to the lower prevalence of this pregnancy complication seen in post-bariatric pregnant women.

At delivery, in women without previous bariatric surgery, the pilot study also showed a negative correlation between adiponectin levels and BW and a positive association between GIP and BW.

H1 NMR data used in a novel, longitudinal study of maternal metabolomic profiles showed that, at 30-33 and 35-37 weeks gestation, post malabsorptive bariatric surgery (gastric bypass) is associated with a lower serum level of unsaturated lipids, isobutyrate, leucine, isoleucine and N-acetyl glycoprotein. Isobutyrate is a proxy for the presence of particular gut microbial. NMR data also showed a higher level of glutamine and of ketone body D-ß-hydroxybutyrate indicating a ketotic state.

The thesis also included a study of non-bariatric women at 28 weeks gestation and analysed the effect of obesity in pregnancy on the lipoprotein profile. Obese women had higher levels of HDL4 Triglyceride, VLDL1 Phospholipid and VLDL1 Cholesterol and lower levels of HDL, HDL1 cholesterol, LDL2, LDL3 cholesterol and HDL1 phospholipid, compared to women with normal BMI. The role of lipoprotein sub-fractions is yet to be fully defined, however the differing contents elude to their different functions which, for example, may be of more clinical relevance than the cholesterol efflux capacity alone. These particular changes reflect a similar pattern seen in the context of increased IR and diabetes outside pregnancy and therefore, it is reasonable

to conclude that the subclinical raised IR in obese pregnant women is responsible and linked to this atherogenic lipoprotein picture.

7.4 FUTURE WORK

Based on the findings of this thesis, future work could include:

(i) Comprehensive assessment of maternal glucose homeostasis using HGM or continuous glucose monitoring during pregnancy of women with different types of bariatric surgery and investigation of the association between maternal glucose control and BW or pregnancy outcomes overall.

Analysis of diurnal variation would allow a global assessment of the integrity of post bariatric glucose homeostasis. Extending the study to assess glucose homeostasis throughout pregnancy could expose any earlier or later changes in IR in all post bariatric women, which may have clinical implications on BW and pregnancy outcomes.

(ii) Investigation of the role of gut hormones and adipokines in maternal glucose control in post-bariatric pregnancies, according to the type of surgery performed.

(iii) Investigation of the role of gut hormones and adipokines in pregnancy outcomes of women with different types of previous bariatric surgery. More emphasis could be given to the role of the above markers in determining BW and the development of PE.

(iv) Maternal and neonatal metabolomic profiling of other types of bariatric surgery such as sleeve gastrectomy in an attempt to establish the best type of bariatric surgery as far as maternal and offspring, short and long term health is concerned.

Findings from this thesis have shown that it is likely that other factors are at play such as alterations in the maternal microbiome following bariatric surgery. Future work should also compare the bariatric and non-bariatric microbiome by analysing

maternal urine, stool and vaginal discharge to identify an association with pregnancy outcomes and birthweight.

(v) Investigation of lipoprotein profile in pregnant women with previous bariatric surgery and its association with pregnancy outcomes.

Future work should also involve stratifying the types of bariatric surgery to acknowledge those women who have had both restrictive and malabsorptive procedures and revision of procedures prior to pregnancy. Identifying the effect of this would be of great gain since it will provide a true reflection of this heterogeneous cohort of women.

7.5 CONCLUSION

Overall, this body of work has uncovered novel data on the effects of bariatric surgery on maternal IR, biomarkers and metabolome. It has also shown how obesity impacts the lipoprotein profile in pregnancy.

The aetiology and clinical application of these findings, particularly in relation to pregnancy outcome and birthweight still needs to be fully realised. Future work is needed to gain more insight into assertions of pathogenesis and clinical relevance in order to inform guidelines of care.

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APPENDIX

Supplementary Table Abbreviations

BDS	Biliopancreatic diversion/duodenal switch
BMI	Body mass index
BPL	Extended biliopancreatic limb
BPD	Biliopancreatic diversion
BIB	Bio enteric intragastric balloon
BS	Bariatric Surgery
BW	Birth weight
CD14	Cluster of differentiation 14
CG1	Control group 1
CG2	Control group 2
CRP	C reactive protein
CS	Caesarean Section
DJB-SG	Duodenal-Jejunal Bypass with sleeve gastrectomy
DM	Diabetes Mellitus
DSIT	Diverted sleeve gastrectomy with ileal transposition
EFW	Estimated fetal weight
F	Female
GS	Gastric sleeve
GT	Glucose tolerance
GB	Gastric Band
GBP	Gastric Bypass
GDM	Gestational Diabetes Mellitus
GLP -1	Glucagon-like peptide – 1
GLP - 2	Glucagon-like peptide – 2
HDL	High density lipoprotein
HOMA – IR	Homeostatic Model Assessment of Insulin resistance
HOMA – B	Homeostatic Model Assessment of Beta cell function
HMW	High Molecular Weight
Hs-CRP	High sensitivity C reactive Protein
HT	Hypertension
ICAM-1	Intercellular adhesion molecule -1
IGF-1	Insulin growth factor - 1

IGT	Impaired glucose tolerance
IL-6	Interleukin - 6
IOL	Induction of labour
IR	Insulin resistance
IUGR	In utero growth restriction
LAGB	Laparoscopic adjustable gastric band
LBW	Low birth weight
LCGP	Laparoscopic greater curvature plication
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein - cholesterol
LGA	Large for gestational age
LPS	Lipopolysaccharide
LSG	Laparoscopic sleeve gastrectomy
M	Male
MCP-1	Monocyte Chemoattractant Protein-1
MDC	Multidisciplinary diabetes care
MGB	Mini gastric bypass
MMP-9	Matrix metallopeptidase - 9
MMT	Mixed meal tolerance test
MO	Morbidly Obese
MS	Malabsorptive surgery
NGT	Normal glucose tolerance
NICU	Neonatal intensive care unit
NO	Nitric Oxide
NS	Non-significant
NVD	Normal vaginal delivery
OGTT	Oral glucose tolerance test
PAI-1	Plasminogen activator inhibitor type-1
PET	Preeclampsia
PIH	Pregnancy induced hyperrtension
PPH	Postpartum haemorrhage
PROM	Preterm rupture of membranes
PTH	Parathyroid hormone
PTX3	Pentraxin-3

PYY	Peptide YY
RANTES	Regulated upon activation, normal T cell expressed and secreted
RBC	Red blood cell
RBP4	Retinol binding protein 4
RYGB(P)	Roux en Y Gastric Bypass
SAT	Subcutaneous adipose tissue
SCBU	Special care baby unit
SGA	Small for Gestational Age
SG	Sleeve gastrectomy
sICAM	Soluble intracellular adhesion molecule-1
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
TLR 2	Toll Like Receptor 2
TLR 4	Toll Like Receptor 4
TNF-a	Tumour necrosis factor alpha
TRACP 5a	Tartrate-resistant acid phosphatase 5a
VAT	Visceral adipose tissue
VLCD	Very low calorie diet
VSG	Vertical sleeve gastrectomy
VTE	Venous thromboembolism

Supplementary Table 1: Literature review of pregnancy outcomes following bariatric surgery

Author, Journal	Study Design	Subjects (N)	Method	Results
Ben-Porat T et al. Obes Surg. 2020	Retrospective cohort study	121 pregnant women with history of laparoscopic sleeve gastrectomy	Medical records of women who underwent SG and delivered during 2010-2018 in a single University hospital were reviewed	68 (56.2%) had evidence of anaemia (Hb<11.0 g/dL) pre- delivery. ↑ Blood transfusion rate
Maric T et al. Acta Obstet Gynecol Scand. 2020	Prospective, longitudinal, observational study	189 pregnant women; Previous BS (n=63) No surgery (n=126)	Fetal arm and thigh volume were obtained at 30-33 and 35-37 weeks' gestation. A 75 g, 2h OGTT was done at 28-31 weeks of gestation	↓ Fetal arm and thigh volume, post-BS, and were positively correlated with the maternal fasting/post-prandial (2 h) glucose levels, at both time points (p<0.01)
Machado BM et al. Obes Surg. 2020	Matched case control study	Singleton births with previous RYGB, n=58 CG1: No surgery, BMI <35kg/m^2 (n=58) CG2: No surgery, BMI >35kg/m^2 (n=58)	Data was retrieved using patient's hospital registration and telephone interviews between years 2000–2010	Compared to CG1: ↓ Gestational weight gain ↓ BW & ↑CS Compared to CG2: ↓ BW, cephalic perimeter, ↓ Macrosomia, hypertension, and GDM

Cruz S et al. Obes Surg. 2020	Longitudinal, retrospective study	119 women who underwent RYGB Not pregnant (n=79) Pregnant (n=40)	At pre-op, 1 year or >1 year (max 2 years) post op: Serum Vitamin D, calcium, PTH was assessed. Gestational and neonatal complications were recorded	Women who became pregnant within 1 year of BS were more likely to develop a urinary tract infection which, in turn, was associated with Vitamin D inadequacy (p=0.02)
Jacamon AS et al. Surg Obes Relat Dis. 2020	Retrospective matched cohort study	52 pregnant women with previous BS Pre-BS BMI, age & parity matched (n=104); Pre-pregnancy BMI, age & parity matched (n=104)	From 1 April 2015- 31 January 2019; maternal and neonatal records assessed	↓ Risks of excessive fetal growth and GDM ↑ Risk of SGA
Różańska-Walędziak A et al. J Clin Med. 2020 (424)	Retrospective Case control	107 women who conceived after BS 345 non-BS women who delivered at tertiary perinatal centre	Data was collected from 627 female patients after BS, of whom 107 had a history of pregnancy after the surgery, and 345 non-BS patients who had a delivery at a tertiary perinatal centre	Patients after bariatric procedures: ↓ GDM (p=0.04) ↓ PIH (p=0.60) ↓ Preterm birth (p=0.003) ↑ CS rate ↑SGA and ↓ LGA

T Maric et al. BJOG. 2020	Prospective Study	162 pregnant women: Previous BS (n=54) No surgery (n=108) with similar booking BMI	Fetal biometry, estimated fetal weight and fetoplacental Dopplers were measured at 3 time-points in pregnancy. BW was recorded	No difference in the feto-placental Doppler indices between groups. Maternal glucose levels at OGTT were positively correlated with third-trimester EFW and BW
Christinajoice S et al. Obes Surg. 2020	Prospective cohort study	45 women LSG n=40 RYGB n=4 LAGB n=1	Retrospective analysis from a prospectively collected database (June 2013-June 2016)	Pregnancy outcome post BS: Preterm delivery (63.15%) LBW (47.3%) NVD (73.4%) maternal anaemia (26.3%)
Malik S et al. Surg Obes Relat Dis. 2020	Prospective case control study	16 post BS 31 obese controls	Prospective analysis of maternal and fetal outcomes in both groups	Post op pregnancies compared to controls: ↓GDM ↓PIH/PET (p=0.003) ↑ LBW (p=0.016)
West KA et al. Gut. 2020	Prospective, longitudinal study	Pregnant women with previous RYGB (n=25) or restrictive (n=16) procedures and matched controls with no surgery (n=70)	A parallel metabonomic and gut bacterial profiling approach was used to determine maternal longitudinal phenotypes associated BS compared with matched controls. Metabolic profiles of	Post malabsorptive BS: ↓ Leucine, isoleucine and isobutyrate levels; ↑ Protein putrefaction metabolite excretion & shift in gut microbiota

			offspring at birth were also analysed	↑ Maternal and neonatal urinary phenylacetylglutamine p=0.001 and p=0.021 respectively
Bozkurt L et al. Obes Facts. 2020	Prospective cross-sectional study	25 women post RYGB No surgery controls: Obese and normal weight (n=19 each)	Women were assessed at 24th-28th gestational week for determination of fasting lipids with follow-up in a subgroup after delivery. Data on neonatal biometry were additionally assessed	After RYGB: ↓ Ultrasensitive C-reactive protein ↓Total-cholesterol, ↓ LDL-C, non-HDL-C & triglycerides vs obese mothers BW percentiles were associated with maternal lipid profile except TG and non-HDL
Maslin K et al. J Hum Nutr Diet. 2020	Retrospective Cohort study	46 pregnancies following BS	Routine clinical information was collected retrospectively from the medical notes of women who had BS and subsequently delivered (January 2012- November 2018)	Suboptimal maternal iron 56.1% and Vitamin D statuses 64.6%

