

Lifestyle Mediators of Dysglycaemia in Pregnancy: Towards Novel Strategies for Diabetes Management in Pregnancy

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Chapter 3

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Chapter 4

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Chapter 5

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Abstract

Women with diabetes in pregnancy (i.e., pre-gestational type 1- and type 2- and gestational diabetes mellitus [DIP]), struggle to control glucose levels during pregnancy resulting in high risk of pregnancy complications. Current dietary guidelines and methods using carbohydrate content of meals for achieving and quantifying (postprandial) glucose responses are suboptimal, as personal and physiological factors beyond the characteristics of food have been implicated to play an important role.

This PhD project aimed to: (i) examine (dietary) mediators – including personal, physiological and environmental parameters – of (postprandial) glucose control in DIP, and (ii) examine possible nutritional and lifestyle strategies associated with (postprandial) glucose control in DIP.

Study 1, a systematic review and meta-analysis, found that nutritional supplements, diet, and exercise play a prominent role in the management of gestational diabetes (GDM), improving measures of glycaemia, but evidence for pre-gestational diabetes is lacking. The observational secondary data analysis in study 2 concluded that glycaemia varies across the day, with morning glycaemia demonstrating the greatest level of variability, and that increased dietary protein may assist in improving glucose control in GDM. Study 3 was designed to assess the role of diet as mediator of dysglycaemia throughout pregnancy in pre-gestational diabetes and the moderating effects of personal, physiological and environmental parameters. However, recruitment was delayed with no results yet obtained. Therefore, study 4 was designed and conducted using dietary metabolite data from the Born in Bradford cohort and found that meat consumption could be characterised by a distinct metabolite profile using total self-reported meat-intake as criterion. Future analyses exploring other criteria for identifying a distinct metabolite profile and linking meat intake (a major source of protein) to postprandial glucose response in pregnancy are warranted.

This thesis provided new insights into the factors (e.g., timing and protein intake) driving (postprandial) glycaemia in DIP. Future work should aim to better understand the relationship of these factors to aid in the development of more personalised recommendations for improving DIP management.

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Abbreviations

ADA	American diabetes association
AUC	Area under the curve
BiB	Born in Bradford
BMI	Body mass index
CGM	Continuous glucose monitoring
CHOICE	Higher-complex carbohydrate/lower-fat
CSII	Continuous subcutaneous insulin infusions
CV	Coefficient of variation
CVB	Coxsackievirus
CVD	Cardiovascular disease
DASH	Dietary Approaches to Stop Hypertension
DIP	Diabetes in pregnancy
ER	Endoplasmic reticulum
FA	Fatty acids
FFQ	Food frequency questionnaire
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
GI	Glycaemic index
GIP	Gastric inhibitory polypeptide
GL	Glycaemic load
GLP-1	Glucagon-like peptide-1
GLUT4	Glucose transporter type 4
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GV	Glycaemic variability
GWAS	Genome-wide association studies
HAPO	Hyperglycaemia and Adverse Pregnancy Outcome study
HbA _{1c}	Glycated Haemoglobin

HDL	High-density lipoprotein
HLA	Human leucocyte antigen
HOMA-IR	Homeostatic Model of Assessment – Insulin Resistance
hPL	Human placental lactogen
HR	Hazard ratio
HRA	Health Research Authority
IADPSG	International Association of Diabetes in Pregnancy Study Group
IDF	International diabetes federation
IgM	Immunoglobulin M
iAUC	Incremental area under the curve
IMD	Index of multiple deprivation
IR	Insulin resistance
IRS-1	Insulin receptor substrate-1
isCGM	Intermittently scanned continuous glucose monitoring
Kcal	Kilocalories
LDL	Low-density lipoprotein
LGA	Large for gestational age
LMIC	Low- and middle income country
LSEQ	Leeds Sleep Evaluation Questionnaire
LTHT	Leeds Teaching Hospitals Trust
MAGE	Mean amplitude of glycaemic excursions
mAHEI	Modified Alternative Healthy Eating Index
MaGiC	Maternal Glucose in Pregnancy
MD	Mean difference of effect
MDI	Multiple-dose insulin
MMS	Metabolic meat-intake score
MUFA	Mono-unsaturated fatty acids
NGA	Normal for gestational age
NHS	National Health Service
NICE	National Institute of Clinical Excellence
NICU	Neonatal intensive care units
NMR	Nuclear magnetic resonance

OR	Odds ratio
OGTT	Oral glucose tolerance test
pCGM	Professional continuous glucose monitoring
PI 3	Phosphatidylinositol-3
PLS	Partial least squares
PPAQ	Pregnancy Physical Activity Questionnaire
PPI	Patient and Public Involvement
PPG	Postprandial glucose
PPGR	Postprandial glucose response
PUFA	Poly-unsaturated fatty acids
RCT	Randomised Controlled Trial
RDI	Recommended daily intakes
RoB2	Risk of bias tool
RR	Relative risk
rtCGM	Real-time continuous glucose monitoring
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SES	Socioeconomic status
SGA	Small for gestational age
SMBG	Self-monitoring of blood glucose
SRMA	Systematic review and meta-analysis
SFA	Saturated fatty acids
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TAR	Time-above-range
TBR	Time-below-range
TIR	Time-in-range
TNF- α	Tumour necrosis factor-alpha
VIP	Variable Importance in Projection
VLDL	Very low-density lipoprotein

WHO World Health Organisation

Chapter 1

General Introduction & Literature Review

Diabetes is a serious condition that occurs when the body is unable to produce sufficient insulin to manage healthy glucose levels (Van Belle et al., 2011; Nolan et al., 2011; International Diabetes Federation, 2021). Since the first Diabetes Atlas edition in 2000 presented by the International Diabetes Federation (IDF), the global prevalence of diabetes in adults has more than tripled, leading to an estimate of 537 million in the age group of 20-79 years (10.5% of adults in this age category) in 2021 (International Diabetes Federation, 2021). Future projections are equally alarming with an estimated increase of 46% by 2045 (International Diabetes Federation, 2021). These projections are the sum of three main types of diabetes mellitus, type 1 diabetes (T1DM), type 2 diabetes (T2DM), and gestational diabetes mellitus (GDM) (World Health Organization, 2006). While T1DM and T2DM are chronic conditions that can develop at any point affecting the health of the individual. Diabetes mellitus can also develop during pregnancy, this is referred to as GDM. Women who were diagnosed with T1DM or T2DM before pregnancy or diagnosed with GDM during pregnancy can be defined as diabetes in pregnancy (DIP). The IDF estimated 16.7% of live births to women in 2021 – compared to 15.8% in 2019 – were affected by some form of DIP (International Diabetes Federation, 2019; International Diabetes Federation, 2021). Of these, 80.3% were diagnosed with GDM, while 19.7% were due to pre-existing T1DM or T2DM (International Diabetes Federation, 2021). The IDF 2021 Atlas also reports a difference in prevalence of GDM in low-, middle- and high-income countries, which is 12.7%, 9.2% and 14.2%, respectively.

Pregnant women with DIP are at greater risk of adverse pregnancy outcomes compared to pregnant women with within range glucose levels (i.e., normoglycaemia), including a three-fold higher rate of perinatal mortality (Schaefer-Graf et al., 2018; McCance, 2015; Moncrieff, 2018). Other potential adverse maternal and foetal outcomes, include pre-eclampsia (~30% increased odds), need for caesarean section (~50% increase odds), preterm delivery (~20% increased odds), large for gestational age (LGA; ~30% increased odds), and birth injury (e.g., shoulder dystocia; ~10% increased odds) (Moncrieff, 2018; McCance, 2015; Feig and Palda, 2002). Alterations of the maternal environment

can not only impact the intrauterine development of the foetus, but also influence the offspring's health risk over the life-course (Dabelea and Crume, 2011). The Exploring Perinatal Outcomes among Children (EPOCH) study found that exposure to maternal GDM in utero was associated with higher BMI (i.e., 1.3 kg/m²), waist circumference (i.e., 4.2 cm), visceral and subcutaneous adipose tissue (i.e., 3.6 and 34.7 cm², respectively) in 6- to 13-year-old multi-ethnic youth, which has been corroborated in further studies (Dabelea et al., 2011; Crume et al., 2011; Dabelea et al., 2008). Other studies in offspring of mothers with T1DM and GDM showed a significantly higher prevalence of impaired glucose tolerance than a control group matched for age and sex – combined exposure to maternal diabetes and obesity in utero accounted for 47% of the T2DM risk in the offspring (Silverman et al., 1995; Clausen et al., 2008). The body of literature shows that offspring exposed to DIP are at higher risk for diabetes in later life, which in itself is a major health concern, perpetuating transgenerational risk of diabetes from mothers to their offspring. These adverse health outcomes highlight the importance of optimal glycaemic control for women with DIP.

Diabetes UK report that almost £10 billion of National Health Service (NHS) costs is attributable to diabetes and around 80% of this is spent on complications (UK, 2014). The IDF report details that patients with diabetes have medical costs two to three times higher than age and sex matched individuals without diabetes (International Diabetes Federation, 2019). Likewise, DIP imposes a burden on healthcare services, the total annual costs of managing pregnancy and delivery in women with T1DM in the UK is estimated to approach almost £24 million. Moreover, complicated deliveries often associated with maternal diabetes result in additional cost (£3357 for complicated and £1957 for normal delivery) (Murphy et al., 2019; Webber et al., 2015). Furthermore, GDM diagnosis is predicted to cost healthcare services an additional 25% compared to a normoglycaemic pregnancy (Xu and Ye, 2020; Kolu et al., 2011). DIP rates and costs continue to rise globally, highlighting the urgent need for effective methods of managing DIP and GDM prevention.

1.1 Types of Diabetes in Pregnancy (DIP)

1.1.1 Type 1 Diabetes Mellitus (T1DM)

T1DM is a chronic autoimmune and lifelong disease in which the pancreatic β -cells are deleteriously damaged, resulting in insulin deficiency and hyperglycaemia (Van Belle et al., 2011). This type of diabetes is characterised as 'insulin-dependent' diabetes (Zaccardi et al., 2016). The body is still able to breakdown carbohydrates from dietary intake into glucose; however, there is insufficient or no insulin available for glucose uptake into the cells, resulting in build-up of blood glucose levels and hyperglycaemia (American Diabetes Association, 2010; International Diabetes Federation, 2021; International Diabetes Federation, 2019). If hyperglycaemia is left unchecked, these high blood glucose levels will result in health risks (i.e., heart, eye, feet and/or kidney damage) (International Diabetes Federation, 2021; International Diabetes Federation, 2019). This type of diabetes, accounts for approximately 5–10% of diabetes cases (International Diabetes Federation, 2021; International Diabetes Federation, 2019).

Onset usually occurs in people younger than 30, although it can develop at any age. The exact cause of T1DM remains unknown, but a genetic predisposition with environmental factors triggering the autoimmune reaction have been implicated (International Diabetes Federation, 2021). Common early symptoms are excessive thirst, urination, and hunger, when these symptoms occur a urine sample will be taken and blood glucose levels are monitored (e.g., random glucose test) (Van Belle et al., 2011; NIDDK, 2017a). Diabetes mellitus can also be diagnosed when fasting blood glucose is higher than 7 mmol/L (126 mg/dL), 2-hr oral glucose-tolerance test of ≥ 11.1 mmol/L (200 mg/dL), or any blood glucose of ≥ 11.1 mmol/L (200 mg/dL) with symptoms of hyperglycaemia (American Diabetes Association, 2020a). To determine which type of diabetes (i.e., T1DM) is present, healthcare professionals will test for biomarkers, including c-peptide and islet-specific autoantibodies in the bloodstream (NIDDK, 2017a). More recently, the American Diabetes Association (ADA) modified the guidelines for diabetes diagnosis to include glycated haemoglobin (HbA_{1c}) of $\geq 6.5\%$ (American Diabetes Association, 2020a).

T1DM in pregnancy is associated with an increased risk of congenital malformations (e.g., high cardiovascular and urogenital anomalies; three-fold increased risk), obstetric complications (e.g., prematurity and caesarean section; four-fold increased risk), and neonatal morbidity (e.g., high incidence of neonatal hypoglycaemia ~60%) (Evers et al., 2004; Inkster et al., 2006). In summary, T1DM occurs when glucose levels are elevated in insulin-responsive people, who are unable to produce insulin due to deleteriously damaged pancreatic β -cells as consequence of an autoimmune response.

1.1.1.1 Pathophysiology of T1DM

T1DM is the result of an autoimmune mediated destruction of insulin-producing pancreatic β -cells in the islets of Langerhans in the pancreas (Zaccardi et al., 2016). In longstanding T1DM, the pancreas' insulin-producing cells are severely damaged or devoid and remaining β -cells are incapable of regeneration (Atkinson et al., 2014). Consequently, insulin production is reduced and eventually eliminated. In predisposed individuals, early-life environmental factors (such as infections, nutrition, chemicals) are able to trigger and 'activate' self-targeting immune cascades (Zaccardi et al., 2016). In the initial phases, the progressive destruction of β -cells is not associated with changes in blood glucose concentrations, as the pancreatic 'reserve' is sufficient to maintain euglycaemia. Subsequently, further β -cell destruction occurs, resulting in loss of insulin production and an accompanying increase in blood glucose levels. When most of the β -cells are destroyed, overt diabetes develops. Achieving glycaemic control after diagnosis is of predominant importance, as 'near-to-normal' glucose has been shown not only to reduce the risk of diabetic complications, but also to safeguard any remaining β -cell function/mass (Zaccardi et al., 2016). Both humoral and cellular immunity is involved in T1DM pathogenesis, T lymphocytes (which mature in the thymus) play a key role in cell-mediated immunity and are predominant in islet lesions, with lower concentrations of other immunological cells (i.e., macrophages, B lymphocytes and plasma cells) (Zaccardi et al., 2016). The body of literature stipulates that a series of functional defects in the bone marrow, thymus, immune system, and β -cells, induced by genetic and environmental factors, contribute to the pathophysiology of T1DM (Atkinson et al., 2014; Van Belle et al., 2011; International Diabetes Federation, 2019).

The major susceptibility markers for T1DM are the human leucocyte antigen (HLA) class II genes on chromosome 6p21 (which account for ~30-50% of genetic T1DM risk), although more than 40 non-HLA susceptibility gene markers have been confirmed to contribute disease risk with smaller effects (Atkinson et al., 2014; Steck and Rewers, 2011). HLA genes are critical for regulating the immune response, as these genes encode for cell surface proteins involved in the antigen presentation and self-tolerance (Zaccardi et al., 2016). Genetically determined variations of these proteins can, therefore, alter the range of presented peptides and result in the loss of self-tolerance, clarifying the autoimmune response in T1DM individuals (Zaccardi et al., 2016).

Although there is numerous evidence to suggest the association between viruses (e.g., enteroviruses) and T1DM, evidence of a definitive causative relationship resulting in the initiation or progression of islet autoimmunity is limited (Giwa et al., 2020; Van Belle et al., 2011). Enteroviruses (e.g., coxsackievirus [CVB]) are small non-enveloped RNA (ribonucleic acid) viruses and the most common viruses causing human diseases, usually, leading to mild or asymptomatic infections (Tauriainen et al.). Epidemiological studies among children diagnosed with T1DM found that 64-67% of them had positive CVB immunoglobulin M (IgM) serology (Giwa et al., 2020; Clements et al., 1995; Friman et al., 1985). The exact mechanism of viral infections' contribution to the deterioration of islet cells remains unknown. However, suggested mechanisms include molecular mimicry, inflammation, endoplasmic reticulum (ER) stress, and activation or suppression of T cells, which all are detrimental to β -cell function and survival (Giwa et al., 2020).

Interestingly, seasonality and vitamin D deficiency have also been proposed as an environmental trigger of T1DM onset (Giwa et al., 2020; Yang et al., 2013; Hyppönen et al., 2001). Some theories suggest a seasonal variation in the blood glucose and insulin levels due to reduced level of activity in the winter months and seasonal viral infections (Giwa et al., 2020). Other evidence that demonstrates an association between vitamin D signalling and regulating immune responses, where a deficiency in vitamin D is associated with increased risk of autoimmune diseases including T1DM (Yang et al., 2013). Furthermore, the intestinal microbiome is known to influence lipid and glucose metabolism, as well as immunity and systemic inflammation outside of the intestine by formation

of a coherent barrier separating luminal bacteria (Giwa et al., 2020; Van Belle et al., 2011). Thereby, making it more difficult for viruses to cross the intestinal barrier. All in all, evidence shows that pathogenesis of T1DM is multifactorial and genetic as well as environmental factors play an important role in disease development.