Ibiebele I et al. BJOG. 2020	Population-based record linkage study.	All women giving birth in New South Wales, Australia between 1994-2015 (n = 1606737)	Pregnancy and birth outcome records were compared between first and second pregnancies. Bariatric and non-bariatric groups were also compared	Women who had BS between a first and second pregnancy: ↓ Hypertension ↓ Spontaneous preterm birth ↓ LGA infants ↓ Admission SCBU or NICU in the second pregnancy
Auger N et al. Am J Clin Nutr. 2019	Retrospective cohort study	2,194,348 pregnancies that occurred between 1989 and 2016 in Quebec, Canada	Records were compared between women who had BS before pregnancy and non-obese women with no surgery	Compared with no surgery or obesity, BS is associated with: ↑ Risk of birth defects (heart and musculoskeletal) in subsequent pregnancies. This association is no longer present when folic acid was administered
Neovius M et al. JAMA. 2019	Matched cohort study,	Women With RYGB (n = 2921) Matched Controls (n = 30,573)	Records of singleton live births 2007-2014 were obtained from Swedish Medical Birth Register. Included women receiving RYGB and to women without BS	Women with RYGB had ↓ Risk of major birth defects than infants born to matched control women

Balestrin B et al. Obes Surg. 2019	Retrospective Cohort study	Women who had previous BS (n=93) and obese women (BMI ≥ 30kg/m^2) without surgery (n=205)	Interviews were performed, and the patients' medical records and antenatal information cards were evaluated	Pregnant women who had undergone BS compared to no surgery obese women: ↓ Hypertensive diseases ↓ Diabetes ↔ Prematurity, delivery mode, postpartum complications ↑ SGA ↓ LGA
Watanabe A et al. Arch Gynecol Obstet. 2019	Single-centre retrospective case–control study	24 who conceived following BS: LAGB (n=6) LSG (n=5) MS (n=13)	All singleton pregnancies during the postoperative period (2005-2014) were reported, and questionnaire surveys completed by the mothers re: perinatal outcomes	Comparing LAGB and Malabsorptive surgery: ↑ Gestational HT (LAGB) ↔ Neonatal BW ↑ Maternal anaemia (MS), these women had lowest neonatal BW

Maric TM et al. Metabolism. 2019	Prospective study	41 post-bariatric and 82 pregnant women with no surgery and similar early pregnancy BMI	Maternal IR, at 28 weeks of gestation during 2-hour 75 g OGTT, neonatal IR from umbilical cord venous blood, and neonatal BW and body fat composition at birth were evaluated. IR was assessed using the homeostasis model assessment of IR	Pregnancy following BSy: ↓ Maternal IR ↓ BW ↓ Neonatal adiposity No improvement in cord IR
Rottenstreich A et al. Surg Obes Relat Dis. 2019	Retrospective case-control study	66 women with twin gestation were analysed: Post-BS (n=22) Matched control parturient (n=44)	Maternal and perinatal outcomes studied during 2006 through 2017	Compared with control group, post BS twin pregnancy: ↓ GDM (p=0.02) ↓ Gestational HT (p=0.01); ↓ Haemoglobin (p<0.01) ↔ BW ↔ Proportion of SGA infants ↑ Degree of BW discordance (p<0.01)

Blume CA et al. Obes Surg. 2018 (425)	Retrospective case control study	96 women n=32 each group: Post RYGB Controls, no BS: BMI < 35kg/m^2 BMI ≥ 35kg/m^2	Singleton births of women who underwent RYGB between 2000 and 2010 were matched to two control births by maternal age, delivery year, and gender	Post RYGB vs obese controls: ↓ GDM ↓ Hypertensive disorders ↓ BW (p=0.02) ↓ Offspring obesity
Feichtinger M et al. Ultraschall Med. 2020	Longitudinal cohort study	43 singleton pregnancies after RYGB compared to 43 BMI-matched controls	Intrauterine fetal growth development and birth anthropometry of foetuses assessed by ultrasound throughout pregnancy.	After maternal RYGB: ↓ Growth percentiles from 2^{nd} to 3^{rd} trimester (95 %CI 0.9-5.3, p= 0.007)/ four gestational weeks
Cruz S et al. Obes Surg. 2018	Longitudinal, and retrospective study	42 pregnant women who previously underwent RYGB	Concentrations of Vitamin D3, calcium, and PTH were assessed in all trimesters. Maternal anthropometric variables were collected preoperatively and over the trimesters of pregnancy	A total of 97.1% had Vitamin D3 inadequacy at some point in pregnancy

Carolis SD et al. Obes Surg. 2018	Cohort retrospective single-centre study	65 women before and after BS	The data were collected during the period January 1996 - October 2017. on singleton pregnancies. Data on previous pregnancies, before they underwent to BS, were collected	Post BS: ↓ Diabetes ↓ Hypertensive disorders ↓ Macrosomia & LGA ↑ Preterm births (14.5 vs 4.0%) ↑ LBW infants (28.9 vs 0%) ↓ BW lower after than before BS ($p<0.01$)
Basbug A et al. J Matern Fetal Neonatal Med. 2019	Retrospective observational study	23 pregnant women who underwent LSG at a tertiary hospital in Turkey	Maternal and perinatal outcomes were evaluated, including GDM, pregnancy-associated hypertensive disorders, preterm birth, mode of delivery, SGA, LGA and congenital malformations	LSG may reduce obesity-related gestational complications, such as GDM and LGA
Hammeken LH et al. Eur J Obstet Gynecol Reprod Biol. 2017	Retrospective matched cohort study.	151 pregnant women who underwent prior RYGB. Matched 1:1 with pregnant women non-RYGB	Followed in outpatient obstetric clinic and gave birth between 1 January 2010- 31 December 2013	↑ Risk of SGA birth and maternal anaemia for the RYGB vs the non-RYGB group

Gascoin G et al. Surg Obes Relat Dis. 2017	Case control study	56 newborns of mothers with prior RYGB and 56 newborns of nonobese healthy mothers (controls)	Women were followed between 3 January 2008-31 October 2012	RYGB vs Control group ↑ SGA ($p<0.01$) ↓ Cord blood levels of Iron, Zinc, Vitamin A, Calcium ↑ Cord blood levels of magnesium, Vitamin E, Vitamin D, Vitamin B12
Parent B et al. JAMA Surg. 2017	population-based retrospective cohort study	Post BS mothers and their infants (n = 1859) Controls (no surgery) women and their infants matched by delivery year (n = 8437)	From 1 January 1980- 30 May 2013 in Washington State, data were collected from birth certificates and maternally linked hospital discharge data	Post BS vs Controls: ↑ Prematurity ↑ NICU admission ↑ SGA and low Apgar score
Chagas C et al. J Womens Health (Larchmt). 2017	Analytical, prospective, and longitudinal study	30 pregnant women with prior RYGB	Women were followed for 2 years from surgery until delivery	Most common pregnancy complications post RYGB: Anaemia (73.3%), Urinary tract infection (33.4%), Dumping syndrome (33.4%).
de Alencar Costa LA et al. J Perinat Med. 2016	Retrospective cross-sectional study	63 women who had undergone RYGB and 73 obese women (control).	Demographic data, the characteristics of the BS, and the maternal and perinatal results were evaluated.	Previous RYGB vs Controls: ↑ Anaemia ↓ Macrosomia ↓ Prematurity

Abenhaim HA et al. J Matern Fetal Neonatal Med. 2016	retrospective cohort study	8 475 831 births during the study period (221 580 (2.6%) in morbidly obese women and 9587 (0.1%) in women with BS	Using the healthcare cost and utilization project - Nationwide Inpatient Sample (2003-2011) comparing outcome of births among women who had undergone BS with births among women with morbid obesity	BS vs Morbidly Obese women: ↓ Hypertensive disorders, ↓ PROM, ↓ Chorioamnionitis, ↓ CS ↓ Instrumental delivery, ↓ PPH & postpartum infection ↑ IOL, postpartum blood transfusions, VTE, IUGR ↔ Preterm births, fetal deaths, or congenital anomalies
Machado SN et al. Obes Surg. 2016	cross-sectional study	G1: 80 pregnant women without previous RYGB G2: 40 pregnant women with previous RYGB	We used high-performance liquid chromatography with UV detector for quantification of retinol and β-carotene, and the functional evaluation of vitamin A deficiency was performed through standardized interview validated for pregnant women	↑ Serum retinol and β-carotene means in G1 compared to G2 ($p < 0.001$)

Johansson K et al. N Engl J Med. 2015	Retrospective longitudinal case control study	627,693 singleton pregnancies Previous BS (n=670) matched with 5 controls	Swedish Medical Birth Register analysed from 2006 through 2011 for maternal and perinatal outcomes	Pregnancies post BS vs matched controls: ↓ GDM (p<0.001) ↓ LGA infants (p<0.001). ↑ SGA infants (p<0.001) and ↑ Stillbirth or neonatal death (p=0.06) ↔ Congenital malformations
Adams TD et al. Int J Obes (Lond). 2015	Retrospective, matched-control cohort study	G1:295 women with pregnancies before and after RYGB G2:764 women with pregnancies after RYGB Matched no surgery controls	Perinatal outcomes were derived using State-wide birth certificate data	Previous RYGB vs no surgery: ↓ LGA neonate ↑ SGA neonate ↓ PIH and GDM
Berlac JF et al. Acta Obstet Gynecol Scand. 2014	Retrospective, matched-control cohort study	415 women giving birth after RYGB matched with women with similar and normal BMI and no surgery	All women undergoing RYGB (1996-2011) and subsequently giving birth	Gastric bypass vs: normal BMI: ↑ HT in pregnancy ↑ GDM ↑ Acute abdominal pain

				Similar BMI: ↓ Preeclampsia ↓ Emergency CS ↓ Neonatal asphyxia ↓ BW ↑ NICU admissions
Nørgaard LN et al. PLoS One. 2014	Retrospective Case control cohort study	387 Danish women, who had laparoscopic or open RYGB surgery prior to a singleton pregnancy	January 2008-June 2011. Data from Danish National Registry of Patients and Danish National Birth Registry, Pregnancy Complications and Abortion-clinical quality database and the Danish Fetal Medicine Database	Post RYGB vs background population: ↓ Fetal growth index No correlation was found between the surgery-to-conception interval
Mead NC et al. Surg Obes Relat Dis. Nov-Dec 2014	Retrospective cohort study	113 women who gave birth to 150 children after biliopancreatic diversion, RYGB and sleeve gastrectomy	Pregnancy outcomes analysed (June 1994-December 2011)	Post BS ↑ Maternal anaemia ↓ B12, albumin ↓ Average BW (but more than 2500g)

Roos N et al. BMJ. 2013	Population based matched cohort study	For each birth to a mother with a history of BS (n=2562),	1,742,702 singleton births identified in the Swedish medical birth register (1992- 2009)	Post BS vs matched controls: ↑ Preterm birth – iatrogenic and spontaneous ↑ SGA ($p<0.001$) ↓ LGA ($p<0.001$) ↔ Stillbirth or neonatal death
Amsalem D et al. Surg Obes Relat Dis. May-Jun 2014	Retrospective study	109 women, and therefore, 327 paired pregnancies: 109 pregnancies preceded and 218 followed restrictive BS	A retrospective study comparing consecutive pregnancy outcomes of the same women, who conceived before and twice after a restrictive BS, was conducted	Post BS: ↓ Hypertensive disorders ↓ GDM ↓Macrosomia ($p =0.02$)
Shai D et al. J Matern Fetal Neonatal Med. 2014	A retrospective population-based study	326 women who had one pregnancy before and after a BS and 1612 obese women who had at least two consecutive deliveries.	Pregnancy outcome of patients compared following bariatric with the obese population was conducted	Post BS vs Obese controls ↓ GDM ↓ Fetal macrosomia ↑ Maternal anaemia