1.1.1.2 Management and treatment of T1DM

Due to an inability to produce adequate insulin, individuals diagnosed with T1DM need daily insulin injections to maintain a glucose level in euglycaemic range (Atkinson et al., 2014). With daily insulin treatment, regular blood glucose monitoring, education and support, individuals with T1DM can live healthy lives and delay or prevent many diabetes-associated complications (International Diabetes Federation, 2019). Therapeutic strategies for optimising glycaemic control via insulin therapy include: (i) multiple-dose insulin (MDI) regimens with long-acting insulin analogues that mimic physiological insulin release, as basal insulin for overnight and between-meal control, plus bolus doses of rapid-acting insulin analogues to cover ingested carbohydrate loads and treat hyperglycaemia, or (ii) continuous subcutaneous insulin infusions (CSII), known as insulin pumps (Atkinson et al., 2014; DiMeglio et al., 2018).

Maintaining optimal glucose control in T1DM affected pregnancies can be more difficult as hormone levels change and women may experience morning sickness. Therefore, continuous glucose monitoring (CGM) or flash monitoring is offered to help these women controlling their blood glucose levels during pregnancy (Webber et al., 2015). Lately, new methods have been designed to combine insulin pumps and CGM with a computer algorithm (i.e., an integrated closed-loop system, or artificial pancreas). With an artificial pancreas system, glucose levels are monitored continuously (via CGM) and computer algorithm improves blood glucose control by automatically adjusting the amount of insulin delivered, to keep blood glucose levels in range and avoiding hypo- and hyperglycaemia (NIDDK, 2021). Healthcare professionals can monitor insulin doses remotely and recommend dosage adjustments for patients who need closer supervision. The artificial pancreas automates the insulin delivery resulting in more optimal glucose control compared with current available treatments (Stewart et al., 2016). In T1DM affected pregnancies, the artificial pancreas was safe to use and it improved management of blood glucose levels (i.e., ~75% or

~18 hours per day in target range compared to ~60% or 14 hours per day for those using the insulin pump without an artificial pancreas) (Stewart et al., 2016). Future development of these methods strives to improve real-time and accurate insulin delivery to support healthy glucose control, ultimately improving the quality of life for patients with T1DM.

Focussing more closely on management of T1DM in pregnancy, intervention before pregnancy is of importance to ensure optimal glycaemic control throughout the time of conception and at the critical early stage of gestation (Egan et al., 2015). Therefore, there is increasing emphasis on preconception care in the UK (Webber et al., 2015; Egan et al., 2015). Women with pre-existing diabetes planning to become pregnant should establish adequate glycaemic control before conception and continue this throughout pregnancy to reduce the risk of adverse pregnancy outcomes (Webber et al., 2015). The UK National Institute of Clinical Excellence (NICE) guidance suggests aiming for pre-breakfast glucose of 5–7 mmol/L and 4–7 mmol/L before other meals, post-meal targets of 5–9 mmol/L are also included (Webber et al., 2015). Preconception HbA_{1c} target is set at <48 mmol/mol (i.e., <6.5%) and considers any reduction towards this level as helpful (Webber et al., 2015). Despite these preconception guidelines, there are barriers for women meeting preconception targets. Known reasons and barriers for not meeting the preconception targets are: i) up to 50% of pregnancies are unintended; ii) lack of health insurance, a regular primary care or obstetric provider reduces contact with the health care system, this is also seen in women planning to become pregnant; iii) women and health care providers may be unaware of the existence, the importance of preconception care or it is not seen as a high priority; iv) social and economic challenges, including lack of child care, transportation, geographic isolation, and distrust of health care providers (Kendrick, 2004; Khan et al., 2019; Korenbrot et al., 2002; Owens et al., 2006). Solutions to these issues have been suggested (Khan et al., 2019; Owens et al., 2006): i) increasing women's and health care provider's awareness about the importance of preconception care programs, ii) providing culturally and linguistically appropriate diabetes-related health information and education, iii) reminding women about scheduled health care visits, iv) better communication between providers and women in terms of patients' needs.

Upon pregnancy, immediate contact with a joint diabetes and antenatal clinic should be facilitated, allowing for optimal care as the pregnancy progresses (Webber et al., 2015; Egan et al., 2015). These clinics may differ in terms of structure, but women should be reviewed every 1–2 weeks by their diabetes clinical care team (i.e., in-person or via virtual/telephone calls) (Egan et al., 2015). Blood glucose levels should be monitored daily including fasting, pre-meal, 1-hour post-meal and bedtime levels (Webber et al., 2015). Recommended target for fasting glucose is <5.3 mmol/L and for 1-h post prandial is <7.8 mmol/L and women who are taking insulin should maintain their capillary plasma glucose level above 4 mmol/L; however, goals should be individualised and safe (Webber et al., 2015). Women with T1DM are also advised to test for ketones (urinary or capillary) if they become unwell or hyperglycaemic (Webber et al., 2015). Higher levels of HbA_{1c} >6.0 – 6.5% may be used as marker of poor glycaemic control and for increased risk, but HbA_{1c} does not reliably reflect changes in mean blood glucose in pregnancy, particularly in the late stages of gestation (Egan et al., 2015).

For pharmacological therapy, rapid-acting insulin analogues (i.e., aspart and lispro) should be considered, as these have advantages over soluble human insulin during pregnancy (Webber et al., 2015). CSII, also known as insulin pump therapy, is offered to pregnant women with insulin-treated diabetes; i) who are using MDIs of insulin and ii) who do not achieve blood glucose control without significant disabling hypoglycaemia (Webber et al., 2015). To improve blood glucose targets during pregnancy and neonatal outcomes, real-time continuous glucose monitoring (rtCGM) is offered to all pregnant women with T1DM (Webber et al., 2015). If women are unable to use rtCGM or express a clear preference for other types of CGM monitors, intermittently scanned continuous glucose monitoring (isCGM, or 'flash') is offered (Webber et al., 2015).

Finally, to monitor risk of adverse pregnancy outcomes, ultrasound monitoring of foetal growth and amniotic fluid volume every 4 weeks from 28 to 36 weeks is conducted (Webber et al., 2015). Although significant progress has been made in the management of T1DM in pregnancy, these women and their offspring remain at risk of multiple adverse complications. Structured guidelines and management programmes using novel technologies (i.e., CGM and artificial

pancreas) may facilitate the ultimate goal of ensuring an outcome closer to a pregnancy unaffected by diabetes.

1.1.2 Type 2 Diabetes Mellitus (T2DM)

T2DM is a metabolic disorder, characterised by hyperglycaemia and modified lipid metabolism, induced by pancreatic β -cells inability to secrete sufficient insulin in response to over-nutrition, inactivity, overweight or obesity, and insulin resistance (Nolan et al., 2011). This type of diabetes is characterised as 'non-insulin-dependent' diabetes (Zaccardi et al., 2016). In T2DM, production of insulin is suboptimal, leading to decreased glucose uptake by the cells and to increased blood glucose levels, also known as insulin resistance (IR) (NIDDK, 2017b). IR is clinically defined as 'the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual compared to an individual with normal insulin tolerance' (Lebovitz, 2001). T2DM is the most common type of diabetes and accounts for 90–95% of all diabetes cases (American Diabetes, 2010).

Over the years, T2DM is seen at younger ages translating into an increasing number of pregnant women being affected, this has led to increased cases of gestational hyperglycaemia and subsequently higher rates of perinatal mortality (~5%), major congenital malformations (~7%), preeclampsia (~11%), preterm birth of (~20%), birth weight >90th percentile (~32%, and caesarean section (~42%), these rates are comparable between women of Caucasian or ethnic minority origin (Nolan et al., 2011; Feig et al., 2002; Groen et al., 2013). Therefore, awareness maternal dysglycaemia is important and screening women early in pregnancy for overt diabetes is advised (International Association of Diabetes Pregnancy Study Group, 2010).

Healthcare professionals are recommended to routinely screen for T2DM if an individual has certain risk factors, such as age of ≥ 35 years, ethnicity (Black, Southeast Asian, Hispanic/Latino, etc.), overweight or obesity with at least one other risk factor, and previous diagnosis of gestational diabetes. Children can also develop T2DM, and it is recommended to test children and teens between the ages of 10 and 18, who are overweight or have obesity with at least one more risk factor (e.g., low birth weight or family history of diabetes) (NIDDK, 2022).

Diagnostic criteria for T2DM and T1DM are identical – diabetes is diagnosed when fasting blood glucose is higher than 7 mmol/L (126 mg/dL), 2-hr oral glucose-tolerance test of ≥ 11.1 mmol/L (200 mg/dL), an HbA_{1c} of $\geq 6.5\%$ (48 mmol/mol), or any blood glucose of ≥ 11.1 mmol/L (200 mg/dL) with symptoms of hyperglycaemia (American Diabetes Association, 2020a). To identify which type of diabetes follow-up tests – i.e., genetic testing for monogenic diabetes and autoantibody testing for T1DM – are conducted (International Diabetes Federation, 2021; NIDDK, 2022). In summary, T2DM occurs when glucose levels are elevated in insulin-unresponsive people, who insufficiently or are unable to produce insulin due to modifications in the pancreatic β -cells in response to over-nutrition, inactivity, overweight or obesity, and insulin resistance.

1.1.2.1 Pathophysiology of T2DM

T2DM develops when pancreatic β -cells are unable to compensate for (i) the fuel surplus, (ii) impaired expansion of subcutaneous adipose tissue (SAT) and inflammation of adipose tissue, (iii) increased glucagon secretion and reduced incretin response, (iv) augmented endogenous glucose production, and (v) occurrence of IR. Subsequently, T2DM occurs when normal concentrations of insulin produce a less than normal biologic response unable to regulate blood glucose levels within a healthy range (Kahn, 1978; Nolan et al., 2011).

Current understanding of insulin resistance and secretion in the course of T2DM onset and progression stipulate the display an initial state of IR compensated by β -cell hypersecretion of insulin (i.e., hyperinsulinaemia) (Zaccardi et al., 2016). However, this pancreatic ‘functional’ reserve is eventually unable to cope with the required insulin secretion. Predisposed normoglycaemic individuals have $\sim 30\%$ reduced insulin sensitivity, compared to control individuals (Zaccardi et al., 2016; Jallut et al., 1990). These predisposed individuals show increased insulin secretion to maintain normal glucose tolerance (i.e., euglycaemic hyperinsulinaemia). Furthermore, the increased demand of insulin synthesis and secretion results in β -cell dysfunction (Zaccardi et al., 2016). ‘Stressed’ β -cells may excite local inflammation and modify the balance between α - and β -cell mass and function within the Langerhans islets. Insulin exerts negative action on α -cells, thus limiting the secretion of glucagon by the α -cells (Zaccardi et al., 2016). Consequently, the lack of insulin leads to higher levels of glucagon, which further increase blood glucose levels (i.e., hepatic

gluconeogenesis). Additionally, gut 'messengers' (i.e., incretins, glucagon-like peptide-1 [GLP-1] and gastric inhibitory polypeptide [GIP] are known to stimulate insulin secretion and GLP-1 can also reduce glucagon secretion (Drucker, 2006).

Eventually, these individuals experience a further reduction in insulin sensitivity that is no longer associated with this compensatory hyperinsulinaemia, resulting in increased blood glucose levels (i.e., hyperglycaemic hyperinsulinaemia) – this is when T2DM is likely to be diagnosed (Zaccardi et al., 2016; Jallut et al., 1990). The body is able prevent T2DM onset by increasing insulin production; however, if IR worsens and β -cells are no longer sufficient in secreting insulin, accompanied by manifestation of overt hyperglycaemia, T2DM develops (International Diabetes Federation, 2021; Zaccardi et al., 2016). The body of literature stipulates a combination of multi-gene predisposition and environmental triggers (i.e., chronic energy excess and inflammation) as primary cause of T2DM (International Diabetes Federation, 2021; Nolan et al., 2011; Zaccardi et al., 2016).

Ahlqvist et al. (2011) concluded that existing genetic markers explain only a modest <15% of T2DM susceptibility (Ahlqvist et al., 2011). Although, the lifetime risk of developing T2DM is 40% for individuals who have one parent with T2DM (with higher risk from the maternal side) and nearly 70% when both parents are affected (Groop et al., 1996). New developments including genome-wide association studies (GWAS) have demonstrated over 40 loci associated with T2DM, of these loci, TCF7L2, which is associated with β -cell dysfunction and increased fasting glucose (+0.023 mmol/L, $p = 0.004$), is the strongest susceptibility locus for T2DM (OR: 1.40, 95% CI: 1.34 , 1.46) (Dupuis et al., 2010).

More recent research focusses on environmental factors and the pathways leading to β -cell 'failure' and T2DM (Zaccardi et al., 2016). The liver and muscles are recognised as major contributors of systemic insulin resistance (DeFronzo, 2004). T2DM has been linked to a Western lifestyle consisting of processed, energy-dense foods (high in sugar and saturated fat) and reduced physical activity (Nolan et al., 2011). A positive energy balance due to excess calorie intake and a lack of physical activity leads to fat accumulation in the subcutaneous tissue initially (DeFronzo, 2004). When this storage capacity is surpassed, fat storage is diverted to 'ectopic' compartments such as the liver, pancreas, muscles – referred to as 'spill-over' (Sattar and Gill, 2014).

Fat accumulation in hepatic and muscle tissues proceeds to impaired insulin mediated glucose uptake due to impairment of insulin signalling, contributing to T2DM onset (DeFronzo, 2004). In perspective, previous studies found ~16-21% increased risk of T2DM associated with the Western diet pattern, whilst light physical activity was significantly associated with ~35% decreased risk (Beigrezaei et al., 2019; Panagiotakos et al., 2005). Moreover, in utero exposures (maternal hyperglycaemia and excessive growth of the foetus) are affecting phenotypic outcomes through modification of the epigenome of the developing foetus, this process is called “epigenetic programming” (Dabelea et al., 2011; Nolan et al., 2011).

Epigenetic alterations in response to overnutrition may lead to permanent alterations or metabolic imprinting in genes involved in the regulation of energy homeostasis (e.g., genes for lipid metabolism, glucose transporters, and endocrine disorders) (Melzner et al., 2002; Yokomori et al., 1999; Stupecka-Ziemilska et al., 2020). A Danish study comparing the offspring of mothers with and without diabetes found that adjusted odds ratios (OR) were elevated for prediabetes or T2DM in the offspring of women with GDM (7.8%, 95% CI: 2.6 , 23.4) or women who had pre-gestational T1DM (4.0%, 95% CI: 1.3 , 12.3) (Clausen et al., 2008). These findings suggest that a pattern of uncommon genetic defects, intrauterine and postnatal environments, or a combination of these factors is important for unravelling the pathogenic mechanism involved in T2DM development. All in all, showing that pathogenesis of T2DM is multifactorial; however, environmental factors play the most prominent role in disease development.

1.1.2.2 Management and treatment of T2DM

Treatment of T2DM is largely structured around dietary modifications and increasing physical activity with focussing on maintaining optimal glycemic control (International Diabetes Federation, 2021). Oral medication can be initiated if lifestyle modifications are not sufficient to control blood glucose levels. Metformin is used as the first-line pharmacological treatment (International Diabetes Federation, 2021). However, if metformin is unable to improve glucose control or when treatment with metformin is not an option, a range and combination of pharmacological treatment options are available (e.g. sulphonylureas, dipeptidyl peptidase 4 inhibitors, GLP-1 agonists and sodium

glucose co-transporter 2 inhibitors). Insulin injections are commenced if non-insulin medications fail to achieve glycaemic control (International Diabetes Federation, 2021). T2DM management and therapy strategies aim to reduce chronic energy excess and enhance energy expenditure via lifestyle modifications and pharmacological treatment (if needed), thereby improving glycaemic control, which protects islet β -cells from progressive failure, improves adipose tissue dysfunction, and restores α -cell function and regulation of hepatic glucose production (Nolan et al., 2011). T2DM diabetes management and treatment is pivotal in achieving optimal glycemic control and reducing risk of complications (International Diabetes Federation, 2019).

Pre-existing T2DM diabetes in pregnancy is a common medical complication and preconception planning is an essential component of care for affected women of childbearing age (Webber et al., 2015; Egan et al., 2015). Despite awareness of preconception planning, there are barriers for women meeting preconception targets (Kendrick, 2004; Khan et al., 2019; Korenbrot et al., 2002; Owens et al., 2006): i) women may be undiagnosed; ii) up to 50% of pregnancies are unintended; iii) reduced contact with the health care system; iv) women and health care providers may be unaware of the existence or the importance of preconception care or it is not seen as a high priority; v) social and economic challenges. All contributing to women not meeting the intended preconception guidelines. Solutions to these barriers have been previously discussed (see '1.1.1.2 Management and Treatment of T1DM').

Once pregnant, structured clinical care in a multidisciplinary team setting is necessary to ensure optimal neonatal outcomes (Webber et al., 2015; Egan et al., 2015). Management strategies for pre-gestational T1DM and T2DM in the UK follow the same structure, which has been described in detail (see '1.1.1.2 Management and Treatment of T1DM'). However, there are some unique differences in monitoring of blood glucose. The NICE guidelines advise that pregnant women with T2DM (on a MDI injection regimen) test their fasting, pre-meal, 1-hr post-meal and bedtime blood glucose levels daily (Webber et al., 2015). When T2DM is managed with (i) diet and exercise changes alone, or (ii) taking oral therapy (with or without diet and exercise changes), or (iii) single-dose intermediate- / long-acting insulin, these women are advised to test their fasting and 1-hr post-meal blood glucose levels daily (Webber et al., 2015). Furthermore,

rtCGM is recommended if they have (i) problematic severe hypoglycaemia (with or without impaired awareness of hypoglycaemia), or if they have (ii) unstable blood glucose levels that are causing concern despite efforts to optimise glycaemic control (Webber et al., 2015). Although significant progress has been made in the management of T2DM in pregnancy, comparably to T1DM in pregnancy these women and their offspring remain at risk of multiple adverse pregnancy complications (Egan et al., 2015). Therefore, future studies should focus on strategies for achieving optimal glycaemic control, thereby advancing current guidelines and management programmes facilitating improved pregnancy outcomes.

1.1.3 Gestational Diabetes Mellitus (GDM)

GDM is a common complication of pregnancy that manifests in advancing gestation. This type of diabetes is defined as hyperglycaemia that is first recognised during pregnancy (Piper et al., 2017; Coustan, 2013). The majority of GDM cases (~80%) present as β -cell dysfunction on a background of chronic IR, to which there is an additive effect of IR transpiring in pregnancy (Buchanan and Xiang, 2005). These women are likely to present an even greater degree of IR than 'healthy' pregnant women, resulting in further reductions in glucose utilisation and increased glucose production (Catalano et al., 1999). The β -cells degrade due to excessive insulin production in response to insulin resistance and excess energy consumption, exhausting the cells over time, closely resembling the pathology of T2DM (see '1.1.3.2 Pathophysiology of GDM' for details) (Plows et al., 2018). GDM is considered as one of the most common pregnancy complications and accounts for 70-90% of all incidences of hyperglycaemia during pregnancy (Johns et al., 2018). Similarly to T1DM and T2DM, GDM is associated with adverse maternal and offspring health risks, including preeclampsia (OR: 1.78, 95% CI: 1.43 , 2.11), caesarean section (OR 1.48, 95% CI: 1.33 , 1.65), LGA (>90th percentile birthweight adjusted for gestational age and sex; OR: 2.19, 95% CI: 1.93 , 2.47), childhood overweight/obesity (OR: 2.09, 95% CI: 1.25 , 3.50) and later-life overt diabetes (abnormal glucose / T2DM; OR: 7.76, 95% CI: 2.58 , 23.39) (Metzer et al., 2008; Clausen et al., 2008). Usually, GDM is associated with no or mild symptoms, such as women being thirstier than normal or having to urinate more regularly (Webber et al., 2015). In the UK, guidelines recommend screening for GDM in women at 24 to 28 weeks' gestation

with any of the following risk factors – BMI >30 kg/m², previous macrosomic baby weighing ≥4.5 kg, previous GDM, family history of diabetes (a first-degree relative with diabetes) or an ethnicity with a high prevalence of diabetes (e.g., Southeast Asian; see ‘Screening and Diagnosis of GDM’ for details) (Webber et al., 2015). If there is a strong suspicion of GDM due adverse symptoms or if women had previous GDM, testing will be offered an earlier point in pregnancy. In summary, GDM occurs when maternal glucose levels are elevated in pregnancy, these women insufficiently or are unable to compensate for changes in glucose metabolism during pregnancy, possibly due to an underlying predisposition.

1.1.3.1 Screening and Diagnosis of GDM

Screening and diagnosis of GDM can be performed in early pregnancy (i.e., first trimester or at the initiation of antenatal care) or later pregnancy (i.e., between 24 and 28 weeks’ gestation) (Johns et al., 2018). Early screening is generally recommended to exclude pre-existing diabetes in women at high risk. Guideline recommendations for at risk individuals differ per guideline bodies, these are most common risk factors: BMI >30 kg/m², hypertension 140/90 mmHg, previous macrosomic baby ≥4.5 kg, previous GDM, family history of diabetes (i.e., first-degree relative), high risk race/ethnicity (e.g., Southeast Asian, Middle-Eastern, and Caribbean) (Johns et al., 2018; Webber et al., 2015). Screening guidelines differ per guideline body, but is performed using an oral glucose tolerance test (OGTT) and can follow a ‘one-step’ (i.e., 2-hr 75g or 100g OGTT) or an alternative ‘two-step’ approach (i.e., glucose challenge test followed by an OGTT) (Johns et al., 2018; Coustan and Carpenter, 1998). Implementation of the ‘one-step’ compared to the ‘two-step’ approach results in higher percentages of women, who receive GDM diagnosis and eventually treatment, though no significant differences were found in clinically important pregnancy outcomes (Hillier et al., 2021; Davis et al., 2021).

Following screening, there is no international consensus on diagnostic criteria for GDM (Piper et al., 2017). The breakthrough Hyperglycaemia and Adverse Pregnancy Outcome study (HAPO) described the risks of adverse pregnancy outcomes associated with maternal hyperglycaemia (Metzer et al., 2008). Based on findings from the HAPO study, the International Association of Diabetes in Pregnancy Study Group (IADPSG) produced new guidelines in 2010 recommending lower FPG thresholds (≥5.1 mmol/L) and higher 2-hr value (≥8.5

mmol/L) (International Association of Diabetes Pregnancy Study Group, 2010). Implementation of the one-step IADPSG criteria, was associated with an increased prevalence of GDM but was found to be both clinically and cost effective compared to the two-step Carpenter-Coustan approach (International Association of Diabetes Pregnancy Study Group, 2010; Piper et al., 2017; Coustan et al., 1998). Implementation of the IADPSG criteria resulted in reduced rates of caesarean section, LGA infants and admission to neonatal intensive care units (NICU). The World Health Organisation (WHO) and ADA both have adopted the IADPSG criteria, though ADA also give the option using the 'two step' approach for diagnosing GDM (Piper et al., 2017; International Association of Diabetes Pregnancy Study Group, 2010; American Diabetes Association, 2017). The WHO proposes an universal screening method using the IADPSG criteria, however; an international consensus may fail to take into account feasibility and applicability in low- and middle-income countries (LMICs) (Piper et al. 2017; International Association of Diabetes Pregnancy Study Group, 2010; Nielson et al., 2012). The following barriers and challenges to screening and diagnosis in LMICs have been observed (Rani and Begum, 2016; Nielson et al., 2012): i) shortage in health care providers (including female care providers) related to patient load and providers with adequate training to provide quality care; ii) the lack of standard protocols for diagnosis and many women are unable to provide the required information, making it complicated to screen based on risk factors; iii) lack of test consumables and equipment for screening and diagnosis (e.g., laboratory equipment, glucose solution, computers and software for record keeping and administration); iv) lack of health financing for screening and treatment; v) lack of functioning referral systems and feedback mechanisms within and between health care facilities; and vi) transportation to the health centre, both in terms of cost and distance.

The NICE diagnostic criteria in the UK differ from those recommended by the WHO and IADPSG but do follow a 'one-step' approach of 75 g OGTT (Webber et al., 2015; International Association of Diabetes Pregnancy Study Group, 2010). NICE guidelines recommend a higher threshold for FPG (≥ 5.6 mmol/L) and a lower 2-hr value (≥ 7.8 mmol/L) (Webber et al., 2015). A study retrospectively applying the NICE and IADPSG diagnostic criteria, suggested that the NICE criteria would have missed only a small number of women with GDM,

otherwise detected by the IADPSG criteria (approximately 0.5% missed) (Piper et al., 2017). However, this group had a higher risk of having an LGA infant and caesarean delivery compared to women with normal glucose tolerance (Piper et al., 2017).

Given the variation in prevalence based on ethnicity, it may be that a single screening threshold is not always the best approach for diagnosis of GDM in each ethnic group – perhaps ethnic-specific glucose screening thresholds should be used (Yogev et al., 2004; Esakoff et al., 2005). For example, if the goal is to finding the best balance between sensitivity and specificity, optimal screening threshold would be 135 mg/dL for African Americans, 140 mg/dL for Whites and Latinas, and 145 mg/dL for Asians (Esakoff et al., 2005). Ethnic-specific standards may be the way forward for improving the high heterogeneity in GDM diagnosis.

1.1.3.2 Pathophysiology of GDM

During pregnancy several changes in glucose regulation occur to facilitate nutrient supply to the growing foetus (Johns et al., 2018). In women with normal glucose tolerance, the pancreatic β -cells adapt to these changes by increasing insulin production, allowing normal blood glucose levels to be maintained (Johns et al., 2018). However, in 80% of the cases, GDM results as an inability to effectively control for the increase in IR, and the remaining 20% is possibly attributable to autoimmune disorders, chemically induced diabetes (e.g., as a result of organ transplant or HIV infection) or pancreatic diseases (Plows et al., 2018).

GDM develops when compensatory insulin secretion is insufficient to meet the level of insulin resistance imposed by the metabolic changes of pregnancy, resulting in glucose intolerance and hyperglycaemia (Moncrieff, 2018). This can be due to deficient ability of pancreatic β -cells to compensate for the enhanced level of IR (Powe et al., 2016). Hyperlipidaemia (elevated blood lipids) is also a characterising factor of GDM (Layton et al., 2019). The foetus cannot synthesise fatty acids, which are required for growth and development, thus is completely reliant on the mother. Lipid metabolites and physiological changes in lipid metabolism have been associated with GDM, although the precise mechanism by which hyperlipidaemia influences GDM is unclear. However, it is suspected to

be dependent on the level insulin resistance and the interactions between lipid subtypes (Layton et al., 2019).

GDM is likely to manifest, due to an underlying metabolic vulnerability that results in an overcompensation or inability to withstand additional metabolic stress imposed by pregnancy (Hunt et al., 2014; Moncrieff, 2018). This underlying vulnerability could be due to a single factor or a combination of genetic and environmental factors, including hormonal, inflammatory, autoimmune, and metabolic processes highlighting the potential for complex mechanistic pathways underlying GDM (Moncrieff, 2018; Johns et al., 2018).

GWAS has identified multiple genes associated with pancreatic β -cell development, function, and survival for both T2DM and GDM (Lauenborg et al., 2009; Johns et al., 2018). Consequently, shared genetic pathways between T2DM and GDM have been suggested. Previous research identified several T2DM risk alleles more frequent in women with a history of GDM (Lauenborg et al., 2009; Johns et al., 2018). Women who carry 15 or more T2DM risk alleles have a more than three-fold increased risk of developing GDM compared with women with >9 risk alleles (Lauenborg et al., 2009; Cauchi et al., 2008). A Danish cohort study examined 11 loci, when adjusted for age and BMI, three alleles were significantly associated GDM risk – TCF7L2 (OR: 1.44, 95% CI 1.19 , 1.74, CDKAL1 (OR: 1.22, 95% CI 1 , 1.49), and TCF2 (OR: 1.22, 95% CI 1.01 , 1.48) (Lauenborg et al., 2009). When one or more of the 11 alleles were present, a cumulative effect for GDM risk was observed (OR: 1.18, 95% CI 1.10 , 1.27 per risk allele) (Lauenborg et al., 2009). Furthermore, epidemiological studies have reported that offspring risk of T2DM and GDM is more commonly associated with maternal than paternal diabetes (Johns et al., 2018). These observations raise the possibility that the intrauterine environment contributes to offspring diabetes risk.

GDM exposes the foetus to a pro-inflammatory environment, including elevated levels of inflammatory cytokines (e.g., interleukin-6 [IL-6] and TNF- α), this intrauterine environmental exposure to a pro-inflammatory environment may impact diabetes risk by influencing the foetal epigenome (Johns et al., 2018). These epigenetic alterations in pregnancy may lead to permanent alterations or metabolic imprinting in genes involved in the regulation of energy homeostasis, including genes for leptin and glucose transporters (Melzner et al., 2002;

Yokomori et al., 1999). Previous work has also demonstrated that offspring born to mothers with GDM are less sensitive to insulin than offspring of mothers with normal glucose tolerance, which may contribute to predisposition for GDM or overt diabetes in offspring (Anand et al., 2017).

The effect of pregnancy on these underlying predispositions increases with advancing gestation (as IR increases) and the degree of the underlying conditions will determine the timing and severity of associated pregnancy outcomes (Moncrieff, 2018). All in all, the onset of GDM is multifaceted with various potential pathways leading to hyperglycaemia. If not managed accurately, the condition will lead to prolonged exposure of both mother and foetus to elevated glucose elevating their risk for future health consequences.

1.1.3.3 Management and treatment of GDM

Treatment of GDM essentially focusses on lifestyle interventions comprising dietary modification, physical activity, and weight management and aims to reverse hyperglycaemia to reduce risk of adverse pregnancy outcomes (Johns et al., 2018). To reduce adverse pregnancy risk blood glucose levels should be tightly controlled (Webber et al., 2015). NICE guidelines recommend a fasting blood glucose of ≤ 5.6 mmol/L, 1-hour postprandial ≤ 7.8 mmol/L and ≤ 6.4 mmol/L 2-hr postprandial (i.e., 75g OGTT) (Webber et al., 2015). Dietary recommendations focus on low carbohydrate/low glycaemic index (GI) diet (Webber et al., 2015; Piper et al., 2017). Evidence propose that lifestyle strategies may be sufficient to achieve blood glucose targets in 70–85% of women; however, if targets are not achieved with lifestyle intervention, the addition of pharmacological therapy is warranted.

In Canada and the US, insulin is recommended as the first-line therapy, whereas oral therapy is the preferred in the UK unless blood glucose levels are significantly elevated (Johns et al., 2018; Webber et al., 2015; American Diabetes Association, 2020b). Insulin is typically administered as multiple daily injections, and oral agent used are metformin and glibenclamide (glyburide in the US and Canada). NICE guidelines recommend using metformin if blood glucose targets do not meet the target ranges within 1–2 weeks of prescribed diet and exercise (Webber et al., 2015). Insulin therapy is initiated if metformin is contraindicated, or if target glucose are not achieved with diet+exercise and/or metformin. In the

UK, treatment with insulin is immediately commenced in women with FPG of ≥ 7.0 mmol/L and should be considered with fasting glucose levels of ≥ 6.0 mmol/L if complications of macrosomia are detected (Webber et al., 2015). Glibenclamide is only recommended if insulin therapy is declined, metformin is unable to achieve target glucose levels or metformin is not tolerated (Webber et al., 2015). Additionally, CGM can be used for women who have problematic hypoglycaemia or who have unstable blood glucose levels, to minimise variability or to gain information about variability in blood glucose levels (Hernandez and Barbour, 2013). Differences in management of GDM in LMICs can have a significant effect on the level of management and quality of treatment. Because lack of resources, trained personnel, and other priorities related to reducing maternal, foetal, and neonatal mortality, providing care to women with GDM is not high on the priority lists in many LMICs (Nielsen et al., 2012; Goldenberg et al., 2016). Furthermore, not only is the cost of medication a barrier but equally the cost of following the recommended diet can be challenging (Nielsen et al., 2012). In some cultures the woman herself does not make the decisions concerning her own health - those decisions are generally made by her husband and/or in-laws (Nielsen et al., 2012). All these factors contribute to the management of GDM in LMICs. Lastly, it is important to note that various pathophysiological mechanisms may be involved in GDM, resulting in differing magnitudes of risk and therefore, potentially, in the need for varying degrees of intervention in different individuals (Powe et al., 2016).