Kjær MM et al. Am J Obstet Gynecol. 2013	Matched cohort study	339 women with a singleton delivery after BS (84.4% RYGB). They were matched to 1277 unexposed women	Nationwide register-based matched cohort study of singleton deliveries after BS during 2004-2010	Infants in post BS group: ↓ Mean gestational age ↓ Mean BW ($p<0.001$) ↓ risk LGA ↑ SGA
Ducarme G et al. J Matern Fetal Neonatal Med. 2013	Retrospective multi-centric cohort study	94 neonates in 79 women were included	Pregnancy and neonatal outcome of patients was compared	Significantly lower mean BW (2993 g vs. 3253 g; $p = 0.02$) was observed after RYGB and the mean Z-score for BW was significantly closer to 0 in neonates of the LAGB group than in those of the RYGB group.
Belogolovkin V et al. Arch Gynecol Obstet. 2012	Population-based, retrospective cohort analysis	Women with Previous BS (n=293) No surgery (n = 656,353)	Vital records and hospital discharge data in Florida was analysed during 2004-2007	Non-obese mothers with prior BS: ↑ Anaemia, chronic HT, endocrine disorders & SGA infants Obese mothers without BS:

				↑ GDM, chronic HT, macrosomic infants & prolonged hospital stay compared to non-obese mother without BS
Bebber FE et al. Obes Surg. 2011	Retrospective cohort study	33 women who had undergone previous restrictive malabsorptive BS	Medical records of first pregnancies after BS, with EDD until June 2008 were analysed	↑ CS (69%) ↓ Vitamin B12 (53%)
Stone RA et al. J Womens Health (Larchmt). 2011	Retrospective cohort study	102 women identified, 52 (51%) were obese and 50 (49%) were not obese at conception	From a database of women who received outpatient perinatal services, we identified women with a history of BS who are currently pregnant with a singleton gestation	Maternal obesity (≥30 kg/m^2) post BS was associated with: ↑ CS (p=0.01) ↑ Pregnancy-related HT (p=0.001) vs nonobese women (<30kg/m^2)
Dell'Agnolo CM et al. Obes Surg. 2011	Retrospective, exploratory cohort study	32 women who had a pregnancy following BS	Analysis of medical records (1999 through 2008)	Pregnancy post BS: ↑ Neuropsychiatric disorders, ↑ Post-surgery anaemia ↑ CS ↓ Pregnancy-related HT

Santulli P et al. Obes Surg. 2010	Retrospective cohort study	24 pregnancies, following RYGBP BMI-matched control group (n=120) Normal BMI control group (n=120)	Hospital data were reviewed from all groups in the same 6-year period	RYGBP versus normal BMI and BMI-matched controls: ↔ Perinatal complications ↓ BW ($p<0.001$) RYGBP vs normal BMI: ↑ Pre-labour CS ($p=0.04$)

Supplementary Table 2: Literature review of the effect of bariatric surgery on peptide hormones: Insulin, C-peptide, Glucagon and Ghrelin.

Author, Journal	Study Design	Subjects (N)	Method	Results
Navarro García MI et al. Endocrinol Diabetes Nutr 2020	Prospective, observational, analytic cohort	54 patients GBP (n= 27) VSG (n=27)	At 12-month follow-up period demographic and anthropometric data, comorbidities, weight loss and fasting ghrelin levels were recorded	↑ Ghrelin at 12 months post-op (both procedures)
Bunt JC et al. Int. Journal of Obesity. 2017	Prospective non-randomized	18 Obese participants RYGB (n=10F) LAGB (n= 7F/1M)	Peptide hormones, incretins and pancreatic polypeptide responses to MMT were measured at 4-8 weeks pre- and post-op	↓ C-peptide & insulin MMT profiles post LAGB. ↓ Glucose & insulin, not c-peptide MMT profiles post RYGB
Smeu B et al. Chirurgia(Bucur) 2015	Prospective Study	60 consecutive obese patients with or without T2DM admitted for LSG	Measured BMI, waist circumference & glycaemic parameters at study entry, 10 days and 6 months post-op	Glycemic control improved from D10 post-op. At 6 months post-op: ↓Glycemic levels ($p<0.001$), ↓ HOMA ↓ Insulin ($p<0.001$), ↓ C-peptide ($p<0.001$)

Zhu Y et al. Bioscience Trends. 2017	Retrospective Case control	67 T2DM participants LSG (n=35) RYGB (n=32)	The laboratory and anthropometric data was analyzed pre-surgery and during a 2-year follow-up	↓ HOMA-IR at 3 months post LSG & RYGB ↓ Ghrelin at 1, 3 and 6 months post LSG ↑ Ghrelin post LGB, not statistically significant
Souteiro P et al. Obesity Surgery. 2017	Retrospective cross-sectional	363 Obese, diabetic participants. LAGB (n=95) RYGB (n=203) SG (n=65)	Clinical, anthropometric and analytic measures that included: Fasting glucose, insulin and C-peptide were obtained at pre-op and follow-up visits	Postoperative diabetes remission was achieved in 39.9 % of patients at 1 year post-op
Farey JE, et al. Obesity Surgery. 2017	Prospective Cohort	11 Obese patients underwent BS Matched with 22 non-obese controls	Obese participants' fasting blood samples taken 3 months post-op	3 months post LSG: ↓ Fasting Ghrelin, glucagon
Mazidi M et al. Surgery. 2017	Prospective Cohort Study	152 participants 81% with T2DM	Measured post op insulin secretion and sensitivity, glucose homeostasis, and improvement in diabetic control	Indices of insulin secretion, including serum C-peptide improved at the 3-year follow-up, with a significant improvement in insulin sensitivity and glucose homeostasis

Haruta H et al Obesity Surgery. 2017	Retrospective Cohort	831 respondents LSG (n=501) LSG-DJB (n=149) RYGB (n=100) LAGB (n=81)	Pre-op weight & comorbidities and 1, 3 and 5 years post-op. Diabetes improvement stratified by baseline ABCD score: Age, BMI, C-peptide level and duration of diabetes	3 years postoperative remission rates: Diabetes 78%; Higher ABCD score following complete diabetes remission (6.4±1.6 vs 4.2±2.0, $p<0.05$)
Celik A et al. Obes Surg. 2017	Comparative Observational Cohort	251 Obese patients T2DM for ≥3 years: SG (n=49), MGB (n=93) DSIT (n=109)	Measurements included: fasting and 1-hour postprandial plasma, C-peptide and total insulin levels	Glycaemic control was achieved following DSIT and MGB but not SG. BMI and postprandial C-peptide levels were independent predictors of early glycaemic control following DSIT
Lee WJ et al. World J Surg. 2017	Prospective Cohort	579 obese T2DM patients SG (n=109) GB (n=470)	Remission of T2DM after 1 year post SG or GB was evaluated using ABCD scoring system: Age, BMI, C-peptide level and duration of diabetes	↓ Fasting C-peptide 1 year post GB
Santiago-Fernández C et al. Endocrinol Diabetes Nutr. 2017	Case control cohort	103 morbidly obese subjects underwent RYGB, BPD and SG.	Ghrelin levels were measured before and 6 months post- BS	↑ Ghrelin post RYGB ($p<0.05$) ↔ Ghrelin after BPD ↓ Ghrelin after SG ($p<0.05$)

		And 21 non-obese subjects		
Kalinowski P et al. Surgery for Obesity and related diseases. 2017	Randomised controlled trial	72 morbidly obese patients SG (n=36) RYGB (n=36)	Fasting ghrelin, glucose, insulin, C-peptide, glucagon and HOMA-IR were assessed pre-op then 1, 6 and 12 months post–op	↓ Fasting Ghrelin 1-12 months post SG, increased by 12 months post RYGB ↓ Glucose, insulin, and C-peptide. ↓HOMA-IR in both groups during 12 months
Yadav R et al. Frontiers in Immunology. 2017	Cohort Study	37 obese patients with (n=17) and without (n=20) T2DM undergoing RYGB	Pre op, 6 and 12 months post RYGB - Lipoproteins, insulin resistance, inflammatory markers were measured	6 months post-op: ↓ HOMA-IR
Casella G et al. British Journal of Surgery. 2016	Cohort	10 morbidly obese participants underwent SG	Insulin sensitivity and secretion was measured pre-op, 3, 6 and 12 months post-op	At 12 months post op: ↑ Median Insulin sensitivity ↓ Fasting Insulin sensitivity ↓ Total Insulin secretion
Hansen M et al. Acta Diabetologica. 2016	Case Control	43 participants RYGB (n=16, T2DM) RYGB, no T2DM (n=27)	Hepatic insulin sensitivity and clearance determined at: baseline, post diet-induced weight loss, 4 and 18 months post-op	Hepatic insulin sensitivity improved after RYGB
Wroblewski E et al Cytokine 2016	Prospective Observational	67 Obese patients BIB (n=25) LABG (n=10)	Circulating hormones levels were analyzed pre- and post-	At 50-54weeks: ↓Ghrelin levels

		LSG (n=32)	endoscopic and surgical procedures	
Zachariah PJ et al. Obes Surg. 2016	Retrospective Cohort	46 T2DM obese DJB-SG (n=21) SG (n=25)	Blood glucose, C-peptide, and insulin levels were estimated following MMTT pre-op and at 1 year	Patients with DJB-SG compared to SG group during MMTT: ↓ Post prandial blood glucose ↓ C-peptide levels
Purnell JQ et al. Diabetes Care. 2016	Observational Cohort	606 Obese, diabetic participants RYGBP (n=466) LAGB (n=140)	Metabolic measurements assessed pre- and post-op annually for 3 years	↑ Insulin sensitivity post LAGB and RYGB ↓ Insulin secretion post RYGBP
Papamargaritis D et al. Surgery for Obesity and Related Diseases. 2016	Prospective Cohort	13 Female participants All had SG Pre-op OGTT: 9 high risk and 8 low risk of DM	OGTT was repeated 6 weeks and 6 months post-op	↓ Insulin levels, early insulin secretion, and insulin resistance indices at 6 weeks post op in the high risk DM group and at 6 months post-op in both groups
Kruljac I et al. Clinical Endocrinology 2016	Non-Randomised Cohort	51 participants LAGB (n= 21) LSG (n=15) RYGB (n=15)	Measurements included Ghrelin, insulin & HOMA-IR at baseline and 1, 3, 6 and 12 months	↑ Ghrelin post LAGB (p=0.016) ↓ Insulin and HOMA-IR significantly in LSG and RYGB group
Vrbikova J et al. Obesity Facts. 2016	Prospective cohort	52 Obese, T2DM women BPD (n=16) LAGB (n=16)	Euglycemic clamps and MMT done pre-op then 1 month and 6 months post-op	↓ Basal insulin secretion post- op (all 3 BS)

		Laparoscopic Gastric Plication, (n=20)		↓Total insulin secretion only following the BPD
Ivan Kruljac et al. Clin Endocrinol (Oxf). 2016	Non-randomized study	51 patients LAGB (n=21) LSG (n=15) RYGB (n=15)	Serum ghrelin, insulin, growth hormone, HOMA-IR and HOMA-β was recorded at baseline and 1, 3, 6 and 12 months	↑Ghrelin only in the LAGB group (P = 0·016). ↓Insulin and HOMA-IR after LSG and RYGB ↑ HOMA-β increased after LAGB and LSG (p<0.001 for all changes)
Federico A et al. In Vivo. 2016	Case Control Cohort	47 participants: Bilio-intestinal bypass (n=19) 28 healthy, normal weight controls	Analysis included: Plasma levels of peptide YY, GLP-1/2, ghrelin, orexin and cholecystokinin and anthropometric data	↑ Ghrelin post-op with respect to controls
Salehi M et al. Obesity. 2015	Case Control	15 participants with previous GBP 6 matched obese non-surgical controls and 7 lean individuals	Islet hormones were measured before and after meal ingestion during hyperinsulinemic hypoglycaemic clamps	In GBP subjects: ↓ Fasting β-cell secretion during the insulin clamp ↑ Meal-induced insulin secretion during fixed sub-basal glycaemia ↓ Glucagon responses to hypoglycaemia and meal ingestion
Wentworth JM et al. Obesity Surgery. 2015	Randomised Prospective	44 Overweight, T2DM MDC group (n = 22) LAGB group (n = 22)	OGTT glucose levels were measured	↑ Fasting C-peptide/insulin ratio in LABG group at 2 years