In the 5–10 years following pregnancy, the incidence of T2DM in women with GDM is 70% higher than in the background population (Piper et al., 2017); therefore, screening in the postnatal period is of importance to exclude overt diabetes or impaired glucose tolerance. NICE guidelines recommend testing blood glucose to exclude persisting hyperglycaemia before being transferred to community care – advising that women be offered a fasting glucose test at 6–13 weeks after delivery or HbA_{1c} test after 13 weeks if testing is delayed (Webber et al., 2015).

1.2 Glucose metabolism in pregnancy

1.2.1 Normal glucose tolerance pregnancy

Pregnancy induces a unique state of glucose metabolism resulting in increased IR and decreased insulin sensitivity (Kampmann et al., 2019a; Powe et al., 2019; Salzer et al., 2015b; Catalano et al., 1991). This occurs in both pregnancies with normal glucose tolerance as well as pregnancies affected by diabetes. Increased IR is intended to limit maternal glucose utilisation and to facilitate maternal energy storage for providing an adequate energy supply to the growing foetus – a ~14-fold increase in energy demand is required for foetal growth and development during the second and third trimesters of pregnancy (Kampmann et al., 2019a; Barry and Anthony, 2008).

In pregnancy, maternal tissues become progressively insensitive to insulin, with insulin sensitivity decreasing by 50-60% with advanced gestation (Kampmann et al., 2019a; Powe et al., 2019). Conjointly, insulin secretory response increases two- to three-fold (Powe et al., 2019). Autopsy studies suggest that β -cell mass increases by at least 40% during pregnancy, most likely in response to decreases in insulin sensitivity (Butler et al., 2010; Van Assche et al., 1978). In fact, the predominant theory postulates that the increase in insulin secretory response compensates for the decrease in maternal insulin sensitivity, occurring with advancing gestation (Powe et al., 2019). Powe et al. (2019) reported that the insulin secretory response is augmented in early pregnancy, prior to and independent of any decrease in insulin sensitivity – insulin sensitivity is actually increased in early pregnancy (Powe et al., 2019). The increases in both insulin sensitivity and insulin secretory response may be due to metabolic adaptations for accumulation of adipose tissue, suggesting that that early pregnancy is an anabolic state. The decrease in IR in the first trimester has been attributed to decreased levels of progesterone and thyroid hormones, and an increase in C-peptide and morning sickness could potentially play a role (García-Patterson et al., 2010).

In normal glucose tolerance pregnancies, the change in insulin sensitivity at the end of the first trimester is significantly correlated with maternal BMI ($r = 0.52$) (García-Patterson et al., 2010; Catalano et al., 1998; Catalano et al., 1991). Enhanced insulin sensitivity promotes the storage of energy substrates for later

use in pregnancy, supporting insulin action and shuttling of glucose to the growing foetus (Moncrieff, 2018). However, both insulin secretory response and insulin sensitivity subsequently decrease in late pregnancy (McCance, 2015; Powe et al., 2019). In addition, IR increases with advancing gestation, specifically in the last half of pregnancy (Powe et al., 2019; McCance, 2015; Moncrieff, 2018). In normal glucose tolerance pregnancies, pancreatic islet cells have the ability to adapt to these changes in IR by increased production of insulin, maintaining glucose homeostasis (Salzer et al., 2015b). Consequently, these metabolic alterations in later pregnancy allow for substrates (i.e., amino acids, glucose and lipids) to become available for foetal growth. These metabolic modifications are likely mediated by a combination of maternal adiposity, placental hormones and cytokines, such as leptin and tumour necrosis factor-alpha (TNF- α), although the exact mechanisms are unclear (Powe et al., 2019; Salzer et al., 2015b).

The insulin signalling cascade is of importance in understanding the mechanisms the alterations of glucose metabolism during pregnancy. Firstly, insulin action is initiated by binding to the insulin receptor (Yamashita et al., 2000). The insulin receptor is composed of subunits (i.e., two α -subunits and a β -subunit). Upon binding, insulin causes a conformational change that activates the β -subunit to phosphorylate tyrosine residues and activates the tyrosine kinase activity that leads to increased tyrosine phosphorylation of cellular substrates, causing transmission downstream signals via insulin receptor substrate-1 (IRS-1) and other substrates. IRS-1 and IRS-2 (both common and tissue-specific) may play different roles in insulin signalling cascade. Tyrosine phosphorylation of IRS proteins is crucial for insulin sensitivity. During pregnancy, IRS-1 expression is decreased and IRS-2 expression is increased. Upregulation of IRS-2 may compensate for reduced IRS-1 levels and could preserve pancreatic β -cell function. Furthermore, the binding and activation of phosphatidylinositol-3 (PI 3)-kinase to IRS-1 is important for insulin action in glucose transport. PI 3-kinase activity is linked to insulin-stimulated glucose transport in muscle and fat cells by activating the translocation of glucose transporter type 4 (GLUT4) to the plasma membrane. Importantly, activation of PI 3-kinase by insulin is required for GLUT4 translocation. Glucose uptake by cells occurs through a family of membrane proteins (i.e., GLUT1–GLUT4). The distribution of these glucose transporters is unique per tissue. GLUT4 is insulin-sensitive and cycles between the plasma

membrane and one or more intracellular compartments. Finally, after insulin stimulation, the GLUT4 vesicle exocytosis, resulting in the insulin-stimulated shift of GLUT4 to the cell surface, thereby inducing glucose uptake (Yamashita et al., 2000).

The placenta is another determinant in glucose metabolism during pregnancy, acting as the interface between maternal and foetal environments (Kampmann et al., 2019a). Changes in placental structure and function influence foetal growth and development (Kampmann et al., 2019a). Glucose and other substrates are exchanged between mother and foetus via the placenta, thus playing a pivotal role in foetal development. Placental and reproductive hormones increase with advancing gestation (Yamashita et al., 2000). These hormones are known to induce IR and contribute to altered β -cell function (Yamashita et al., 2000). With advancing gestation, maternal concentrations of cortisol, human placental lactogen (hPL), and prolactin are increased compared to the non-pregnant state (Yamashita et al., 2000; Salzer et al., 2015b). hPL is proposed as one of the hormones primarily responsible for decreased insulin sensitivity with advancing gestation (Salzer et al., 2015b). Moreover, other hormones such as oestrogen, progesterone, prolactin, cortisol, and human placental growth hormone (hPGH) have been postulated as mediators of the change in insulin sensitivity during gestation (Powe et al., 2019; Kampmann et al., 2019a).

Besides the placenta, alterations in cytokine (i.e., leptin, adiponectin, TNF- α and interleukin 6) secretion have been implicated as a potential mechanism involved in the increase of IR during pregnancy (Kampmann et al., 2019a; Powe et al., 2019). IR is known to reduce maternal glucose uptake, the inhibitory effect on glycogen and lipid breakdown (Moncrieff, 2018). This allows mobilisation of glucose and amino acids for placental transfer to the foetus, whilst lipids become the primary fuel for maternal use, as the maternal usage of glucose is decreased (Moncrieff, 2018). TNF- α significantly increases during pregnancy, correlated with the changes in body fat from early to late gestation, and contributing to the decrease in insulin sensitivity in gestation (Yamashita et al., 2000). In pregnancy, TNF- α concentrations are inversely associated with insulin sensitivity (Powe et al., 2019). Insulin signalling defects in skeletal muscle during pregnancy related to TNF- α levels are concurrent with an inflammatory mechanism for decreased

insulin sensitivity (Powe et al., 2019). Furthermore, TNF- α influences IR by increasing phosphorylation of IRS-1 and diminishing insulin receptor tyrosine kinase activity (Kampmann et al., 2019a).

Another cytokine associated is Leptin, this cytokine is secreted by adipocytes in adipose tissue (adipokines) and increased body fat results in elevated concentrations of leptin (Salzer et al., 2015b). Correlations between leptin and serum levels of fasting insulin have been found, making it a marker for insulin resistance. Leptin concentrations are significantly higher during the second and third trimesters in pregnancy compared to the non-pregnant state (Salzer et al., 2015b). The increase in maternal leptin concentration during pregnancy occurs before any significant increases in maternal body fat, thus is most likely secreted from placental sources (Powe et al., 2019). Moreover, Powe et al. (2019) found that maternal leptin levels were associated with maternal insulin secretory response across gestation, independent of both fat mass and insulin sensitivity. In women with normal glucose tolerance, the maternal metabolism changes to overcome decreases in insulin sensitivity and increased in IR by a sufficient rise in insulin production by pancreatic β -cells, these changes in maternal metabolism revert back to non-pregnancy state after delivery (Catalano et al., 1991; Kampmann et al., 2019a). However, in women with diabetes, endogenous insulin secretion is insufficient during pregnancy as result of an underlying predisposition affecting the post-receptor insulin signalling cascade (Powe et al., 2019; Kampmann et al., 2019a).

1.2.2 Diabetes affected pregnancy

Glucose homeostasis in pregnancy can be considered as a continuum, there is a gradual decline from normal glucose tolerance, through altered physiologic state of IR, towards a state of GDM and could end at overt diabetes (Salzer et al., 2015b). In pregnancies complicated by diabetes, insulin production by pancreatic β -cells is insufficient to overcome altered insulin sensitivity and IR (see '1.2.1 Normal Glucose Tolerance Pregnancy' for details) (Kampmann et al., 2019a; Catalano et al., 1991). In this case, IR can become a serious condition with significant implications for adverse pregnancy outcome and long-term morbidity for mother and offspring (Kampmann et al., 2019a). The mechanisms underlying insulin sensitivity and IR during pregnancy are multifaceted involving hormonal, placental, (epi)genetic contributions, and modifications from level of

activity, diet, microbiome and BMI, which other sections of this thesis describe in detail (see '1.1.1.1/ 1.1.2.1/ 1.1.3.2 Pathophysiology of T1DM, T2DM and GDM' for more details). Maternal obesity and diabetes are correlated with placental changes (e.g., increased angiogenesis, increased placental weight, delayed villous maturation, altered specific amino acid transporter proteins), these changes are closely related to the level of glycaemic control in pregnancy (Kampmann et al., 2019a; Huynh et al., 2015). In addition, disrupted glycaemic control may be a consequence of impaired mitochondrial function due to increased oxidative stress induced by pregnancy (Kampmann et al., 2019a; Myatt and Maloyan).

GDM (glucose intolerance evolved during pregnancy) is considered as a consequence of adaptations in the glucose metabolism during gestation; however, decreased insulin-stimulated glucose disposal was found to be abnormal prior to conception and was associated with hyperglycaemia severity during pregnancy (Catalano et al., 1993). Furthermore, a previous study did not observe any differences in the change in insulin sensitivity or insulin secretory response according to GDM status or obesity status across gestation (Powe et al., 2016). These data support the perception that GDM is a pre-existing condition rather than a disease of pregnancy, with symptoms of hyperglycaemia only becoming evident with the increase in IR. However, this is the case for women with known risk factors for GDM. For women without risk factors of GDM, the pathophysiology is uncertain (Powe et al., 2016).

Conjointly, hyperglycaemia severity and the pattern of insulin requirements varies between women with T1DM and T2DM in pregnancy, suggesting a differential effect of pregnancy-mediated IR (Kampmann et al., 2019a). Women with T2DM require a larger increase in insulin dose from the start to the end of each trimester and insulin requirements do not decrease in first and end of third trimester as is the case in T1DM (Padmanabhan et al., 2016; García-Patterson et al., 2010). A potential theory is differences in the decrease of the post-receptor insulin signalling cascade, specifically a decrease in IRS-1 tyrosine phosphorylation, leading to a reduction in the ability to translocate the glucose transporter GLUT 4 and the transportation of glucose into the skeletal muscle cells (Kampmann et al., 2019a). This decrease in tyrosine phosphorylation and

receptor tyrosine kinase activity is seen in both women with normal glucose tolerance and in women with diabetes in pregnancy (Kampmann et al., 2019a).

1.3 Adverse pregnancy outcomes and complications

The literature reports that all types of maternal diabetes (i.e., GDM, and pre-gestational T1DM and T2DM) are associated with adverse pregnancy outcomes; although, complications are more common in women with pre-gestational diabetes (Johns et al., 2018; Malaza et al., 2022; Tinker et al., 2020). These adverse outcomes are closely related to poor glycaemic control including hyper- and hypoglycaemia. Preconception dysglycaemia and longer time of exposure to dysglycaemia in utero is speculated contribute to the complications associated with pre-gestational diabetes, as the first trimester is a critical period for organogenesis (Malaza et al., 2022). However, previous work has also linked GDM to increased risk of adverse pregnancy outcomes (Billionnet et al., 2017). Dysglycaemia, hyperglycaemia particularly, has been documented with range of adverse pregnancy outcomes and complications for the mother and offspring (Johns et al., 2018). Relationships between maternal glycaemia and adverse pregnancy outcomes due to intrauterine dysglycaemia are thought to be linear (Lapolla and Metzger, 2019; Metzger et al., 2008).

Hyperglycaemia (glucose levels above 7.8 mmol/L) plays a pivotal role in the aetiology of complications in diabetic pregnancies (Law et al., 2019; Ceriello and Ilnat, 2010; Tam et al., 2017). Hyperglycaemia leads to β -cell hyperplasia and possibly accelerates the maturation of the β -cell secretion coupling mechanism in the foetus, resulting in increased insulin production by the foetus (Tam et al., 2017; Burlina et al., 2019). In response to hyperglycaemia induced by an excessive mobilisation of glucose across the placenta, foetal insulin acts as a growth hormone promoting foetal growth and adiposity, resulting in macrosomia and LGA. Exposure to intrauterine hyperglycaemia can induce metabolic changes in the offspring leading to DNA methylation and foetal programming, resulting in decreased insulin secretion, increased IR, impaired glucagon suppression, and functional changes in adipose cells (Burlina et al., 2019; Tam et al., 2017). In addition, emerging evidence suggest that rapid glucose swings may play an important role in development of complications, emphasising on the relationship between postprandial hyperglycaemia and other glycaemic excursions (Monnier et al., 2006). Postprandial hyperglycaemia is

shown to induce overproduction of superoxide, thereby activating oxidative stress (Brownlee, 2001; Monnier et al., 2006). Toxic substances are produced and can subsequently lead to endothelial damages, and microvascular and macrovascular complications (Monnier et al., 2006; Brownlee, 2001; Di Flaviani et al., 2011). Moreover, other studies demonstrated that glycaemic variability (GV) during postprandial periods and glucose swings are positively correlated with oxidative stress ($r = 0.86$, $p < .001$), suggesting that interventional trials should target acute glucose swings and aim to minimise GV (Monnier et al., 2006; Monnier et al., 2008).

Striving for euglycaemia possibly increases the risk of hypoglycaemia (glucose levels below 3.9 mmol/L or 3.7 depending on which source is consulted), which could be a barrier for achieving optimal glycaemic control in diabetes affected pregnancies (Nielsen et al., 2008; Shafiee et al., 2012; Law et al., 2019). Hypoglycaemic episodes occur three to five times more frequently in the first trimester compared to third trimester, risk factors for these episodes include hypoglycaemic unawareness, duration of diabetes, and fluctuation in glucose levels (Shafiee et al., 2012). Previous work has demonstrated that lowered maternal glucose leads to increased foetal heart rate accelerations (ter Braak et al., 2002). This higher foetal activity is speculated to reflect increased foetal sympathoadrenal activity, which partly relates to maternal hormonal responses, such as an increase in maternal catecholamines (ter Braak et al., 2002). Several regulatory responses are induced by hypoglycaemia, including a decrease in pancreatic β -cell insulin secretion, and an increase in pancreatic α -cell glucagon secretion, ACTH/glucocorticoids secretion and sympathoadrenal response (Desouza et al., 2010). Furthermore, several indirect changes are induced by hypoglycaemia, including altered inflammatory cytokine secretion and endothelial function (Desouza et al., 2010). Both hyper- and hypoglycaemia can lead to adverse pregnancy outcomes, as described above; these outcomes can be categorised in maternal and offspring complications.

1.3.1 Maternal complications

DIP substantially increases the likelihood of the mother suffering a miscarriage or pregnancy complications, these complications include, increased rate of gestational hypertension, prevalence of pre-eclampsia and rate of caesarean section (Johns et al., 2018; Lapolla et al., 2019; Wendland et al., 2012; McIntyre

et al., 2020). Hypertensive disorders of pregnancy are associated with IR, obesity, and microvascular complications observed in diabetes (Peticca et al., 2009; Johns et al., 2018). The observed risk of developing preeclampsia is 2.5 times higher compared to normal glucose tolerance pregnancies (T1DM; OR: 2.80, 95% CI: 2.20 , 3.50) (Peticca et al., 2009). T2DM and GDM subtypes are quintessentially similar characterized by glucose intolerance, obesity, and insulin resistance, hence the related pathophysiology may explain the comparable increased risk of hypertensive disorders (T2DM; OR: 1.92, 95% CI: 1.32 , 2.70, and GDM; OR: 2.02, 95% CI: 1.77 , 2.31) (Peticca et al., 2009). The risk of caesarean section is increased in for each type of maternal diabetes (T1DM; OR: 3.75, 95% CI: 2.98 , 4.71, T2DM; OR: 1.93, 95%CI: 1.62 , 2.28, and GDM; OR: 1.22, 95% CI: 1.18 , 1.26) (Gualdani et al., 2021).

Not only during the pre- and postnatal period women with DIP are at risk, but also later in life morbidity has been implicated (**Table 1.1**). A woman with GDM is almost 2.5 times more likely to develop CVD in the decade following her diagnosis (Kramer et al., 2019). Similarly, T1DM and T2DM are associated with increased CVD risk (T1DM; myocardial infarction hazard ratio [HR]: 3.26, 95% CI: 2.47 , 4.30; heart failure HR: 2.68; 95% CI: 1.76 , 4.09; ischemic stroke HR 2.61; 95% CI: 1.80 , 3.79 and T2DM; myocardial infarction HR: 1.65, 95% CI: 1.48 , 1.83; heart failure HR: 1.69; 95% CI: 1.50 , 1.90; ischemic stroke HR 1.68; 95% CI: 1.49 , 1.89) (Larsson et al., 2018). In addition to these physiological ramifications, the quality of life and mental health of mothers with DIP may be impacted, with an increased risk of postpartum depression (McIntyre et al., 2019; Kunasegaran et al., 2021). Compared to women with normal glucose tolerance, postpartum depression was more common in women with diabetes (GDM or pre-gestational diabetes; 34.8 vs 16.7%, respectively) (Miller et al., 2016). Recurrence of GDM in a subsequent pregnancy was estimated to be 48% in a meta-analysis and more likely in non White European ethnic groups (e.g., Asian, Hispanic, and African American) and multiple pregnancy (Schwartz et al., 2015). Moreover, a systematic review and meta-analysis has reported a 7-fold risk of T2DM in women following GDM and an incidence of 50% or higher in the 5–10 years following the index pregnancy compared to women with normal glucose tolerance in pregnancy (Bellamy et al., 2009). Higher fasting glucose levels on OGTT has been implicated as the risk factor most closely associated with risk of

progression of GDM to T2DM, although a risk threshold has not yet been identified (Johns et al., 2018). Developing T2DM at a younger age will result in life-long health and quality of life consequences (Johns et al., 2018). Together, these results show strikingly repercussions for women with maternal diabetes, thus research and interventions are warranted for improving quality of life and pregnancy outcomes.

Table 1.1: Risks of adverse maternal pregnancy complications in DIP.

	Type 1 Diabetes	Type 2 Diabetes	Gestational Diabetes
Early-term outcomes			
Pre-eclampsia	Medium/high	Medium	Medium/high
Cesarean section	High	Medium/high	Medium
Later-term outcomes			
Cardiovascular disease	Medium/high	Medium/high	Medium
Abnormal glucose (e.g., T2DM)	NA	NA	Medium/high
Recurrence of GDM	NA	NA	High
Postnatal depression	Medium/high	Medium/high	Medium/high

Risk was interpreted from frequencies and OR of several studies and systematic reviews / meta-analyses (Johns et al., 2018; McIntyre et al., 2020; Peticca et al. 2009; Gualdani et al., 2021; Kramer et al., 2019; Larsson et al., 2018; McIntyre et al. 2019; Miller et al. 2016; Schwartz et al., 2015; Bellamy et al. 2009). NA: Not Applicable. Colours indicate severity of risk (orange = medium, light red = medium/high and dark red = high).

1.3.2 Offspring complications

Offspring born to mothers with DIP are at increased risk of multiple neonatal complications, as result of exposure to intrauterine dysglycaemia (**Table 1.2**). Known complications can be categorised as early- (e.g., prematurity, increased birth weight or size [macrosomia and LGA], birth injury, neonatal hypoglycaemia, and NICU admission) and long-term outcomes (e.g., overweight/obesity, diabetes, and cardiovascular disease) (Johns et al., 2018; Metzger et al., 2008; Wendland et al., 2012; Eletri and Mitanchez, 2022). The excess risk of

prematurity (childbirth < 37 gestational weeks) is a common characteristic for pre-gestational diabetes and GDM, with highest rates among pregnancies with pre-gestational T1DM (OR: 8.86, 95% CI: 7.18, 10.94) compared to pre-gestational T2DM and GDM (OR: 2.60, 95% CI: 2.15 , 3.14 and OR: 1.26, 95%: 1.21 , 1.32, respectively) (Gualdani et al., 2021). The reported risk of LGA is markedly higher in pre-gestational T1DM (OR: 4.75, 95% CI 3.48 , 6.49), and less in pre-gestational T2DM or GDM (OR: 2.07, 95% CI: 1.60 , 2.66 and OR: 1.73, 95% CI: 1.54 , 1.94, respectively) (Gualdani et al., 2021; Shen et al., 2020). Long-term exposure to dysglycaemia could also lead to increased risk of perinatal mortality (relative risk [RR] = 1.55; 95% CI 0.88 , 2.73; $p = 0.13$) (Wendland et al., 2012; Johns et al., 2018). Moreover, a large body of evidence supports an association between dysglycaemia in early pregnancy and increased risk of congenital malformations, due to the impact of hyperglycaemia on embryogenesis during the first eight weeks of gestation (Johns et al., 2018; Gualdani et al., 2021; Shen et al., 2020; Malaza et al., 2022).

Studies in later-life of DIP offspring, comparing DIP with normal glucose tolerance, have shown evidence of increased adiposity (e.g., overweight and obesity), IR, systolic blood pressure, and risk of circulatory disease in childhood (Johns et al., 2018; Krishnaveni et al., 2010). A Danish cohort study found a two-fold increased risk of childhood obesity in offspring from pre-gestational T1DM and GDM pregnancies (T1DM; OR: 1.79, 95% CI: 1.00 , 3.24 and GDM; OR: 2.27, 95% CI: 1.30 , 3.98, respectively) (Clausen et al., 2009). Furthermore, a systematic review and meta-analysis by Kawasaki et al. (2018) found 2-hr post-glucose challenge levels in early adulthood were also higher in GDM offspring (mean difference = 0.43mmol/L, 95% CI 0.18 , 0.69) (Kawasaki et al., 2018). Additionally, cardiovascular disease (CVD) risk is increased in offspring of maternal diabetes (RR = 1.19, 95% CI 1.07 , 1.32) (Yu et al., 2019c). Likewise, a systematic review reported offspring to have a significantly higher systolic blood pressure (mean difference = 1.75 mmHg, 95% CI 0.57 , 2.94) (Pathirana et al., 2020). Maternal hyperglycaemia can lead to overnutrition of the foetus and subsequently an increased risk of metabolic disease later in life (Kampmann et al., 2019a). The intrauterine exposure to hyperglycaemia is believed to affect the offspring sex-specifically, with females being more prone to the effects (Kampmann et al., 2019a). The body of evidence demonstrates that adult

offspring of maternal diabetes have reduced insulin sensitivity and increased risk of prediabetes/T2DM, metabolic syndrome, and higher BMI compared with the background population (Johns et al., 2018; Kelstrup et al., 2013). Epigenetic modifications have been proposed as a possible mechanism of early life exposures resulting in long-term effects (Kampmann et al., 2019a). Intrauterine modifications create an epigenetic memory, thus programming the offspring's later life phenotype. Epigenetic changes have been observed as a consequence of GDM and maternal T2DM, thereby creating a link between early life exposure and later life metabolic disease, resulting in an inter-generational disease cycle (Kampmann et al., 2019a). The large body of evidence reports there are significant associations between DIP and adverse pregnancy outcomes for both mother and child; however, pre-gestational diabetes seems to be more frequently associated with adverse outcomes, but conflicting results have been reported. Thus, a consensus of differences between maternal diabetes type and associated adverse outcomes is lacking.

Table 1.2: Risks of adverse pregnancy complications in DIP offspring.

	Type 1 Diabetes	Type 2 Diabetes	Gestational Diabetes
Early-term outcomes			
Prematurity	High	High	Medium
Birth weight/size (e.g., LGA & macrosomia)	High	Medium/high	Medium/high
Congenital anomalies	Medium	Medium	Medium
Neonatal hypoglycaemia	High	High	Medium/low
NICU admission	High	Medium	Medium
Long-term outcomes			
Obesity/overweight	Medium	High	Medium/high
Abnormal glucose (e.g., T2DM)	Medium	High	High
Type 1 Diabetes	High	Low	Medium/low
Metabolic syndrome	Medium	High	High
Cardiovascular disease (including risk factors)	Medium	Medium/high	Medium

Risk was interpreted from frequencies and OR of several studies and systematic reviews / meta-analyses (Johns et al., 2018; Wendland et al., 2012; Shen et al., 2020; Eletri and Mitanchez, 2022; Gualdani et al., 2021; Clausen et al., 2009; Kawasaki et al., 2019; Pathirana et al. 2020, Kelstrup et al. 2019). NA: Not Applicable. Colours indicate severity of risk (green = low, light orange = medium/low, orange = medium, light red = medium/high and dark red = high).

1.4 Drivers of (postprandial) glucose control

A multitude of person-specific variables have been postulated to contribute to individual glycaemic control (including meal-time glucose responses), these include; genetics (Zeevi et al., 2015a), metabolome (Hall et al., 2018), gut microbiota (Korem et al., 2017), ethnicity (Dickinson et al., 2002) and lifestyle (Dunstan et al., 2012). Poor (mealtime) glucose control is a clinically relevant

problem, increasing the burden on healthcare and the risk of diabetes complications. Previous research has demonstrated that glycaemic control, specifically postprandial glucose response (PPGR), can be person-specific (Zeevi et al., 2015b; Mendes-Soares et al., 2019b; Mendes-Soares et al., 2019c). Data obtained by Zeevi et al. have examined associations of PPGR with known risk factors, and demonstrated PPGR was significantly correlated with several of these known risk factors including BMI ($r = 0.24$, $p < 0.0001$), HbA_{1c}% ($r = 0.49$, $p < 0.0001$), wakeup glucose ($r = 0.47$, $p < 0.0001$), and age ($r = 0.42$, $p < 0.0001$) (Zeevi et al., 2015a). Moreover, assessing PPGR by our research group showed that higher PPGR in individuals with T1DM is positively associated with PPGR variability ($r = 0.70$, $p < 0.001$), BMI ($r = 0.32$, $p < 0.001$), age ($r = 0.28$, $p < 0.001$), and HbA_{1c} ($r = 0.22$, $p = 0.014$) (Dingena et al., 2020). In summary, the body of literature ascribes various number of parameters driving glycaemic control, which will be discussed in more detail below (**Figure 1.1**).

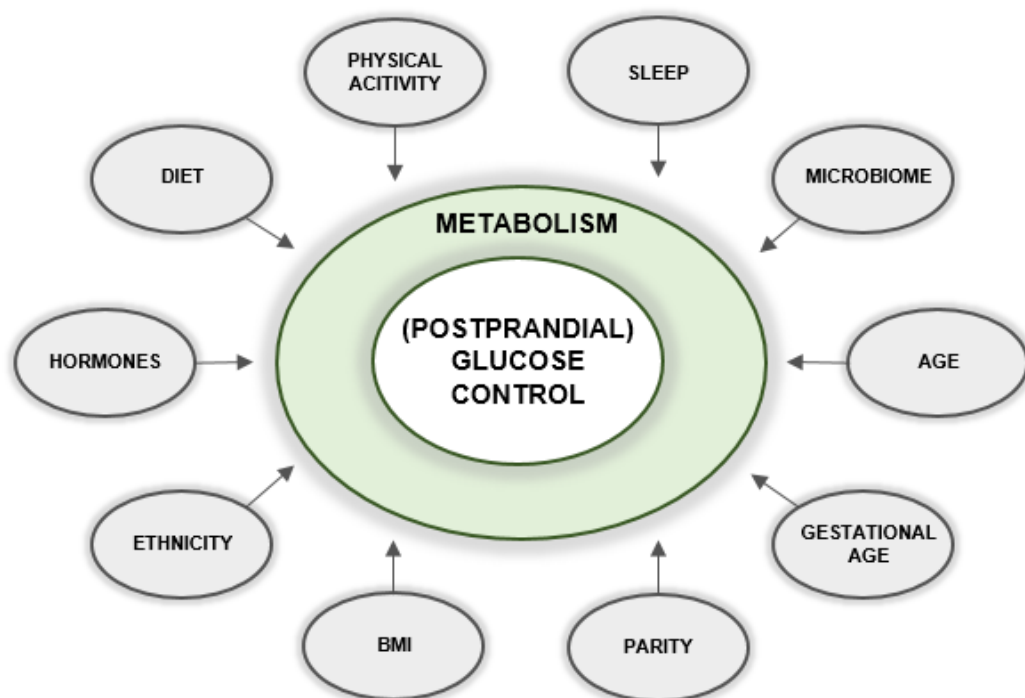


Figure 1.1: Schematic overview of drivers of (postprandial) glucose control in DIP. This scheme provides an overview of potential and known mediators of glycaemic control in maternal diabetes, mainly GDM, as research in pre-gestational T1DM and T2DM is lacking.

1.4.1 Diet

Over the past three decades, research has demonstrated that foods with the same quantity of carbohydrate can elicit widely different glucose responses depending on the form of carbohydrate, other nutrients, and the structure of the food (Jenkins et al., 1981; Vega-López et al., 2007). Jenkins et al. reported inequality in the extent to which different carbohydrate sources raise the blood glucose and indicate that simple carbohydrate exchanges based on chemical analysis do not predict the physiological response (Jenkins et al., 1981). To capture this information a system to classify foods based on glycaemic response has been developed to capture this information, named the glycaemic index (Jenkins et al., 1981). The GI value of a food is determined by monitoring the incremental area under the curve (iAUC) for blood glucose response for 2-hrs, after feeding a 50g carbohydrate portion of both a test food and standard food, and expressing the data as a percentage of the test food relative to the standard food (Jenkins et al., 1984; Jenkins et al., 1981). Implicit to this method is the assumption that the glycaemic response of an individual to a portion of food is similar between individuals regardless of metabolic and physiological factors (Vega-López et al., 2007). GI has been endorsed by organisations and research groups for use as a tool to guide food choices to improve glycaemic control, and reduce chronic disease risk, but not by all (Ludwig, 2002; Nishida and Nocito, 2007; American Diabetes Association, 2004; Matthan et al., 2016). Reluctance to universally recommend the use of GI is driven by uncertainties relating to reproducibility among people and variability in the composition and preparation of individual foods. Additionally, GI does not provide information on the prolonged effect on glycaemia of the food item. Therefore, a separate measure named the glycaemic load (GL) was developed, this method adjusts GI for serving size, thereby providing a more accurate picture of a food item impact on postprandial glycaemia (Vlachos et al., 2020). Methods which aim to estimate PPGR (i.e., GI and GL) have demonstrated limited applicability in assessing PPGR to meals consumed in real-life (145, 147, 151). Corroborated by Matthan et al. (2016), who demonstrated substantial variability in responses to GI/GL value determinations (151).