Campos GM et al. Surgery for Obesity and related diseases. 2014	Prospective Case Control	22 Morbidly Obese non-diabetic participants RYGB (n=12) Diet (n=10)	At 14 days and 6 months post RYGB glucose & pancreatic hormones during an MTT and steady-state insulin concentrations during euglycemic-hyperinsulinemic clamp were measured	14 days post RYGB: Enhanced postprandial glucose, C-peptide, & glucagon responses ↓ Insulin concentration 6 months post RYGB: ↓ Insulin concentrations persisted
Terra X et al. Obes Surg. 2013	Cohort	30 morbidly obese women SG (n = 17) RYGB (n = 13)	Analysed levels of chemerin, ghrelin and leptin at baseline, and after 6 and 12 months post-op	12 months post op: ↑ Ghrelin (p=0.01)
P Cigdem A et al. Minerva Med. 2013	Case control study	20 obese patients who underwent LGB and control group (n=20) healthy, normal-weight	Plasma ghrelin, leptin, orexin-A & glucose was measured before and 1 month post-op and once from the control group	Post op: ↑ Ghrelin (p=0.01) ↓ Insulin resistance
Bužga M et al. Videosurgery and other miniinvasive technique. 2013	Cohort	35 participants who underwent LSG	Parameters of glucose metabolism were measured pre- and 3 and 6 months post-op	At 6 months post LSG: ↓ C-peptide (p<0.02)
Samat A et al. Diabetes, Obesity and Metabolism. 2013	Cohort	9 obese T2DM subjects underwent a mixed meal tolerance test before and at 1 and 12 months post RYGB surgery	Changes in ghrelin, glucose tolerance and insulin sensitivity were measured	MMT 1 and 12 months post RYGB: ↓ postprandial Ghrelin concentrations (p<0.05)

Ramón JM et al. J Gastrointest Surg. 2012	Randomised controlled	7 patients were randomised to LRYGB and 8 to LSG	Pre op and at 3 and 12 months post-op: before, 10 and 60 mins after a standard test meal ingested, blood samples were taken	LSG group: ↓ Fasting ghrelin levels. LRYGB group: ↑ Post prandial GLP-1 LSG group: ↓ fasting ghrelin levels
Jørgensen NB et al. Am J Physiol Endocrinol Metab. 2012	Case control	13 obese subjects with T2DM and 12 matched subjects with normal glucose tolerance underwent RYGB	Examined during a liquid meal before (Pre), 1 week, 3 months, and 1 year post-RYGB	1st week post RYGB ↓ Fasting glucose and insulin in both groups ↑ post prandial glucagon secretion
Jacobsen SH et al. Obes Surg. 2012	Cohort	8 obese non-diabetic patients underwent RYGB	Pre and within 2 weeks post op, OGTT and a liquid mixed meal test (200 mL 300 kcal) were performed on separate days	Post-op findings: ↓↓ Fasting glucose, insulin, ghrelin ↑ Insulin sensitivity Post prandial: ↑ Glucagon;↓ total and active ghrelin
Peterli R et al. Obes Surg. 2012	Prospective, randomized	12 non diabetic obese patients were randomized to LRYGB and 11 to LSG	Pre-op and 1 week, 3 and 12 months post-op: standard test meal was given after an overnight fast. Blood samples collected before, during and after food intake	Post-surgery: Improvement in glucose homeostasis At 12 months, LRYGB ghrelin levels approached preoperative values LSG ghrelin levels were still markedly attenuated

Reed MA et al. Journal of Clinical Endocrinology and Metabolism. 2011	Cross-sectional, non-randomized, controlled	27 participants: Lean controls (n=9) Obese T2DM (n=9) Obese no DM (n=9)	Surgery patients were studied before then 1 week and 3 months post- RYGB	1 week post RYGB: ↓ Insulin secretion ↓ Fasting insulin no different from lean control despite continued elevated glucose in the T2DM patients compared with lean
Jankiewicz-Wika J et al. Endokrynol Pol. 2011	Cohort	28 obese patients with metabolic syndrome	Before and 3, 6, 12, and 24 months post- BS	↔ Fasting glucose, leptin, total cholesterol and LDL-C pre- and post-op ↑ HDL, adiponectin, resistin, and ghrelin post-op
Promintzer-Schifferl M et al. Obesity (Silver Spring). 2011	Case Control	18 participants: Nondiabetic obese group underwent RYGB (n=6) Lean, no surgery (n=6) Obese, no surgery (n=6)	Surgery group before and 7 months post op: Time-courses of glucose, insulin, C-peptide, measured after oral glucose load	Post RYGB: ↓ Fasting plasma insulin and C-peptide ↔ Fasting glucose levels ↑ C-peptide and insulin concentration following glucose ingestion
Lima MMO et al. J Clin Endocrinol Metab. 2010	Cohort	19 obese women with metabolic syndrome underwent RYGB: T2DM (n=6) IGT (n=7) Normal GT (n=6)	Euglycemic-hyperinsulinemic clamp, HOMA-IR assessed at baseline and 4.5 weeks post-op	↓ Fasting glucose decrease $p<0.01$ ↓ Fasting insulin $p<0.01$

Supplementary Table 3: Literature review of the effect of bariatric surgery on Adipokines, Leptin, Visfatin and Resistin.

Author, Journal	Study Design	Subjects (N)	Method	Results
Głuszek S et al. Int J Environ Res Public Health. 2020	Cohort Study	163 morbidly obese patients SG (n=120) GB (n=35) RYGB (n=8)	Metabolic parameters were measured pre and post operatively	12 months post op: ↓ Leptin (p=0.01)
Min T et al. Obes Surg. 2020	Prospective cohort study	19 participants (17 T2DM) undergoing BS: SG (n=10) BPD (n= 6) RYGB (n=2) LAGB (n=1)	Adipokines, inflammatory cytokines and global plasma measures of oxidative stress were analysed 1, 6 months, and 4 years post-op	4 Years post-op: ↓ Leptin (p=0.001)
Garruti G et al. Ann Med Surg (Lond). 2020	Prospective study	27 non-diabetic obese subjects underwent LGB and 10 healthy controls	Before (T0), 3 months (T3), 6 months (T6), and 12 months (T12) after LGB & hypocaloric diet /physical activity: serum Adiponectin and Resistin levels were evaluated	Diet & Compliant exercise group: ↓ Resistin at T12 ↑ Adiponectin at T6 and T12 Diet & Poor compliance exercise group: ↓ Adiponectin at T6 & T12 ↔ Resistin levels
Farias G et al. Obes Surg. 2020	Cohort Study	32 adults with obesity underwent GBP	The anthropometric and biochemical markers were	Post-op: ↓ Leptin ↓ Leptin/adiponectin ratio

			collected pre-op then 6 and 24 months post-op	↓ Resistin levels (p<0.01)
Salman MA et al. Obes Surg. 2020	Prospective Cohort Study	100 morbidly obese. RYGB (n=50) SG (n=50)	12 months post op, serum levels of adipocytokines (leptin and active chemerin) and gastrointestinal hormones	Post LSG and RYGB: ↓ Leptin
O'Rourke RW et al.Int J Obes (Lond). 2019	Multi-centre, observational cohort	2,458 subjects: RYGB (n=1770) LAGB (n=610), SG (n=59) BDS (n=19)	At baseline, 12-, 24-, and 26-months post op serum biomarkers were assessed	Leptin and ghrelin levels were inversely associated with DM prevalence
Unamuno X et al. Nutrients. 2019	Cohort Study	25 obese participants with T2DM undergoing RYGB	Anthropometric and biochemical variables were evaluated before and after RYGB	Post RYGB: ↑ Adiponectin / Leptin ratio (p<0.001)
Stephens JW et al. Surgery for obesity and related diseases. 2019	Prospective Cohort Study	55 participants with impaired glucose homeostasis and T2DM undergoing LSG	Inflammatory cytokines and plasma markers of oxidative stress were measured pre-operatively, 1 and 6 months postoperatively	6 months post LSG: ↑ Adiponectin ↓ Leptin
Wolf RM et al. Journal of Clinical endocrinology and metabolism. 2019	Cross sectional study	37 obese patients 37 lean patients. 25 obese patients post BS	Cytokine levels were evaluated before and after RYGB and VSG	After bariatric surgery ↑ Adiponectin ↓ Leptin

Freitas WR Jr et al. Obes Surg. 2018	Randomised controlled trial	55 severe obese patients underwent RYGB. Control group (n=19)	Fasting levels of tumor necrosis factor alpha (TNF-α), adiponectin and leptin were analysed	6 months Post op ↓ Leptin ↑ Adiponectin
Cigdem A P et al. J Invest Surg. 2018	Case control study	19 morbidly obese patients under went LAGB and 22 healthy control group	Plasma resistin, and visfatin assessed at pre op, 1 and 6 months post-op	Plasma resistin and visfatin were higher in morbidly obese patients compared with the control group. They all decreased post-op
Caparrós EP et al. Nutr Hosp. 2017	Case control study	68 morbidly obese patients underwent GBP and 31 lean subjects were controls	Adiponectin and resistin were assessed pre-op and 1 month post-op	↔ Resistin levels between morbidly obese patients and controls or between obese patients before and after surgery & weight loss
Yadav R et al. Frontiers in Immunology. 2017	Cohort Study	37 obese patients with (n = 17) and without (n = 20) T2DM undergoing RYGB	Pre op, 6 and 12 months post-op RYGB - Lipoproteins, insulin resistance & inflammatory markers were measured	6 months post-op: ↑ Adiponectin
Biagioni MFG et al. Obesity surgery. 2017	Cohort Study	30 obese women undergoing RYGB	Baseline and at 3, 12, 24 months post op adipocyte proteins were measured	3 months post-op: ↓ Leptin ↑ Adiponectin
Kalinowski P et al. Surg Obes Relat Dis. 2017	Randomised controlled trial	72 morbidly obese patients were randomly selected to undergo either SG (n = 36) or RYGB (n = 36)	Fasting ghrelin, leptin, glucose, insulin, C-peptide, glucagon, glycated haemoglobin, and HOMA-IR	↓ Leptin in both groups during 12 months

			were assessed pre-op and at 1, 6, and 12 months post-op	
Hagman DK et al. Metabolism. 2017	Cohort Study	14 obese participants undergoing BS	Fasting blood and subcutaneous abdominal adipose tissue were obtained before (n=14), at 1 month (n=9) and 6-12months (n=14) after BS	At 12months post-op improved systemic inflammation: ↑ Adiponectin (p=0.003).
Sams VG et al. Surgical Endoscopy. 2016	Cohort	25 obese subjects: LRYGB (n=20) LAGB(n=5)	Samples of serum and adipose tissue were collected at the time of surgery, 2 weeks and 6 months post-op	Post-surgery: ↑ Serum & tissue adiponectin
Bitencourt M et al. Internation Journal of Clinical chemistry. 2016	Case control	60 participants: Clinical treatment n = 20 obese RYGB n= 20 obese n = 20 obese,T2DM	Biochemical, inflammatory parameters & biomarkers of oxidative stress measured at 1, 3, 6, and 12 months after surgery and clinical treatment	12 months post RYGB: ↑ Adiponectin
Lips et al. Metabolism: Clinical and Experimental. 2016	Case control	39 female subjects RYGB (n=15) VLCD (n=12). Age matched, lean women, controls (n=12)	Systemic inflammation was assessed one month before and 3 months after intervention	At 3 months after intervention: ↓ CRP and Leptin levels ↑ Adiponectin levels were increased both by RYGB and VLCD