Despite current practice recommending reducing carbohydrate intake, the use of meal carbohydrate content is a deficient predictor of PPGR and there is

limited data regarding the optimal diet for achieving euglycaemia (Jenkins et al., 1984; Jenkins et al., 1981; Vega-López et al., 2007; Webber et al., 2015). For example, a pilot study randomised 12 diet-controlled overweight/obese women with GDM into a higher-complex carbohydrate/lower-fat (CHOICE) diet with 60% carbohydrate, 25% fat, 15% protein or an isocaloric conventional low-carbohydrate/higher-fat diet with 40% carbohydrate, 45% fat, 15% protein (Piper et al., 2017). This study demonstrated that women on the CHOICE diet for 7 weeks had decreased FPG and free fatty acids with improved IR compared to women on the conventional low-carbohydrate diet. Furthermore, Meng et al. (2017) examined the influence of macronutrient preload on mealtime glucose responses and reported that protein preload attenuated the subsequent rise in the PPGR and resulted in glucose iAUC values that were lower than those after carbohydrate or fat preload (Meng et al., 2017b). This reduction of PPGR is possibly the result of protein reducing the rate of gastric emptying and enhancing the insulin response. Other studies have postulated that dietary fibre can increase gastrointestinal content viscosity, to decrease the gastric-emptying rate, and to slow glucose-absorption rates (Meng et al., 2017a).

Additionally, the Mediterranean diet has been the focus of numerous studies, as it has the potential to improve maternal and offspring outcomes by preventing GDM, preeclampsia, and foetal growth restriction (Timmermans et al., 2012; Al-Wattar et al., 2019; de la Torre et al., 2017). A Mediterranean-style diet is rich in mono- and polyunsaturated fatty acids; key components of this diet include high intake of nuts, extra virgin olive oil, fruit, vegetables, non-refined grains, and legumes (Salas-Salvado et al., 2019). A study by Al-Wattar et al. (2019) concluded that a Mediterranean-style diet in pregnancy did not reduce the overall risk of adverse pregnancy outcomes but has the potential to reduce gestational weight gain and the risk of GDM. De la Torre et al. (2017) conducted a prospective, randomized controlled trial to evaluate the incidence of GDM with two different dietary models; a Mediterranean diet supplemented with extra virgin olive oil and pistachios or standard diet with limited fat intake. This study reported that early nutritional intervention reduced the risk of GDM (RR: 0.75; 95% CI: 0.57–0.98; $p = 0.039$) and reduced rates of insulin-treated GDM, prematurity, gestational weight gain, emergency caesarean section, perinatal trauma, and SGA and LGA newborns (all $p < 0.05$) (de la Torre et al., 2017).

Foods are rarely eaten alone, and the macronutrient contents of meals and snacks can differ considerably throughout the day, resulting in differing nutrient preloads and glucose responses, making generalisability and interpretation of diet-based intervention strategies complex. Thus far, no clear dietary recommendations have emerged to improve pregnancy outcomes for women with metabolic risk factors. The differences in interventions and outcome reporting, coupled with the limited availability of strong evidence regarding the effectiveness of the diet can account for this phenomenon (Al-Wattar et al., 2019). Implementation of dietary interventions in practice requires clear definitions of dietary components accompanied by clear guidance to improve adherence to the diet (Al-Wattar et al., 2019). Particularly challenging is to evaluate diet interventions in multi-ethnic pregnant populations comprising women from varied ethnic and socioeconomic backgrounds. All in all, many individuals with diabetes struggle to achieve optimal mealtime glucose control. This is because real-life meals consist of mixed-macronutrients, arbitrary food combinations, varying quantities consumed at different times of the day and may be influenced by the proximity of previous physical activity and meals, factors which current guidelines do not account for (Meng et al., 2017b; Meng et al., 2017a; Matthan et al., 2016; Vega-López et al., 2007).

To assess diet and glucose control associations in DIP, metabolites offer a novel approach to untangle this association and better understand the role of diet on glucose metabolism in pregnancy. All metabolites are part of the metabolome, this is the complete set of metabolites formed by the cell in association with its metabolism (Nielsen and Oliver, 2005). The metabolome provides insights of the biological molecules in the individual at the time of sample collection and into the biological mechanisms of metabolic diseases (Wang et al., 2021). Furthermore, the metabolome is influenced by various factors (i.e., diet, medication, microbiome, environmental exposures, and an individual's health status), besides it reflects both genetic and environmental factors making it an interesting and valuable component for investigating the relationship between diet and disease progression (McIntyre et al., 2020; Li et al., 2020). As a result, metabolites are ideal components to study metabolic dysregulation and glycaemic control in DIP. Indeed, diabetes in pregnancy study groups have called

for more research regarding the metabolome and DIP (Wang et al., 2021; Schaefer-Graf et al., 2018).

Dietary parameters explain almost 50% of the observed variation in serum metabolite levels, making it more influential than other personal parameters, such as age, sex, the microbiome, and other lifestyle factors (e.g., exercise and sleep) (Bar et al., 2020). In fact, various metabolites (e.g., lipoproteins, amino acids, pyruvate, and carbohydrates) have been associated with glycaemic control and GDM, albeit with only few reproducible results (Wang et al., 2021). Even though, there is a body of evidence regarding the role of dietary metabolites in glucose metabolism and GDM development; (i) carbohydrates (e.g. hexose and tricarboxylic acid metabolites) increased in early pregnancy and post-partum are linked to the dysregulation glycolysis and promotion of lipid synthesis, (ii) amino acids (e.g., branched chain amino acids [BCAAs]) increased in early pregnancy are linked to increased IR and inhibit β -cell function, and (iii) lipids (e.g., fatty acid metabolites) strongest observation during the third trimester are linked to increased hyperglycaemia, insulin sensitivity, inflammation and oxidative stress (Vladu et al., 2022; Li et al., 2020; Wang et al., 2021; Zhao et al., 2019; Hosseinkhani et al., 2021). Therefore, the metabolome offers a unique opportunity to investigate the heterogeneity of effect of diet and these other drivers on glucose control and DIP, but also potentially providing opportunities for prevention of GDM and management of pre-gestational diabetes by aiding the development of tailored dietary interventions.

Nutrients, foods and dietary patterns are all drivers of PPGR and glycaemic control; however, previous evidence has identified other factors beyond the characteristics of food as contributors to maintaining glucose control in normal range, such as placental hormones and genetics (Kampmann et al., 2019a; Powe et al., 2019), metabolic parameters (Zeevi et al., 2015a), gut microbiota (Zeevi et al., 2015a; Korem et al., 2017), and lifestyle (Dunstan et al., 2012). Unfortunately, evidence in pre-gestational diabetes for most of these parameters is deficient. Therefore, future studies should not only focus on GDM pregnancies but also include pre-gestational T1DM and T2DM. Ultimately, identifying these mediators could have novel implications for management and treatment of DIP.

1.4.2 Physical activity and exercise

Past research has established that exercise or physical activity has significant influence on glycaemic control, for instance via stimulating the glucose transporters onto the surface of skeletal muscle cells, thereby improving glucose uptake and reducing IR (Kampmann et al., 2019a; King et al., 1988; Richter and Hargreaves, 2013). Recently, a systematic review and meta-analysis (SRMA) demonstrated reduced blood glucose concentrations in women with and without diabetes in pregnancy after acute exercise (i.e., a single exercise session; $n = 26$; MD -1.42 mmol/L, 95% CI -1.69 , -1.16 , $I^2 = 8\%$ and $n = 285$; MD -0.46 mmol/L, 95% CI -0.60 , -0.32 , $I^2 = 62\%$, respectively) (Davenport et al., 2018). For chronic exercise interventions (i.e., usual activity), FPG was reduced only in women with diabetes ($n = 70$; MD -2.76 mmol/L, 95% CI -3.18 , -2.34 , $I^2 = 52\%$). In addition, a recent RCT demonstrated a reduced incidence of GDM in the exercise group (i.e., stationary cycling for 30 minutes three times per week) compared to the control (22.0% in the exercise group and 40.6% in the control group; OR: 0.412, 95% CI: 0.24 , 0.71), this represents a clinically important reduction of 45.8% in the incidence of GDM (Wang et al., 2017). Furthermore, evidence shows that moderate-to-vigorous physical activity (measured via an accelerometer) was associated with an improved insulin sensitivity (Matsuda index: 3.54, 95% CI: 0.46 , 6.62) and insulin response in overweight and obese pregnant women (first-phase insulin response: -879.8 , 95% CI: -1466.5 , -293.1 and second-phase insulin response: -211.4 , 95% CI: -369.1 , -53.8) (van Poppel et al., 2013). In general, the level of physical activity (i.e., energy expenditure), has been associated with a decreased risk in development of T2DM in women (age 34-59 years) (Manson et al., 1991). Thus, physical activity, as in energy expenditure or exercise bouts, can be considered as a key strategy to manage glycaemia in DIP and long-term health.

1.4.3 Sleep

More recently, reduced sleep duration has gained significant attention for its possible contribution to metabolic dysfunction. Currently, it is recognised that sleep reduction can adversely impact glycaemic control (O’Keeffe and St-Onge, 2013; Spiegel et al., 1999). During pregnancy, sleep architecture is altered and trimester-specific, in the second trimester less time is spent in rapid eye movement sleep (Lee, 1998; Lee et al., 2000). Shorter sleep duration is

associated with higher fasting glucose levels, elevated post-breakfast glucose, and increased levels of cortisol and inflammation (e.g. IL-6, TNF- α and C-reactive protein), the latter are known to be associated with decreased glucose tolerance and insulin sensitivity (Andrews and Walker, 1999; Spiegel et al., 1999). The reduction in glucose tolerance that accompanies all pregnancies may result in the childbearing mother being more susceptible to the adverse effects of disturbed sleep on glucose metabolism, thus future work focussing on investing and promoting sleep quality to improve glycaemic control in DIP is warranted.

1.4.4 Personal and physiological parameters

Several personal and physical parameters have been implicated as modifiers of glucose control. Ethnicity has been associated with onset of diabetes and glucose intolerance severity (Johns et al., 2018; McIntyre et al., 2019; Dickinson et al., 2002). For example, previous research has reported that Southeast Asians and Chinese have the highest postprandial dysglycaemia (iAUC_{glucose} 100% and 50% higher, respectively) and lowest insulin sensitivity compared to White Europeans (HOMA-IR: 21.1 \pm 2.1 and 13.1 \pm 0.8 mmol/L·pmol/L, respectively), these are known to be the most insulin sensitive and carbohydrate tolerant (Dickinson et al., 2002). Furthermore, obesity, multiple pregnancies, advanced maternal age are all associated with increased risk of hyperglycaemia (Negrato et al., 2022). The HAPO study confirmed maternal age, BMI, gestational age, and parity to be confounders of HbA_{1c}, thus showing associations with glucose control (Lapolla et al., 2019). Women of advanced maternal age (AMA, \geq 35 years) are more likely to have pre-existing T2DM in pregnancy because glucose intolerance increases with age (Waites et al., 2022). A recent retrospective study, reported an increase in diabetes from 5.5% of pregnancies at 20–34 years to 11.5% and 15.4% at 35–39 years and at 40 years and over, respectively (Mills and Lavender, 2014). Furthermore, Koren et al. (2012) demonstrated a significant change in the gut microbiota with loss of bacterial richness and an increase in the beta-diversity in pregnant women from early to late pregnancy; thus, the gut microbiota may contribute to maternal metabolic changes (Koren et al., 2012). Likewise, a Danish study, investigating gut microbiota profiles in GDM and women with normal glucose tolerance pregnancy, found that GDM was associated with an altered gut microbiota in third trimester compared to pregnant women with normal glucose tolerance (Crusell et al., 2018). Altogether, these studies reveal the complexity of

maternal glycaemic control and that numerous personal factors are associated with (postprandial) glycaemic control.

1.5 Monitoring glucose control

Optimal glycaemic control in the first and second trimester is imperative for the prevention of adverse pregnancy outcomes (Schaefer-Graf et al., 2018; Hunt et al., 2014). HbA_{1c} is the widely accepted hallmark measure of glucose control, as it reflects time-averaged glucose levels over a previous 8-12-week period and is derived from a composite of fasting and mealtime (postprandial) glucose responses (Jeffcoate, 2004). Recommendations state to aim for HbA_{1c} levels of <6.5% (ideally, below 6.1%) (Webber et al., 2015). Results from a UK cohort showed that <10% of UK women with diabetes had HbA_{1c} <6.1% at the first antenatal visit, and 22% of women had HbA_{1c} levels of ≥7% at 34 weeks' gestation (McCance, 2015). These results show that dysglycaemia is persistent in diabetic pregnancies and there is scope for improvement. Moreover, previous studies have demonstrated a linear trend between increasing second and third trimester HbA_{1c} values, independently of first trimester HbA_{1c}, and adverse pregnancy outcomes (McCance, 2015; Kampmann et al., 2019a; Jeffcoate, 2004).

Women with DIP are advised to self-monitor their blood glucose levels throughout pregnancy, examples of self-monitoring techniques are self-monitoring of blood glucose (SMBG) and continuous glucose monitoring (CGM) (Webber et al., 2015; Jones et al., 2019). For the SMBG method, a glucose meter is used to measure capillary glucose at multiple times during the day (usually around meal times). An important limitation of this method is that it provides only a single value and does not allow for continuous, longitudinal monitoring; hence, hypo- and hyperglycaemic events may go undetected (Jones et al., 2019; Yogeve et al., 2003).

To counter this issue, a new technology has been developed, namely CGM. CGM uses a device, placed on the upper arm of the individual, measuring interstitial glucose levels in subcutaneous tissue (Feig and Bonomo, 2020). Glucose values obtained with CGM have been shown to correlate with laboratory measurements of plasma glucose levels and with glucose meter values measured at home (Jones et al., 2019). CGM could be used to guide more

precise therapeutic approaches. This method provides detailed data on the direction and rate of change of glucose levels, measuring interstitial glucose levels up to 288 times per day (Jones et al., 2019; Feig et al., 2020; Law et al., 2015). Thereby, assessing the dynamic glucose signals of daily life.

There are three types of CGM, “professional” (pCGM), “real-time” (rtCGM), and “flash” glucose monitoring (isCGM) (Feig et al., 2020). pCGM is often used as a research, diagnostic, and educational tool, as it uses blinded measurements only retrospectively available. rtCGM is an actual therapeutic tool, dedicated to optimising self-management of therapy measuring glucose in real-time, while using alarms when glucose concentrations are out of range, this is the most commonly used CGM tool in the UK (Webber et al., 2015). isCGM measures the interstitial glucose concentration semi-continuously, glucose values and trends are not automatically available to the patient, but can be accessed in real-time with scanning of the sensor, using a specific reader or smartphone, plus a retrospective analysis of the results is available (Feig et al., 2020).

Murphy and colleagues (2007) were first to document the changes in glycaemic patterns throughout pregnancy using CGM in women with pre-existing diabetes (Murphy et al., 2007). This study demonstrated clear differences between the level of glycaemic control achieved by women with T1DM and T2DM, which were not apparent from mean blood glucose or HbA_{1c} measurements (59). Women with T2DM spent only two-thirds of the amount of time hyperglycaemic compared to T1DM (ratio of proportion of time with blood glucose level >140 mg/dl, 0.69 [95% CI: 0.53 , 0.89]) (Murphy et al., 2007). Another study comparing SMBG with CGM found that CGM detected substantial episodes of hyperglycaemia (>3 hours per day) and overnight hypoglycaemia missed by conventional glucose monitoring (Yogev et al., 2003). In a UK trial of pregnant women with T1DM and T2DM, the use of the CGM was associated with both reduced HbA_{1c} (by 0.6%) and reduced risk of macrosomia (OR: 0.36; 95% CI: 0.13 , 0.98) compared to SMBG (Murphy et al., 2008). Furthermore, a multicentre English and Danish research group (2015) explored the CGM results of pregnant women with a new statistical technique called functional data analysis, they investigated the association between distinct temporal glycaemic patterns and the occurrence of LGA infants in diabetes affected pregnancies (Law et al., 2015). The results showed that LGA was associated with lower mean

glucose in the first trimester (7.0 vs. 7.1 mmol/L; $P < 0.01$), yet higher mean glucose in the second and third trimester (7.0 vs. 6.7 mmol/L; $P < 0.001$ and 6.5 vs. 6.4 mmol/L; $P < 0.01$, respectively). Furthermore, glucose was significantly lower midmorning (from 09:00 to 11:00h) and early evening (from 19:00 to 21:30h) in the first trimester, and significantly higher during the evening (from 20:30 to 23:30h) in the third trimester in women with LGA infants (Law et al., 2015). Together, these studies show that CGM is a promising and validated technique to aid in the management of glycaemic control and to improve neonatal health outcomes attributed to the exposure of maternal dysglycaemia. However, further research is warranted to identify distinct glycaemic profiles in diabetes during pregnancy and improve maternal glucose control.

The Advanced Technologies & Treatments for Diabetes panel have written an international consensus statement on recommendations for standard reporting of CGM data (Danne et al., 2017). Fourteen key metrics should be utilised to assess and document glycaemic control, these include mean glucose, percentage of time spent in range (3.9 – 7.8 mmol/L), hypoglycaemia (<3.9mmol/L) and hyperglycaemia (>7.8mmol/L), HbA_{1c}, AUC_{glucose}, and glycaemic variability (**Figure 1.2**). Glycaemic variability (GV) can be considered a third component of dysglycaemia, this measure has been reported to be a novel parameter besides hypo- and hyperglycaemia (Monnier et al., 2008). To assess the GV, the standard deviation (SD) and the coefficient of variation (CV) are considered the “gold standard” metrics (Danne et al., 2017). Another method estimating the GV is mean amplitude of glycaemic excursions (MAGE) (**Figure 1.2**). MAGE is the arithmetic average height of glucose excursions that exceed the standard deviation for a 24-hr period (Monnier et al., 2008; Ceriello et al., 2010).

GV is implicated to have more detrimental effects than sustained hyperglycaemia in the development of diabetes complications as both postprandial glucose peaks and interprandial glucose nadirs can activate oxidative stress (Monnier et al., 2008; Ceriello et al., 2010). For example, the urinary excretion rate of 8-iso-PGF₂ α , which is a reliable marker of oxidative stress, was found to be strongly and positively correlated ($r = 0.86$, $p < 0.001$) with glycaemic variability estimated using CGM (Monnier et al., 2008). Studies have shown that PPG values appear the most effective for the determination of the

likelihood of adverse pregnancy outcomes, e.g. FPG levels only explain 12% of the variation of birth weight, and PPG approximately 40% (Ben-Haroush et al., 2004; Dalfra et al., 2011). Acute glucose fluctuations including PPG excursions from peaks (highest values) to nadirs (lowest values) can be described by two components, the duration of excursions, and the magnitude of rise (Monnier et al., 2008). The duration of PPG increment relates to sustained hyperglycaemia, while the magnitude reflects GV. To assess the entire PPGR phenomenon, PPG area under curve above the pre-prandial glucose value can be utilised, this is referred to as the incremental AUC_{glucose} ($iAUC_{\text{glucose}}$) (Cheng et al., 2018). Concluding, glycaemia can be measured in numerous ways all having relevant implications for DIP outcomes. Future work is needed to distinguish the most important variables for measuring glycaemic control and associations of pregnancy outcomes; however, remaining variables should not be disregarded.

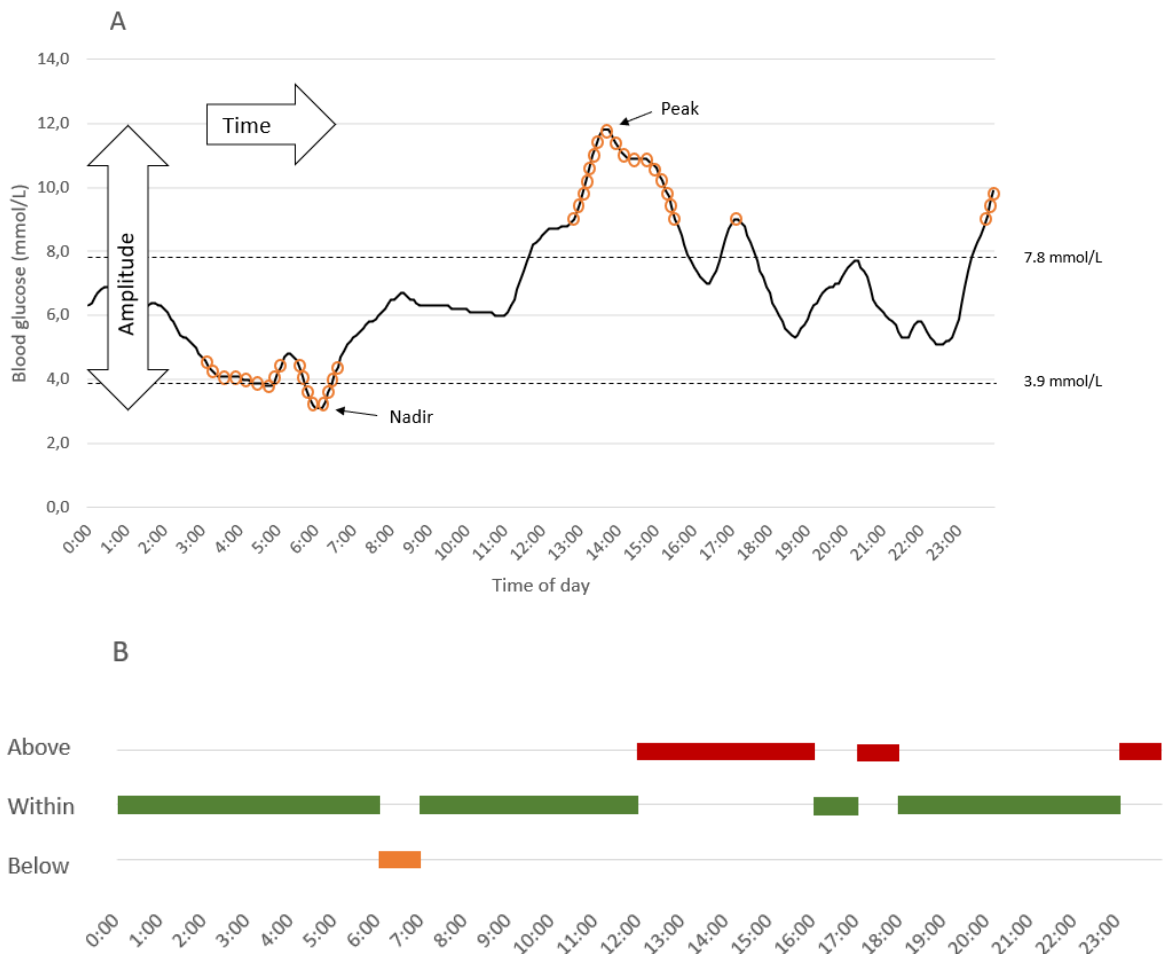


Figure 1.2: Principal components of glycaemia. (A) Glucose fluctuations are a process in time that has two dimensions, amplitude and time. CV, SD and MAGE are metrics measure projected along the amplitude axis. Peak and nadir are the maximum and minimum excursions. The orange circles denote upward and downward glycaemic excursions 1SD away from the 24-hour mean blood glucose as an example. (B) Projected along the time axis are temporal characteristics, such as time within target range (3.9–7.8 mmol/L) and time spent in hypo- or hyperglycaemia. The CGM measurements were obtained from a random participant with GDM in the observational study described in Chapter 4.

1.6 Summary

DIP places a great risk on public health due to its prevalence and association with adverse short- and long-term pregnancy outcomes for both mother and offspring. Healthcare bodies have developed dietary and lifestyle guidelines to achieve optimal (mealtime) glycaemic control. In reality, women with DIP struggle to achieve (postprandial) glycaemic control resulting in dysglycaemia (primarily hyperglycaemia) increasing the risk of adverse pregnancy outcomes significantly. The body of literature has shown that glycaemia in DIP is influenced by numerous factors, including personal (e.g., age, ethnicity, and lifestyle) and

physiological/clinical parameters (e.g., HbA_{1c}, plasma lipid and inflammatory profile), and that it changes throughout pregnancy. Mealtime glucose represents a key target for improving long-term glycaemic control, as this is the main period of time that blood glucose levels go out of target range, and the more time spent above target range is associated with more adverse pregnancy outcomes. Current methods focusing on reducing carbohydrate intake and using carbohydrate counting for quantifying PPGR are suboptimal in assessing glucose responses to meals consumed in real-life, as personal and physiological factors beyond the characteristics of food have been postulated to play an important role. Therefore, it is of importance to examine mediators of (postprandial) glycaemia and establish data on phenotypic responses in different populations.

This thesis aims to contribute to the scientific literature and aid in the understanding of mediators of (mealtime) glycaemic control in DIP by (i) evaluating the current evidence regarding the role of lifestyle (dietary and exercise) interventions in improving glycaemia in DIP; (ii) analysis of CGM data to identify the times during the day in which women with GDM struggle maintaining glycaemic control; (iii) preliminary analysis of a longitudinal study with an embedded RCT on (postprandial) CGM glucose profiles throughout the course of the pregnancy and how they are associated with personal characteristics and physiological parameters in DIP, and (iv) assessing associations of self-reported meat intake with plasma metabolic markers and glycaemic control in pregnancy. Identifying these characteristics will aid in determining phenotypic differences and gaining important insights into individual factors involved in PPGR and long-term glycaemic control. Approaches reflecting the individuality of (postprandial) glycaemia may have exceptional clinical utility in individuals, who normally rely on carbohydrate estimation and reducing carbohydrate intake for self-managing mealtime glucose levels. Ultimately, aiding in the development of new nutritional strategies for optimal glycaemic control in DIP.

Chapter 2

PhD Objectives

Women with diabetes in pregnancy are at increased risk of adverse pregnancy outcomes and have a three-fold higher rate of perinatal mortality compared to women with normal glucose tolerance in pregnancy. Moreover, offspring of DIP pregnancies have increased risk of childhood obesity and developing T2DM in later life, this contributing to an inter-generational disease cycle. Current methods using carbohydrate content of meals for quantifying (postprandial) glucose responses are suboptimal, as personal and physiological factors beyond the characteristics of food have been implicated to play an important role. Therefore, this PhD aims to contribute to the understanding of mediators – including dietary, personal, physiological and environmental parameters – of (postprandial) glucose control and which management strategies could improve these mediators and glycaemic control in DIP.

General aim 1: Examine possible nutritional and lifestyle strategies associated with (postprandial) glycaemic control in DIP.

- Objective 1: To investigate the independent and combined effects of diet, dietary supplements and exercise on management of dysglycaemia in diabetes in pregnancy (Chapter 3).
- Objective 2: To characterise glycaemic control over 24-hrs, using continuous glucose monitoring metrics, and evaluate the effect of clinical care – diet (i.e., macronutrient intake) and pharmacological treatment (i.e., metformin) – on dysglycaemia in a diverse population of women with gestational diabetes (Chapter 4).

General aim 2: Examine (dietary) moderators of (postprandial) glycaemic control in DIP.

- Objective 3: To assess the role of diet as a mediator of dysglycaemia in early, mid, and late pregnancy and the moderating effects of personal, physiological and environmental parameters (Chapter 5).
- Objective 4: To assess the association between metabolic markers of meat intake and glycaemic control in a diverse cohort of pregnant women with varied levels of dysglycaemia (Chapter 6).

Chapter 3

Nutritional and Exercise-Focused Lifestyle Interventions on Glycaemic Control in Maternal Diabetes: a Systematic Review and Meta-analysis

Authors' original report of the study published as Dingena, C. F., Arofikina, D., Campbell, M. D., Holmes, M. J., Scott, E. M. and Zulyniak, M. A. 2023a. Nutritional and Exercise-Focused Lifestyle Interventions and Glycemic Control in Women with Diabetes in Pregnancy: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. *Nutrients*, **15**(2), p323. <https://doi.org/10.3390/nu15020323>

What do we know? Current management of DIP to a large extent focusses on lifestyle changes to improve maternal glucose control, with pharmacological therapy initiated if dysglycaemia persists.

Key issues: The literature review in *Chapter 1* shows that women with DIP still struggle to achieve optimal (mealtime) glycaemic control and that maternal glucose metabolism is complex, with many factors beyond the characteristics of food implied to play a role.

Aims: This systematic review and meta-analysis was conducted to explore the current literature on the most effective lifestyle intervention strategies for pre-gestational diabetes as well as gestational diabetes (i.e., maternal diabetes or DIP).

Thesis implications: The results and conclusions of this review were used to inform the direction and design of the other studies of this PhD project.

3.1 Abstract

Globally, diabetes disrupts 1 in 7 pregnancies, conferring immediate and long-term health risks to the mother and her child. Diet and exercise are commonly prescribed to control dysglycaemia during pregnancy, but its effectiveness across populations and types of maternal diabetes (i.e., pre-gestational T1DM / T2DM, or GDM) is uncertain. To inform health strategies, this review evaluated the independent and combined effects of nutritional supplements, diet and exercise on management of gestational dysglycaemia across diverse populations.

Scientific databases (e.g., PubMed, Scopus etc.) were systematically searched for RCTs that investigated the effect of diet and/or exercise on glycaemia in pregnant women with diabetes. Random effects models were employed to evaluate effect sizes across studies and anticipated confounders (e.g., age, ethnicity, BMI). Quality of the included studies was assessed using Cochrane Collaboration's tool for assessing risk of bias (RoB2) and Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool. The SRMA was registered with the PROSPERO database – CRD42021268977.

Twenty-six studies – 8 nutritional supplements, 12 dietary, and 6 exercise interventions – were included (of 4845 records retrieved). Furthermore, all studies were conducted in patients with GDM. Overall, supplement- and exercise-based interventions reduced FPG (-0.30 mmol/L; 95%CI: -0.55 , -0.06; p=0.02; and -0.10 mmol/L; 95% CI= -0.20 , -0.01; p=0.04); and supplement- and diet-based interventions reduced HOMA-IR (-0.40; 95% CI= -0.58 , -0.22; p<0.001; and -1.15; 95% CI= -2.12 , -0.17; p=0.02). Subgroup analysis by confounders only confirmed marginal changed effect sizes or heterogeneity, yet maternal age, gestational age, and country of study were most commonly reported. Evidence for FPG in dietary- and supplement-based interventions was graded low and moderate in exercise-based interventions. For HOMA-IR, the evidence was graded moderate in dietary- and supplement-based interventions.

This meta-analysis highlights the key role of nutritional supplements, diet, and exercise in the management of GDM and shows promising advantageous effects on measures of glycaemia. However, the results underline a lack of evidence in ~20% of other diabetes-related pregnancies (i.e., women with pre-gestational diabetes). Future studies should focus on improving the study design and including all types of diabetes in pregnancy.

3.2 Background

DIP is one of the most common complications during pregnancy, with in 2021 an estimated 16.7% of live births to be affected (International Diabetes Federation, 2021; Chivese et al., 2021). GDM comprises 80.3% of all cases of DIP, while 19.7% were the result of diabetes detected prior to pregnancy (including pre-gestational T1DM and T2DM) (International Diabetes Federation, 2021; Chivese et al., 2021). Women with DIP are at a three-fold higher risk of adverse maternal and infant pregnancy outcomes – including foetal macrosomia, stillbirth, neonatal metabolic disturbances, preeclampsia, and caesarean delivery – and are at long term risk of comorbidities compared to women without DIP (Modder, 2006; Allehdan et al., 2019; Vargas et al., 2010; Temple and Murphy, 2010). Moreover, these women are at risk of developing T2DM, while their offspring are at increased risk of obesity and glucose intolerance in later life (Allehdan et al., 2019; Yamamoto et al., 2018). The literature hypothesises that the adverse intrauterine environment causes epigenetic changes in the foetus that contributes to metabolic disorders throughout life and future generations, referred to as the ‘vicious cycle of diabetes’ (Yamamoto et al., 2018).