Kruljac I et al Clin Endocrinol (Oxf). 2016	Non randomised study	51 patients, LAGB (n=21) LSG (n=15) RYGB (n=15)	Serum ghrelin, leptin, insulin, growth hormone, HOMA-IR and HOMA-β was recorded at baseline and 1, 3, 6 and 12 months	↓Serum leptin all groups
Tam CS et al. J Clin Endocrinol Metab. 2016	Parallel-arm, prospective observational study	30 Obese adults RYGB (n=5), SG (n=9), LAGB (n=7) LCD (n=9)	At baseline, 8 weeks and 1 year blood samples taken to compare inflammatory markers	1 year after RYGB or SG: ↑ Adiponectin, HDL ↓ TGs and CRP
Wroblewski E et al. Cytokine 2016	Case control	67 obese subjects BIB (n=25) LABG (n = 10) LSG (n = 32) 72 non-obese controls	Circulating hormone levels were analysed before and after endoscopic and surgical procedures	Post intervention: ↓ Leptin ↑ Adiponectin levels to the levels observed in non-obese
Lindegaard KK et al. Diabetol Metab Syndr. 2015	Case Control	13 obese T2DM subjects and 12 obese, non-diabetic controls underwent RYGB	Subjects were examined before, one week, three months and one year post-op	One year after surgery: ↓ Leptin ↑ Adiponectin
Netto BD et al. Obes Surg. 2015	Cohort Study	41 extremely obese who underwent RYGB	Anthropometric and clinical data and biochemical markers of inflammation	Pro-inflammatory biomarkers decreased: Leptin ($p<0.01$)

			were collected prior to surgery and 6 months post-RYGB	Resistin ($p<0.01$)
Major P et al Wideochir Inne Tech Maloinwazyjne. 2015	Prospective Cohort Study	35 patients LSG (45.8%) LRYGB (54.2%)	Serum GLP-1, PYY, leptin, and ghrelin was measured at baseline and 12 months post-op	12 months post-op ↓ Ghrelin ↓ Leptin level ↑ GLP-1
Iaffaldano L et al. Obes Surg. 2014	Case Control	20 obese individuals and 10 matched controls	Serum inflammatory marker levels were evaluated before (T0) and after LAGB (T1)	Post LAGB: ↓CRP, triglycerides, leptin, leptin/adiponectin ratio homeostasis model assessment ($p<0.05$)
Mallipedhi A et al. Surg Obes Relat Dis 2014	Non-randomised prospective study	22 participants with impaired glucose homeostasis and T2DM undergoing SG	Serum inflammatory markers, leptin and adiponectin were recorded pre-op, 1 and 6 months post-op	At 1 – 6 months post SG: ↓ Leptin ($p<0.01$)
Gumbau V et al. Obes Surg. 2014	Cohort	20 obese patients to the study (40% T2DM). All underwent SG	Clinical, anthropometric, and inflammation parameters were analysed at pre-op visit, 1st and 5th days, 1st and 6th months and 1 year post-op	1-year post-intervention: the average levels of ↑ Adiponectin (NS) ↓ Leptin (significantly)
Auguet T et al. Obesity (Silver Spring). 2014	Case Control	30 morbidly obese women LSG (n = 17) RYGB (n = 13)	Adipocytokine levels were measured at 3 time points:	Post-surgery:

		60 normal-weight controls	before surgery (baseline) at 6 and 12 months post-op	↓ Visfatin and CRP compared to baseline ↑ HMW adiponectin was higher
Umemura A et al. Endocr J. 2014	Case control	23 LSG patients and 23 non-obese patients undergoing elective abdominal surgery were enrolled	6 months post SG, serum adipokines and adipokines from omentum-derived adipocytes and VAT were assessed	↑ Mean serum leptin levels & PAI-1 levels ($p<0.001$) ↓ Adiponectin levels ($p=0.006$)
Hosseinzadeh-Attar MJ et al. Obes Facts. 2013	Cohort Study	35 severely obese patients LAGB (n=14) LTGVP (n=14) GBP (n=7)	Anthropometric and biochemical parameters including adiponectin and visfatin were analyzed before and 6 weeks after weight reduction	After bariatric surgery, ↓ Serum visfatin, HDL-C, LDL-C, and TG levels ↑ Adiponectin
Sdralis E et al. Obes Surg. 2013	Parallel-arm, prospective observational study	31 obese patients Randomized into two groups: SG alone or with omentectomy	Metabolic profile, adipokine secretion, inflammatory status were measured before surgery and at 7 days, and 1, 3 and 12 months post-op	↑ Adiponectin and HDL cholesterol levels ($p<0.01$) in both groups
Shrestha C et al. Int J Endocrinol 2013	Cohort	33 T2DM patients with BMI 22-30 kg/m^2 underwent LRYGB	Plasma levels of adiponectin, sICAM-1, fasting glucose, glycated hemoglobin, and fasting insulin and serum levels of visfatin were	Postoperative: ↑ Adiponectin level ($p<0.01$) ↓ Visfatin ($p<0.01$) ↓ sICAM-1 ($p<0.01$)

			measured before and at three months after LRYGB	
Terra X et al. Obes Surg. 2013	Cohort	30 morbidly obese women SG (n = 17) RYGB (n = 13)	Analysed levels of ghrelin and leptin at baseline, and after 6 and 12 months post-op	12 months post-op: ↓ Leptin ($p<0.001$)
Cigdem A P et al. Minerva Med. 2013	Case control study	20 obese patients who underwent LGB and control group (n=20)	Plasma ghrelin, leptin & glucose was measured before and 1 month post-op and once from the control group	Post op: ↓ Leptin ($p=0.01$)
Siejka A et al. Cytokine. 2013	Cohort	14 obese participants with metabolic syndrome underwent vertical banded gastroplasty	Levels of glucose, insulin, leptin, soluble leptin receptor, obestatin, ghrelin, omentin-1, and RBP4 before and 3, 6, 12, 24 months after BS	After surgery: ↓ Leptin ↑ Leptin receptor & ghrelin
Terra X et al. Clin Endocrinol (Oxf) 2012	Case and control	133 women: 40 lean controls 93 MO; 31 T2DM; 62 nondiabetic	Adipo/cytokines from all participants then follow up samples at 6 and 12 months after laparoscopic GBS from 30 MO patients	↓ Visfatin levels were reduced significantly over 12 months. Visfatin expression in SAT and VAT was similar, but significantly higher in MO compared to controls and independent of the presence of DM
Ramón JM et al. J Gastrointest Surg. 2012	Randomised prospective study	15 patients randomised to: LRYGB (n=7) LSG (n=8).	Patients were assessed: After 10 and 60 min of a standard test meal ingestion	LRYGB group: ↓ Fasting & postprandial Leptin

			and then at 3 and 12 months post-op	
Illán-Gómez F et al. Obes Surg. 2012	Cohort study	60 morbidly obese women	Adiponectin, C-reactive protein, tumour necrosis factor-alpha and interleukin-6 were measured at 3, 6 and 12 months after RYGB	At 12 months post BS: ↑ Adiponectin ($p<0.001$) and HDL-C ($p<0.01$) ↓ IL-6, hs-CRP, Cholesterol, TG, LDL-C, glucose, insulin and homeostasis model assessment
Woelnerhanssen B et al. Surg Obes Relat Dis 2011	Prospective randomised trial	23 non-diabetic morbidly obese patients randomised to: LRYGB (n=12) LSG (n=11)	Fasting glucose, insulin, lipids, and adipokines were analysed pre-op and 1 week, 3 and 12 months post-p	↓ Leptin by 50% 1 week post-op until 12 months ↑ Adiponectin progressively No difference between LRYGB and LSG groups
Marantos G et al. World J Surg. 2011	Case Control	20 morbidly obese women GBP (n=13) GS (n= 7) 20 lean controls	Anthropometric and metabolic parameters were analysed with changes in leptin, adiponectin, resistin, IL-6 before surgery and 6 and 12 months post-op	12 month post op: ↓ Leptin, resistin, IL-6 ↑ Adiponectin
Jankiewicz-Wika J et al. Endokrynol Pol 2011	Cohort	28 obese patients with metabolic syndrome	Before and 3, 6, 12, and 24 months after BS	↔ Fasting glucose, leptin, total cholesterol and LDL-C before or after surgery. ↑ HDL, adiponectin, resistin and ghrelin post-op

Bose M et al. Obesity (Silver Spring). 2010	Cohort	20 participants GBP (n=11) GB (n=9)	Oral glucose challenge pre – op (T0), after a 12 kg weight loss (T1) and 1 year post-op (T2) – assessed PYY(3-36), ghrelin, GLP-1 and leptin	Post op: ↔ Ghrelin ↓ Leptin (GBP only)
Pardina E et al. Obes Surg. 2010	Case control Cohort	34 morbidly obese patients underwent RYGB 22 matched controls – non obese	Levels of CRP, NO, leptin, adiponectin and IGF-1 were measured before and 1, 6, and 12 months after RYGB	12 months post-op: ↓ CRP and leptin to non-obese values

Supplementary Table 4: Literature review of the effect of bariatric surgery on pro-inflammatory biomarkers:

Author, Journal	Study Design	Subjects (N)	Method	Results
Min T et al. Obes Surg. 2020	Prospective cohort study	19 participants (17 T2DM) undergoing BS: SG (n=10) BPD (n= 6) RYGB (n=2) LAGB (n=1)	Adipokines (adiponectin, leptin), inflammatory cytokines (CRP, IL-6, IL-10) and global plasma measures of oxidative stress 1 and 6 months, and 4 years post-op in subjects with obesity and impaired glucose regulation	4 Years post op: ↓ CRP (p<0.001) ↓ IL-6 (p<0.001)
Salman MA et al.Obes Surg. 2020	Single-arm prospective study	62 patients underwent one anastomosis GBP	The serum levels of selected adipocytokines were monitored pre- and 12 months postoperatively	12 months post-op: ↑ MCP-1 (p=0.01). ↓ hs-CRP and IL-6 (p<0.01) ↔ IL-8 (p=0.12) ↔ TNF-α (p=0.84)
Casimiro I et al. Obes Sci Pract. 2020	Cohort	12 obese women who were previously scheduled to undergo laparoscopic VSG	Evaluate adipocyte size and macrophage activation in women before and 3	Post VSG: ↓ Interleukin (IL)-6 cytokine mRNA expression in SAT

			months after laparoscopic VSG	
Farias G et al. Obes Surg. 2020	Cohort Study	32 adults with obesity underwent gastric bypass.	The anthropometric and biochemical markers were collected pre-op then 6 and 24 months post-op	Post-op: ↓ CRP, PAI-1 levels ↓ IL-6 and ICAM-1 ($p<0.01$)
Stephens JW et al. Surgery for obesity and related diseases 2019	Prospective Cohort Study	55 participants with impaired glucose homeostasis and T2DM undergoing LSG	Inflammatory cytokines and plasma markers of oxidative stress were measured pre-op, 1 and 6 months post-op	6 months post LSG: ↓ IL-6, CRP, Leptin
Stolberg CR et al. Atherosclerosis. 2018	Randomised control trial	60 patients approved for RYGB	Patients were assessed pre-surgery, 6, 12, and 24 months post-op 6 months post-op, they were randomized 1:1 to an intervention (exercise) group or a control group	RYGB markedly improved markers of inflammation: ↓IL-6, CRP ($p<0.001$)
Coimbra S et al. J Investig Med. 2018	Cohort study	20 obese patients underwent LAGB	Before (T0) and 13 months after LAGB intervention (T1) inflammation, iron bioavailability and RBC biomarkers were evaluated	↓TNF-α, IL-6 and CRP

Mossberg KE et al. Surg Obes Relat Dis. 2017	Cohort	12 obese patients with and without T2D (n = 6) who were scheduled for GBP	Plasma PAI-1 antigen was measured by enzyme-linked immunosorbent assay (ELISA) pre-op and at 4 and 42 days after GBP	↓ PAI-1 by 53% (p=0.02) in early phase, non-significant decrease in the late phase
Yadav R et al. Front Immunol. 2017	Cohort	37 obese patients: T2DM (n = 17) No T2DM (n = 20) Underwent RYGB	Lipoproteins, insulin resistance, mediators of systemic and vascular inflammation, were measured before and 6 and 12 months after RYGB	These parameters improve mostly 6 months post-op in obese patients with and without diabetes ↓ HOMA-IR, MCP-I, CRP
Linkov F et al. Gynecol Oncol. 2017	Case control cohort study	107 female BS patients vs 74 age-matched non-obese women	Blood samples were collected pre-op and 6months post-op	Post-op: ↓ CRP, leptin, IL-1Rα, and IL-6 ↑ Adiponectin
Farey JE et al. Obes Surg. 2017	Prospective cohort study	15 patients undergoing LSG	34 plasma protein biomarkers thought to be associated with cancer processes were analyzed at baseline and following successful weight loss at 12 weeks using a multiplex bead-based assay	12 weeks post-op: ↓ IL-6, PAI-1 and other inflammatory markers

Lylloff L et al. Obes Facts. 2017	Case control study	48 subjects who underwent RYGB – Groups: Control – non T2DM T2DM regressed or persisted post-op	Inflammatory markers including IL-6 and diabetes-related markers were measured pre- and post-op	↓IL-6 in the group with diabetes remission and in the control group, but not in the group with persistent diabetes
Hagman DK et al. Metabolism 2017	Cohort Study	14 obese participants undergoing BS	Fasting blood and subcutaneous abdominal adipose tissue were obtained before (n=14), at 1 month (n=9), and 6-12months (n=14) after BS	At 12 months post-op improved systemic inflammation: ↓ CRP (p=0.002) ↑ Adiponectin (p=0.003)
Sams VG et al. Surgical Endoscopy 2016	Cohort	25 obese subjects: LRYGB (n=20) LAGB (n=5)	Samples of serum and adipose tissue were collected at the time of surgery, 2 weeks and 6 months post-op	Post-op: ↓ MCP-1
Bitencourt M et al. Internation Journal of Clinical chemistry 2016	Case control	60 participants: Clinical treatment n = 20 obese RYGB n= 20 obese n = 20 obese,T2DM	Biochemical, inflammatory parameters & biomarkers of oxidative stress measured at 1, 3, 6, and 12 months post surgery and clinical treatment	12 months Post RYGB: ↓ IL-6, interleukin-1, ↓TNF-α and resistin ↑Adiponectin
Cepeda-Lopez AC et al. Am J Clin Nutr. 2016	Cohort	43 obese subjects who underwent LSG	Erythrocyte incorporation of iron isotopic labels, body composition, iron status,	After 6 months post-op: ↓IL-6 (p<0.005)

			hepcidin, and inflammation was compared at 2 and 8 months post-op	
Lips et al. Metabolism: Clinical and Experimental 2016	Case control	39 female subjects RYGB (n=15) VLCD (n=12). Age matched, lean women, controls (n=12)	Systemic inflammation was assessed 1 month before and 3 months after intervention	At 3 months after intervention: ↓ CRP
Shih KC et al. Clin Chim Acta. 2016	Cohort study	93 obese patients underwent BS: Non-diabetic (n=69) Diabetic (n=24)	Anthropometry, insulin resistance, inflammatory markers and serum TRACP 5a were measured at baseline and 3, 6 and 12 months post- op	3-6 months post-op in DM grp ↓ CRP ↓ IL-6 In non-DM group: ↔ CRP and IL-6
Barazzoni R et al. Surg Obes Relat Dis. 2016	Case control study	24 morbidly obese individuals (BMI >40) underwent RYGB Control groups: 56 age- and sex-matched normal-weight and 44 obese individuals (BMI 31)	Before and 3, 6, and 12 months after LRYGB plasma PTX3, CRP, and cytokines, including TNF-a and IL-6 were measured	Plasma CRP and proinflammatory cytokines declined during LRYGB-induced weight loss
Gómez FI et al. Nutr Hosp. 2016	Cohort	79 morbidly obese patients who underwent GBP	measured the levels of sICAM1, PAI-1, high-sensitivity CRP and IL-6 at	12 months post op: ↓ PAI-1 ($p<0.05$), ↓ hs-CRP ($p<0.001$)

			baseline and 3, 6 and 12 months post-GBP	↓ IL-6 ($p<0.001$) ↓ HOMA ($p<0.001$)
Lindegaard KK et al. Diabetol Metab Syndr. 2015	Case Control	13 obese T2DM subjects & 12 obese, non-diabetic controls underwent RYGB	Subjects were examined before, 1 week, 3 months and 1 year post-op	One year after surgery: ↓IL-6, TGF-β and leptin
Montecucco F et al. Thromb Haemost. 2015	Case Control	Morbid obese subjects (n=11) underwent GBP Controls of normal and overweight (n=20)	Insulin resistance, circulating and SAT levels of endocannabinoids, adipocytokines and CC chemokines were assessed pre- and post-GBP and compared to the control group	GBP induced: ↓ C-reactive protein, leptin, and CCL2 levels. ↓adipocytokines and CC chemokines (CCL2 and CCL5)
Netto BD et al. Obes Surg. 2015	Cohort Study	41 extremely obese who underwent RYGB	Anthropometric and clinical data, and biochemical markers of inflammation were collected pre-op and 6 months post-op	Pro-inflammatory biomarkers decreased: PAI-1 ($p<0.01$),CRP($p<0.01$), ICAM-1 ($p<0.01$), leptin ($p<0.01$) and resistin ($p<0.01$)
Lupoli R et al. Blood Transfus. 2015	Cohort study	156 obese subjects GBP (n=77) SG (n=79)	Haemostatic factors, fibrinolytic variables and natural anticoagulants were evaluated pre- and 2 months post-op	↓ 20% in PAI-1 ↓ Vit K dependent coagulation factors

Gumbau V et al. Obes Surg. 2014	Prospective Cohort study	20 obese patients underwent SG	The variations of different molecules related to inflammation during the first year following SG were assessed	1-year op period: ↓ Leptin ↓ MCP-1, IL-6, CRP and PAI-1
Nestvold TK et al. Metab Syndr Relat Disord. 2014	Case Control	97 morbidly obese patients who underwent BS and 17 lean subjects (control group)	Anthropometric measurements as well as fasting blood samples were obtained at first admission, pre-op and 1 year post-op	↓IL-6, and IL-13 ↓ fibrinogen and plasminogen activator inhibitor-1 ↓ Leptin and insulin
Iaffaldano L et al. Obes Surg. 2014	Case Control	20 obese individuals who underwent LAGB and 10 controls with normal BMI	Serum analyte levels were measured before (T0) and after surgery LAGB (T1)	At T1 vs T0: ↓Inflammation marker IL6 ($p<0.05$)
Mallipedhi A et al. Surg Obes Relat Dis 2014	Non-randomised prospective study	22 participants with impaired glucose homeostasis and T2DM undergoing SG	Serum levels of IL-6, IL-10, leptin, adiponectin and CRP pre-op, 1 and 6 months post-op	At 1 – 6 months post SG: ↓ IL-6 at 6 months ($p=0.001$)
Kim MK et al. Int J Endocrinol. 2013	Cohort study	57 patients with type 2 diabetes underwent RYGB	Serum levels various inflammatory markers, were measured pre- and 12 months post-op	↓PAI-1 at 1 year after RYGB. DM remission group had lower inflammatory markers compared to non-remission group post-op

Viana EC et al. Obes Surg. 2013	Cohort study	48 obese patients underwent: RYGB (n = 24) SG (n = 24)	IL-6 and TNF-α levels, as well as routine anthropometric and biochemical values, pre- (serum and adipose tissue levels) and 1 year post-BS	↓ IL-6 and TNF-α following surgery in both groups ($p<0.05$)
Thomsen SB et al. J Obes. 2013	Case control	Ten obese patients with T2D and 10 subjects with NGT	Subjects examined in the fasting state and after a standard meal prior to and post- (1 week, 3 months and 1 year) RYGB	Fasting state MCP-1 levels decreased after RYGB in both groups ($p<0.001$)
Bachmayer C et al.Exp Clin Endocrinol Diabetes. 2013	Case control	51 obese patients with metabolic syndrome, 20 obese patients without metabolic syndrome; 21 pre- and post-BS	Obesity-associated factors (hsCRP, MCP-1, sICAM, sVCAM, IGF-BP3, RBP 4 and adiponectin) were assessed	Post BS vs Obese controls ↓ Inflammatory mediators
Pardina E et al. Obesity (Silver Spring). 2012	Cohort study	34 severely obese patients underwent GBP	Various plasma parameters implicated in the intrinsic and extrinsic coagulation pathway were analysed before and 1, 6, and 12 months post-op	↓ PAI-1 plasma protein and PAI-1 mRNA levels in liver and adipose tissue
Tschoner A et al. Nutr Metab Cardiovasc Dis. 2012	Cohort Study	Thirty-seven obese adults underwent BS	Plasma PAI-1 levels examined before and 18 months after surgery	↓PAI-1 levels by 3.2 ± 5.6 ng/ml (all $p \leq 0.015$).

Cugno M et al. Intern Emerg Med. 2012	Case control study	25 women with isolated obesity underwent gastric banding. 25 healthy women served as a baseline control group	Pro-thrombotic and inflammatory markers were evaluated prior to, as well as 3, 6 and 12 months subsequent to gastric banding	12 months post op: ↓ PAI-1 (p=0.03)
Monte SV et al.Surgery. 2012	Cohort study	15 morbidly obese subjects with T2DM underwent RYGB	Morning of surgery and at 180 days fasting bloods taken to assess changes in glycemia, insulin resistance, LPS, mononuclear cell nuclear factor (NF)-κB binding and mRNA expression of CD14, TLR-2, TLR-4, and markers of inflammatory stress	↓ Inflammatory mediators CRP, MMP-9, and MCP-1 ↓ All other parameters studied
Terra X. et al. Clin Endocrinol (Oxf). 2012	Case control	133 women: 40 lean (C) 93 MO who underwent BS	Blood samples at pre-op, 6 and 12 months post-op BS from 30 MO patients	Circulating visfatin levels were positively related to IL6 and CRP levels
Illán-Gómez F et al. Obes Surg. 2012	Cohort study	60 morbidly obese women	Adiponectin, CRP, TNF-a and IL-6 were measured at 3, 6 and 12 months post-GBP	At 12 months post BS: ↓ IL-6, hs-CRP, Cholesterol, TG, insulin HOMA-IR

Dalmas E et al. Am J Clin Nutr. 2011	Case control	14 normal-weight women and 51 obese women	2y after RYGB. Multiplexed proteomics were used to simultaneously assay 27 cytokines and growth factors in serum	After 1 year: ↓ MCP 1, RANTES, Interleukins 8,9 ,10 and other cytokines
Marantos G et al. World J Surg. 2011	Case Control	20 morbidly obese women (premenopausal) GBP (n=13) GS (n= 7) 20 lean controls	Anthropometric and metabolic parameters were analysed pre- surgery, 6 and 12 months post-op	12 month post-op: ↓ IL-6
Brethauer SA et al. Surg Endosc. 2011	Cohort	15 patients (11 female) were enrolled and underwent RYGB	Pre-op and at 3 and 6 months post-op metabolic and inflammatory mediators were quantified.	↔ IL-6 post op ↓ PAI-1 and CRP (p=0.01)
Lima MMO et al. J Clin Endocrinol Metab. 2010	Cohort	19 obese women with metabolic syndrome underwent RYGB: T2DM (n=6) IGT (n=7) Normal GT (n=6)	Euglycemic-hyperinsulinemic clamp, HOMA-IR, nonesterified fatty acids, leptin, ultrasensitive CRP, adiponectin and IL-6 were assessed at baseline and 4.5 (0.9) wk post-op	↓Fasting glucose decrease p<0.01 ↓ Fasting insulin (p<0.01) ↓ Leptin and CRP ↔ IL-6 and adiponectin

Supplementary Table 5: Literature review of the effect of bariatric surgery on Incretins: Glucose-dependent Insulinotropic Polypeptide (GIP) and Glucagon-like Peptide- 1 (GLP-1)

Author, Journal	Study Design	Subjects (N)	Method	Results
S L Prior et al. Obes Surg. 2020	Prospective Cohort study	55 participants with impaired glucose homeostasis and T2D undergoing SG	Serial measurements of glucose, insulin, C-peptide, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic hormone (GIP) were performed during oral glucose tolerance testing preoperatively and 1 and 6 months postoperatively.	↓Glucose, insulin, C-peptide and HOMA ↑GLP-1 response as early as 6 months postoperatively
Roushdy A et al.Surg Laparosc Endosc Percutan Tech . 2020	Randomized study	Forty patients (38 female) with morbid obesity associated with comorbidities.	Randomly assigned to 2 groups: group I underwent SG and group II underwent OAGB.	↓Ghrelin and GLP-1 levels postoperatively at 6 and 12 months in group I compared with group II.
Alexiadou K et al.BMJ Open Diabetes Res Care. 2020	Prospective Cohort study	19 patients with obesity and pre-diabetes/diabetes undergoing RYGB.	Glucose, insulin, GLP-1, glucose-dependent	Post op:

			insulinotropic peptide (GIP), oxyntomodulin, glicentin and glucagon responses to a mixed-meal test (MMT) before and 1, 3 and 12 months after surgery was assessed.	↓Fasting glucose and glucose tolerance ↑Insulin response to MMT ↑Secretion of postprandial GLP-1, oxyntomodulin and glicentin. ↔ GIP secretion ↓ Fasting Glucagon
Jensen CZ et al. Am J Physiol Gastrointest Liver Physiol. 2020	Randomized, crossover study	10 RYGB operated and 8 healthy weight-matched control subjects	Subjects were given 4 different isocaloric (200 kcal) liquid meal tests containing either glucose, protein, or fat. Responses of gut and pancreatic hormones, bile acids, and fibroblast growth factor-21 was assessed.	Post RYGB: ↑ responses of GLP-1, GIP, glicentin, FGF-21, and C-peptide after glucose compared with the other meals.
Min T et al. Obes Surg. 2020	non-randomised	10 participants undergoing LSG and 6 participants undergoing BPD.	Oral GTT pre-operatively and 1 month, 6 months and at approximately 4-7 years post-operatively. Glucose,	↑post-glucose GLP-1 secretion were observed

	prospective study		insulin, C-peptide, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) levels were assessed.	at 1 and 6 months, not maintained at 4 years. ↑post-glucose GIP response at 1 month and 6 months and 4 years.
Salehi M et al. Gut 2019	Case control cohort study	Ten non-diabetic subjects with GB, and 9 body mass index (BMI)-matched and age-matched non-surgical controls (CN) with normal glucose tolerance	Subjects had blood glucose clamped at ~7.8 mM on three separate days. Stepwise incremental infusions of GLP-1 GIP or saline were administered from 90 to 240 min and insulin secretion measured.	Post GB group: ↓ Incretin-stimulated Insulin secretion rates compared to controls.
Svane MS et al. Gastroenterology . 2019	cross-sectional case control study	36 patients: SG (n=12) RYYGB (n=12) No surgery (n=12)	Underwent MMT during continuous infusion of glucose, glycerol, phenylalanine, tyrosine, and urea before. Blood samples were taken at 10 - 60 min intervals, for 6h and analyzed.	After RYGB: ↑ Insulin secfretion, ↑glucagon-like peptide 1, compared with RYGB and controls.

Sridharan K et al. Diabetes Metab Syndr. May-Jun 2019	Prospective Cohort study	28 participants underwent either laparoscopic sleeve gastrectomy or Roux-en Y gastric bypass	Measured the change in insulin resistance, beta cell function, GLP-1 and calcitonin levels before and 2 weeks after bariatric surgery.	After surgery: ↔Fasting GLP-1 ↑ peak GLP-1, and area under curve for GLP-1
Fernandes G et al. Surg Obes Relat Dis. 2019	Cohort study	Eleven patients with obesity and diabetes underwent RYGB with a gastrostomy performed in the excluded gastric remnant.	Preoperative assessments of glycemic and enterohormone profiles and an oral GTT were compared with early postoperative assessments after oral and gastrostomy route administrations.	Incretin improvement, mediated ↑glycogen-like protein 1 increased only in the postoperative oral route. ↓ GIP for both routes.
Honka H et al. Endocr Connect. 2018	Case control study	10 morbidly obese subjects with T2DM underwent bariatric surgery. 10 lean controls.	Subjects given mixed-meal and a glucose-dependent insulinotropic polypeptide (GIP) infusion before and within 3 months bariatric surgery. Hepatic blood flow and volume (HBV) measured.	↑GLP-1 secretion,

Wallenius V et al. Obes Surg. 2018	Cohort study	Eighteen LRYGB and 15 LSG patients were included in the study	Glucose, insulin, GLP-1, and GIP levels were monitored during a modified 30 g oral GTT before surgery and 2 days, 3 weeks, and 12 months after surgery.	↑GLP-1 levels similarly at 2 days, but were higher in LRYGB at 3 weeks ↔ GIP levels
Patrício BG et al. Int J Obes (Lond). 2019	Cohort study	20 non-diabetic weight-stable subjects previously underwent classical RYGB (n = 9) or long BPL RYGB (n = 11)	The gut hormone responses to a liquid mixed meal after RYGB with one of the two different BPL lengths was compared.	The long BPL RYGB group: ↑fasting & post-prandial GLP-1 ↓Responses of GIP, insulin and C-peptide compared to classical RYGB.
Pop LM et al. Diabetes Obes Metab . 2018	Cohort Study	10 patients with type 2 diabetes scheduled to undergo RYGB.	10-day inpatient supervised dietary intervention followed by diet and RYGB period. Metabolic assessments during a 6-hour mixed-meal challenge test, with stable isotope glucose tracer infusion performed before and after each intervention.	Diet and RYGB intervention: ↑↑post-meal glucagon-like peptide-1 (GLP-1) and glucagon levels.

Purnell JQ et al. Diabetologia . 2018	Longitudinal cohort study	Forty participants with type 2 diabetes and 22 participants without diabetes	Islet secretory response and GI hormone secretion after both intravenous glucose and a mixed meal (MM) prior to and up to 24 months after RYGB.	Post –op: ↑x8-fold in postprandial glucagon-like peptide 1 levels during mixed meal.
Yang J et al Surg Obes Relat Dis . 2018	nonrandomized prospective study	20 patients in this study, 10 underwent LSG, and 10 underwent LRYGB.	Fasting plasma levels of insulin, glucagon, ghrelin, gastric inhibitory peptide, glucagon-like peptide (GLP)-1, and GLP-2 were measured preoperatively and at 1, 3, 6, and 12 months after surgery.	↑Fasting GLP-1 in both groups, more post LRYGB. ↓GIP levels after LRYGB but not after LSG.
Tharakan G et al.Eur J Endocrinol . 2017	Case control study	18 symptomatic postprandial hypoglycaemia (PPH). 19 controls: 9 obese no surgery 10 RYBG no PPH	Continuous glucose monitoring to characterize altered glycaemic variability. Also mixed meal test (MMT) done and measured gut hormone concentrations.	↑Insulin, GLP-1 and glucagon in patients who had hypoglycaemia in response to an MMT (MMT Hypo) relative to those that did not (MMT Non-Hypo).
Farey JE, et al. Obesity Surgery. 2017	Prospective Cohort	11 Obese patients underwent BS Matched with 22 non-obese controls.	Obese participants' fasting blood samples taken 3 months post-op.	3 months post LSG: ↓Fasting GLP-1, glucagon

Bunt JC et al. Int. Journal of Obesity. 2017	Prospective non-randomized	18 Obese participants RYGB (n=10F) LAGB (n= 7F/1M)	Peptide hormones, incretins and pancreatic polypeptide responses to mixed meal test (MMT) were measured at 4-8 weeks pre and post op.	↑active GLP-1 responses following MMT post RYGB
Gong K et al. Surg Endosc. 2017	Cohort study	31 patients with T2DM underwent RYGB surgery	The fasting plasma glucose (FPG), HbA1c, C-peptide, fasting insulin (FINS) and glucagon-like peptide-1 (GLP-1) was pre-op and at 1, 3, 6 months post-op.	↑Mean GLP-1 after surgery ($P < 0.05$).
Berggren J et al Surg Obes Relat Dis. 2017	Case control cohort study	9 normoglycemic and 10 T2D patients underwent RYGB	Insulin, glucose, active glucagon-like peptide 1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP) measured at intervals following MMT & calorie restricted diet pre and post RYGB.	Post RYGB: ↑Insulin and GIP immediately. ↑GLP-1 delayed compared with the GIP response.

Griffo E et al. Obes Surg. 2016	Prospective cohort study	19 obese T2DM patients: SG (n=10) RYGB (n=9)	Pre-op and 2 years after BS, clinical parameters and the response of lipid and incretin hormones to a mixed meal (MM) were assessed.	Post op ↑Meal-stimulated GLP-1 postoperatively in both groups although to a greater extent after RYGB ($p < 0.001$ vs. SG). ↓GIP decreased after both procedures, especially after RYGB ($p = 0.003$).
G Nosso et al. Horm Metab Res. 2016	Cohort study	33 morbidly obese type 2 diabetic (T2DM) patients: RYGB (n=14) VSG (n= 19)	Insulin sensitivity, insulin secretion, and the gastrointestinal (GI) hormone response to a mixed meal test (MMT) were evaluated before and one year after BS	↑Meal-stimulated GLP-1 levels after both procedures. Significant after RYGB (p=0.0001). ↓GIP response to MMT after the 2 interventions (p=0.977).
Casella G et al. Br J Surg. 2016	Cohort Study	Sleeve gastrectomy (n=10)	12 months after surgery following assessed: 1. Insulin sensitivity	↑ AUC for GLP-1 180 min at 12 months after sleeve gastrectomy ($P < 0.001$).

			2. Insulin secretion - 3. Time course of GLP) 1, as a marker of insulin secretion following OGTT.	
Lindegaard KK et al. Diabetol Metab Syndr. 2015	Case Control	13 obese T2DM subjects & 12 obese, non-diabetic controls underwent RYGB	Subjects were examined before, one week, three months, and one year after surgery.	One year after surgery: ↑ Postprandial GLP-1
Major P et al Wideochir Inne Tech Maloinwazyjne . 2015	Prospective Cohort Study	35 patients LSG (45.8%) LRYGB (54.2%)	Serum glucagon-like peptide 1 (GLP-1), peptide YY (PYY), leptin, and ghrelin was measured at baseline and 12 months post op.	12 months post op ↑GLP-1
Gandolfini MP et al. Obes Surg. 2015	Cohort study	34 patients (BMI 46 ± 6 kg/m(2),	Cardiac and biochemical parameters were assessed before and 1 year after GBP.	↑ Postprandial (PP) GLP-1 ↓ BP was independently associated with the increase of PP GLP-1 level.
Wölnerhanssen BK et al. Surger Obes Relat Dis. 2015	Cohort Study	LRYGB (n=8) 10±.4 weeks post-op.	All subjects received 10 g and 25 g of oral glucose.	Post LRYGB: ↑GLP-1, GIP

		12 Lean Controls 12 Obese Controls	Assessed; plasma glucose, insulin, GLP-1, GIP, and peptide tyrosine tyrosine concentrations;	
Rhee NA et al. Diabetologia. 2015	Case control study	12 T2DM and 11 age and BMI matched controls	Mucosal biopsies taken during surgery and enteroscopy were immunohistochemically stained for hormone expression of small-intestinal enteroendocrine cells	Post RYGB: ↑density of GLP-1, GIP ↓gene expression GIP
Fellici AC et al. Obes Surg. 2015	Prospective cohort study	36 mildly obese subjects (19 males) with type 2 diabetes using oral antidiabetic drugs with (n = 24) or without insulin (n = 12) underwent RYGBP.	At baseline and 3, 6, 12, and 24 months post-surgery, insulin sensitivity, beta-cell secretory function, and incretin secretion was assessed following MTT.	3 months post-surgery, ↑ GLP-1 AUCi (P = 0.000), ↓GIP AUCi (P = 0.004).
Mallipedhi A et al. Surg Obes Relat Dis. Sep-Oct 2014	Non-randomized prospective study	37 diabetic, morbidly obese participants underwent: SG (n=22)	Serial measurements of glucose, insulin, C-peptide, glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic	↑postprandial GLP-1 response post SG

		BPD (n=15)	hormone (GIP) were performed during oral glucose tolerance testing preoperatively and 1 and 6 months postoperatively.	
Tom Gerner et al. Scand J Clin Lab Invest. 2014	Prospective Case control study	12 participants 3 years post RYGB: > 40% weight loss (n=6) < 25%weight loss (n=6) Control group (n=6)	A 300 kcal mixed meal test was given with blood sampling before and thereafter at 30-min intervals in 180 min.	In RYGB group: ↑Early postprandial GLP-1 response & highest in those with largest weight loss.
Bradnova O et al. Obes Surg . 2014	Prospective Cohort study	13 morbidly obese T2DM women underwent LGCP	MMT preop and at 1- and 6-month follow-up. Plasma levels gut hormones and parameters of glucose metabolism were taken.	↑Postprandial GIP at 1 and 6 months post op ($p < 0.0001$), ↔ Meal-induced GLP-1 response ($p > 0.05$).
E Griffo et al. Obes Surg. 2014	Cohort study	25 obese T2DM patients SG (n=15) GBP (n=10)	Lipid and incretin hormone concentrations were evaluated for 3 h after ingestion of a liquid meal	↑Meal-stimulated response of active GLP-1 ($p < 0.001$).

			before and 2 weeks after BS.	
Kim MJ et al. Asian J Surg. 2014	Cohort study	12 non-obese patients with poorly-controlled diabetes underwent gastric bypass surgery.	GIP and GLP-1 levels were measured before and 1 month after surgery in response to a 75 g oral glucose tolerance test (OGTT).	Post OGTT post op: ↑insulin and GLP-1 levels. ↓GIP levels sharply.
Lips MA et al. Clin Endocrinol (Oxf) . 2014	Case control study	54 obese females NGT: GB (n=11); RYGB (n=16) DM: RYGB (n=15); VLCD (n=12) Normal BMI controls (n = 12)	MMT at baseline and 3 weeks post op	In non-diabetic and T2DM subjects, RYGB: ↑GLP-1 and PYY levels and Low calorie diet and GB: ↑ GIP levels only
M Nannipieri et al. J Clin Endocrinol Metab. 2013	Cohort study	35 patients with T2DM (23 RYGB and 12 SLG).	Mixed-meal test before and 15 days and 1 year after surgery	Post RYGB & SG: ↑GLP-1 meal response. 1 year post op:↑PYY↓PP, amylin, ghrelin, GLP-1

				↑fasting GLP-1 in remitters (P = .04), but flat meal response.
Salinari S et al. Ann Surg. 2013	Cohort study	14 morbidly obese subjects, 7 with normal glucose tolerance and 7 with type 2 diabetes underwent RYGB.	Baseline & 1 month after RYGB studies with euglycemic hyperinsulinemic clamp (EHC), by iv GTT and by OGTT in 3 different sessions.	↑GIP and GLP-1 levels both at fasting and after OGTT mainly in type 2 diabetic subjects.
Jacobsen SH et al Diabetologia. 2013	Cohort study	obese glucose-tolerant individuals	Glucose absorption, metabolism and lipolysis rate before and 3 months after RYGB using the double-tracer technique during a mixed meal.	After RYGB: ↑Post prandial insulin and glucagon-like peptide-1 secretion
Dirksen C et al. Diabetologia . 2013	Cohort study	Eleven severely obese glucose-tolerant individuals underwent RYGB	Hyperglycaemic clamps with arginine bolus and co-infusion of either GLP-1, GIP or saline before, 1 week and 3 months after RYGB. An OGTT was	After OGTT at 3 months: ↑insulin and GLP-1 secretion.

			performed before & 3 months post op.	↔ Insulin and glucagon when iv GIP and GLP-1 given.
Umeda LM et al. Metab Syndr Relat Disord . 2013	Cohort study	10 patients with T2DM (BMI 39.3+2.44) were evaluated before and at 7 and 90 days after Roux-en-Y gastric bypass (RYGB).	A meal test was performed and plasma insulin, glucagon-like peptide-1 (GLP-1), glucose, TG, and adiponectin levels were measured at fasting and at 30, 60, 90, and 120 min postprandial.	90 days post RYGB ↓ TG & glucose fasting levels ↑ Postprandial, adiponectin, GLP-1 and insulin curves.
Werling M et al. PLoS One . 2013	Cross sectional cohort study	14 women from a randomized clinical trial between gastric bypass (n = 7) and VBG (n = 7) were included.	9 years postop patients were assessed. Energy expenditure was measured. Blood samples were analysed for postprandial gut hormone responses.	↑Postprandial peptide YY (PYY) and glucagon like peptide 1 (GLP-1) levels after gastric bypass (both $p<0.001$).
Moran-Atkin E et al. Surg Endosc. 2013	Cohort study	23 morbidly obese patients underwent: RYGB (n=12; 5 DM) GB (n=11; 7DM)	Twenty-three underwent Roux-en-Y gastric bypass (RYGB) or gastric banding. Overall, there were 12 RYGB (5 T2D; 7 nondiabetic) patients and	Postoperative GIP gene expression increased 4.36-fold ($p = 0.02$) in diabetic RYGB patients, whereas diabetic band patients increased 1.4-fold ($p = 0.25$).

			11 gastric band (7 T2D; 4 nondiabetic) patients.	
Jacobsen SH et al. Obes Surg. 2012	Cohort study	8 obese non-diabetic patients underwent RYGB.	Pre and within 2 weeks post op, OGTT and a liquid mixed meal test (200 mL 300 kcal) were performed on separate days.	Post op findings: ↑Post prandial GLP-1, GLP-2 ↔ GIP
Jørgensen NB et al. Am J Physiol Endocrinol Metab . 2012	Case control study	13 obese subjects with T2D and 12 matched subjects with normal glucose tolerance (NGT) underwent RYGB.	Examined during a liquid meal before (Pre), 1 wk, 3 mo, and 1 yr after RYGB.	1st week post RYGB Postprandial ↔GIP secretion ↑↑GLP-1 secretion.
Chronaiou A et al. Obes Surg . 2012	Randomised controlled trial	Twelve patients underwent LRYGBP and 12 patients LRYGBP plus gastric fundus resection (LRYGBP+FR).	All patients were evaluated before and at 3, 6, and 12 months postoperatively. Blood samples were collected after an overnight fast and 30, 60, and 120 min after a standard 300-kcal mixed meal.	Post LRYGBP+FR ↑Postprandial GLP-1 Postoperatively, ghrelin changes correlated negatively with GLP-1 changes.
Anderwald CH et al. Diabetes Care	Case controlled study	6 nondiabetic, morbidly obese patients: 6 Obese controls	Assessed pre RYGB and 7-8 months post op, then OGTT compared with	↑ 29-fold active glucagon-like peptide-1 (GLP-1) dynamic

. 2012		6 Lean controls	matching obese and lean controls	AUC,correlated (r = 0.837, P < 0.001) with 84% increased β-cell secretion.
Dar MS et al Obes Surg. 2012	Cross-sectional study design	N=5 post-RYGB group compared to: lean (n = 9), obese (n = 6), T2DM (n = 10) controls	GLP-1 response to a mixed meal in the 10-year post-RYGB	10-year post-RYGB GLP-1 response: ↑ 0-20 min (p = 0.035) ↓ 20 and 60 (p = 0.041)
Peterli R et al. Obes Surg. 2012	prospective, randomized 1-year trial,	12 non diabetic obese patients were randomized to LRYGB and 11 to LSG.	Pre op and 1 week, 3 months, and 12 months post op: standard test meal was given after an overnight fast. Blood samples collected before, during, and after food intake for GI hormone profiles	Post-surgery: ↑ Postprandial plasma GLP-1 levels (p < 0.05) with ensuing improvement in glucose homeostasis.
Ramón JM et al. J Gastrointest Surg. 2012	Randomised controlled trial	7 patients were randomised to LRYGB and 8 to LSG.	Pre op and at 3 and 12 months post op: before, 10 and 60 mins after a standard test meal	LRYGB group: ↑ GLP-1 levels after test meal.

			ingested, plasma levels of glucose, insulin, ghrelin, leptin, GLP-1 were measured.	
Evans S et al. Surg Endosc. 2012	Case control study	Gastric Bypass (n = 10) 7-day hypocaloric liquid diet matching the post-GBP diet (control, n = 10).	Active GLP-1 was measured fasting and at multiple points after standardized mixed-nutrient and high-fat liquid meals in two matched groups of obese subjects.	↑Mixed-nutrient and high-fat postprandial GLP-1 levels following GBP but not after hypocaloric liquid diet
Umeda LM et al. Obes Surg . 2011	Cohort study	Ten patients with T2DM (BMI, 39.7 ± 1.9) were evaluated before and 7, 30, and 90 days after RYGB.	A meal test was performed, and plasma insulin, glucose, glucagon, and glucagon-like-peptide 1 (GLP-1) levels were measured at fasting and postprandially.	The insulin and GLP-1 curves began to show a peak at 30 min after food ingestion, while there was a progressive decrease in glucagon and blood glucose levels throughout the meal test.
Falkén Y et al. J Clin Endocrinol Metab . 2011	Cohort study	Twelve obese subjects had undergone GBP.	Participants were subjected to a liquid meal without lipids before and 3 d, 2	↓HOMA-IR 2 months post op

			months, and 1 yr after GBP.	↑ Postprandial rise of GLP
Usinger L et al. Obes Surg. 2011	Case control study	8 obese patients underwent LAGB Normal GT (n=3) IGT (n=3) T2DM (n=2)	Underwent a 75 g-oral glucose tolerance test with 1 g acetaminophen before and ~6 weeks after LAGB.	Post LAGB: ↔ Plasma glucose, insulin, C-peptide, glucagon, glucose-dependent insulinotropic polypeptide, or glucagon-like peptide-1 responses to the OGTT.
Promintzer-Schifferl M et al. Obesity (Silver Spring). 2011.	Case Control	18 participants Nondiabetic obese group underwent RYGB. (n=6); Lean, no surgery (n=6) Obese, no surgery (n=6)	Surgery group before and 7 months post op: Time-courses of glucose, insulin, C-peptide, glucagon like peptide-1 (GLP-1) measured after oral glucose load.	Post RYGB: ↑ Postprandial GLP-1 (p 0.01).
Bose M et al. Obesity (Silver Spring). 2010	Cohort	20 participants GBP (n=11) GB (n=9)	Oral glucose challenge pre –op (T0), after a 12 kg weight loss (T1) and 1 year post op (T2) – assessed incretin and peptide hormone levels.	Post op: ↔ GLP-1

Kashyap SR et al. Int J Obes (Lond). 2010	Cohort study	16 obese T2DM patients undergoing either RYGB (N=9) or GR (N=7) surgery.	Pre op,1 and 4 weeks post-surgery glucose, insulin secretion, insulin sensitivity was measured. Response to a MMTT at baseline and 4 weeks post-surgery was also assessed.	Following MMTT: ↑Insulin secretion, glucagon-like peptide-1 (GLP-1) levels and beta-cell sensitivity to glucose only after RYGB ($P<0.05$).