All women diagnosed with DIP require optimal antenatal care to minimise short- and long-term health complications. Glycaemic control may be achieved via a combination of healthy diet, weight management, moderate exercise, blood glucose monitoring, and pharmacological treatments (e.g. metformin or insulin) (Chivese et al., 2021; International Association of Diabetes and Pregnancy Study Groups Consensus, 2010; Webber et al., 2015). In the UK according to the NICE guidelines, pregnant women with any type of diabetes are advised to maintain their plasma glucose below the following target levels – fasting: <5.3 mmol/L, and 1-hr post meals: <7.8 mmol/L or 2-hrs post meals: <6.4 mmol/L (Webber et al., 2015). Key strategies to achieve these targets are enclosed in the promotion of behaviour that include a healthier diet and physical activity – e.g., wholegrains, fruits and vegetables, and regular exercise. These lifestyle behaviours are seen as the cornerstone for managing DIP, which is effective in 70-85% of women with DIP (Webber et al., 2015; American Diabetes Association, 2020b). These NICE dietary guidelines predominantly focus on improving carbohydrate quality by including lower glycaemic index (GI) foods as part of a balanced diet to manage glycaemia during pregnancy (Webber et al., 2015).

Although studies uncovered balanced diets do support the management of overall glucose levels, their effect on reducing episodes of hypo- and hyperglycaemia and ability to reduce maternal and offspring risk of complications is inconsistent and uncertain, highlighting significant heterogeneity in their effectiveness (Perichart-Perera et al., 2012; Moses et al., 2006; Fuller et al., 2022b). Furthermore, preceding studies inferred that increased physical activity (i) improved glycaemic control, (ii) lowered rise in BMI, and (iii) may prevent, reduce, or delay use of pharmacological treatment, lowering risk of adverse pregnancy outcomes (Allehdan et al., 2019; Bung and Artal; Prather et al., 2012; Wang et al., 2015a). However, the effectiveness of these lifestyle strategies are not clearly established. For this reason, an investigation into the generalisability of evidence and key lifestyle moderators (i.e., nutritional supplements, diet and/or exercise) of dysglycaemia in pregnancy is needed.

Since the rise of continuous glucose monitoring technology, growing research has shed light on numerous lifestyle-dysglycaemia associations and novel points of interest for managing dysglycaemia during pregnancy and its associated health risks (Voormolen et al., 2018; Murphy et al., 2007; Perichart-Perera et al., 2012; Moses et al., 2006). However, emerging research has postulated that women with DIP and their offspring continue to be at risk (Law et al., 2019; Scott et al., 2020a; Murphy et al., 2021; Voormolen et al., 2018). Moreover, studies and reviews that focus on the effect of diet and/or exercise interventions on glycaemia beyond GDM are limited. Therefore, this systemic review and meta-analysis aimed to investigate the magnitude and generalisability of the effects of nutritional supplements, diet and/or exercise on measures of glycaemia in women with DIP. To, ultimately, improve glycaemic control and reduce adverse health consequences of the mother and her offspring.

3.3 Methods

The guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were followed for conducting this SRMA (Page et al., 2021). This review was registered with PROSPERO (CRD42021268977) and aimed to investigate the following question:

Do nutritional- and exercise-focused lifestyle interventions improve maternal glucose (i.e., fasting and postprandial glucose levels, glycated

haemoglobin levels and insulin resistance) in women diagnosed with DIP when compared to the control intervention?

3.3.1 Search Strategy and Study Selection

Cochrane, AMED, EMBASE, MEDLINE (via OVID), PubMed, and Scopus were searched from inception to December 21st 2021 to identify randomised controlled trials (RCTs) relevant to 'lifestyle' interventions and glycaemia in DIP. Database searches were limited to original human-based studies written in the English language. The search strategy was structured using PISO (Population, Intervention, Study Type, Outcome) and MESH terms – included key terms and synonyms for maternal diabetes (P); diet, nutritional supplement, and exercise (I); RCTs in humans (S); and glucose control (O). Full search strategy is presented in **Table 3.1**. Additional manual searches were conducted by reviewing reference lists of included articles and relevant reviews.

Table 3.1: Predetermined search strategy.

SEARCH CATEGORIES	USED SEARCH TERMS
1. Population	(diabet*) OR (GDM) OR (IDDM) OR (NIDDM) OR (MODY) OR (LADA)
2. Intervention	(exercise) OR (sports) OR (activity) OR (fitness) OR (training) OR (accelerom*) OR (pedomet*) OR (steps) OR (weightlifting) OR (diet*) OR (dietary) OR (nutrition) OR (nutrient) OR (feeding) OR (food) OR (FFQ) OR (food frequency)
3. Outcome	(insulin) OR (glucose) OR (glycaemic*) OR (glycemic*) OR (blood sugar) OR (glycated haemoglobin) OR (glycated haemoglobin) OR (HbA1c) OR (OGTT) OR (AUC) OR (HOMA)
4. Study type	(randomized controlled trial) OR (random*) OR (placebo*) OR (single blind*) OR (double blind*) OR (triple blind*) OR (clinical trial) AND (humans NOT animals)
5. Combined search	(1) AND (2) AND (3) AND (4)

Screening was performed in duplicate and independently by two authors, with disagreements mediated by a third reviewer, first by reviewing titles and abstracts and finally by reviewing the full-texts to identify all eligible RCTs articles. Included studies were randomized controlled trials and crossover studies either acute (assessing single meal response or intake <2 weeks) or long-term (assessing intake >2 weeks), investigating the effect of nutritional- and exercise-focused interventions in comparison to control on parameters of glycaemic control measured using capillary or venous blood in women diagnosed with DIP (pre-gestational T1DM/T2DM, or GDM). Studies were excluded if they (i) did not report nutritional- and exercise-based interventions; (ii) were focused on children and adolescents (<18 years of age) or women >45 years of age with comorbidities (e.g., cardiovascular disease and cancer etc.); (iii) or if outcome measures of glycaemic control were not reported. The trials included were limited to being published after the year 2000 and peer-reviewed RCTs or crossover studies, available as full-texts in English. Corresponding authors were contacted to request the full-text where articles were not accessible online if no additional data were obtained the study was excluded.

3.3.2 Data Extraction and Quality Assessment

The following data from included studies were extracted: first author and year of publication; publishing journal; country of study; sample and estimated power of sample size; definition of diabetes diagnosis used; design of the study (i.e., RCT vs crossover study); intervention and control (type, dose and format of intervention); study duration; and participant characteristics (i.e., age, BMI [pre-gestational or at enrolment], weeks' gestation at enrolment); outcome (glucose indices reported). The outcome measures of included studies were extracted as means and its variance (e.g., standard deviation (SD), standard error (SE), or confidence interval (CI)) of baseline and post-intervention fasting plasma glucose (FPG - mmol/L), post-prandial glucose (PPG - mmol/L), glycated haemoglobin (HbA_{1c} %), and insulin resistance expressed as Homeostatic Model of Assessment (HOMA-IR). In case data were presented in alternative units (other than mmol/L), they were converted to mmol/L (e.g., mg/dl to mmol/L). For this, the following formula was used:

$$\text{Glucose Concentration (mmol/L)} = \frac{\text{Glucose Concentration (mg/dl)}}{18.0182 \text{ mmol L}^{-1}/\text{mg dL}^{-1}}$$

If data were presented in figure format, values were extracted using Web Plot Digitizer (Drevon et al., 2017).

Bias assessment of the individual studies was conducted using the updated RoB2 (Sterne et al., 2019). With this assessment, the studies were categorised into three categories – high risk, low risk, or some concerns raised – utilising six domains (randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, selection of the reported results and overall bias) (**Appendix Table A.1**). The tool uses an algorithm based on signalling questions to judge risk of bias for each these domains as well as provide an overall risk of bias assessment for each individual study. Publication bias was assessed by visual inspection of funnel plots where ≥ 10 studies were available for a single exposure-outcome analysis.

3.3.3 Data Analysis

Data were analysed using Review Manager (RevMan version 5.4.1; The Cochrane Collaboration, 2020). Trials not reporting uncertainty of effect sizes (e.g., standard deviation, standard error, or confidence interval) were excluded from the meta-analysis. Pooled random effects analyses were performed to estimate the mean difference of effect (MD) of nutritional supplement-, dietary-, or exercise-based trials on DIP participants. Random effects analysis was chosen given the heterogeneity of the outcome and expected heterogeneity of the study populations and their exposures. Effects were estimated for FPG, PPG, HbA_{1c}, and HOMA-IR with 95% CIs between pre- and post-intervention. All analyses were conducted, so that a negative MD was presented as a favourable intervention (i.e., lowering of measures of dysglycaemia). Heterogeneity was assessed using Tau² and I², as well as calculation of prediction intervals. Where heterogeneity was high or of interest due to population/study heterogeneity (I² > 50%), subgroup analysis were performed – if ≥ 2 RCTs were included in the meta-analysis. Planned subgroup analysis included expected confounders: maternal age (< vs > mean age), gestational age (< vs > 28 weeks), maternal BMI (recommended BMI vs overweight), country (Western vs Non-Western), diabetes diagnostic criteria (ADA guidelines vs other), and study duration (acute vs longitudinal). Forest plots were created using R Statistical Software (v2022.07.2+576; RStudio Team 2022).

3.3.4 Grading the Evidence

The GRADE tool was used to improve interpretability of results data, evaluate the certainty of evidence, and determine the strength of the review conclusions (Gradepro, 2015). Evidence of an effect can be graded either 'very low', 'low', 'moderate', or 'high' based on evaluation outcomes in five domains – overall risk of bias, inconsistency, indirectness, imprecision, and other considerations.

3.4 Results

A total of 5304 studies were identified through database searches and other sources. After de-duplication, title- and abstract screening of 4843 records; 57 records were identified for full-text screening, and 6 of these were excluded because full-text articles could not be retrieved. Thus, 51 records were identified for full-text screening, of which 24 records were excluded for not meeting the inclusion criteria (**Figure 3.1**). In total, 24 RCTs and 3 randomized crossover trials were included for the systematic review. The meta-analysis included 23 RCTs and 3 randomized crossover trials, comprising a total of 1653 individuals with gestational diabetes. No studies including other types of diabetes during pregnancy (i.e., pre-gestational T1DM or T2DM) were identified. The RCTs were classified according to the type of intervention – nutritional supplement- (n = 8, **Table 3.2**), dietary- (n = 13, **Table 3.3**), or exercise-based (n = 6, **Table 3.4**) – these studies were summarised and analysed per intervention category.

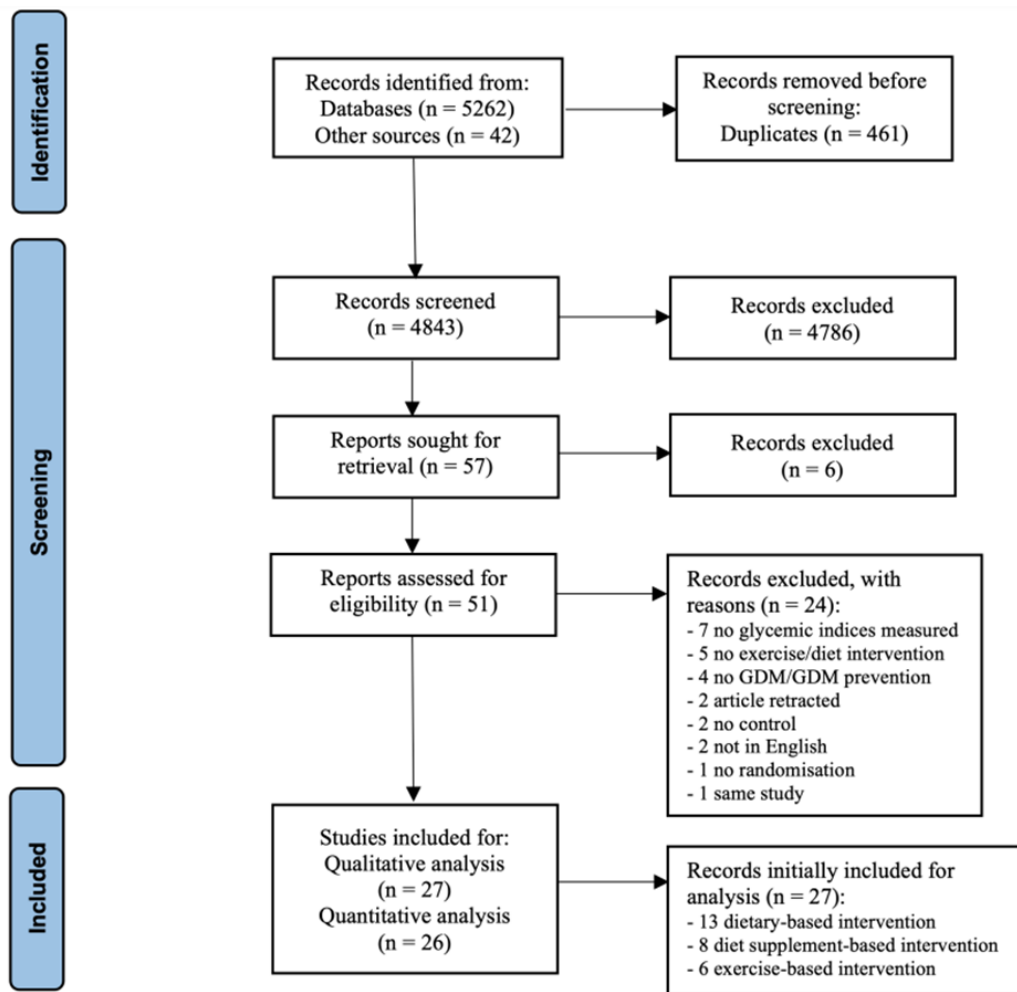


Figure 3.1: PRISMA flow diagram of study selection adapted from Page MJ, et al. (Page et al., 2021). Records were identified via searches in Cochrane, AMED, EMBASE, MEDLINE (via OVID), PubMed, and Scopus databases. These databases were searched from inception until 21/12/2021.

For clarification, nutritional supplements were defined as a product intended for ingestion that contains a "dietary ingredient" (i.e., a concentrated source of a vitamin, mineral or other substance), with a nutritional or physiological effect (alone or in combination) intended to supplement the diet and is sold in dose form. Of the studies retained for analysis, nutritional supplement interventions focused on alpha-lipoic acid, probiotic, ginger, fish oil, or combination zinc and vitamins intake versus a placebo. Dietary interventions primarily focused on higher complex CHO / lower GI, restricted energy intake, and Dietary Approaches to Stop Hypertension (DASH) diets versus a standard care diet. Finally, exercise interventions included brisk walks, resistance exercise, home-based exercises, and moderate intensity aerobics versus standard antenatal care.

Table 3.2: Summary of RCTs investigating effect of nutritional supplement-based interventions on glycaemic indices in GDM

Author, year	Country	N	Estimated sample Size	Definition of GDM (diagnostic criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Aslfalah et al., 2020</i>	Iran	60 (n=30 for both groups)	Not reported	American Diabetes Association guidelines	8 weeks	RCT double-blinded <i>Intervention:</i> received ALA (100 mg/day) <i>Control:</i> received cellulose acetate (100 mg/day)	Age <i>Intervention:</i> 30.96 ± 0.93 <i>Control:</i> 31.1 ± 0.92 Wks of gestation at baseline <i>Intervention:</i> 26.28 ± 0.23 <i>Control:</i> 26.51 ± 0.24 BMI (pre-pregnancy) <i>Intervention:</i> 26.64 ± 0.71 <i>Control:</i> 26.95 ± 0.73	FPG and HbA _{1c}
<i>Fei et al., 2014</i>	China	97 (n=46 for I and n=51 for C)	Not reported	National Diabetes Data group guidelines	8 weeks	RCT <i>Intervention:</i> treated with the combination of insulin, regular diet, and soybean oligosaccharides (SBOS) <i>Control:</i> regular diet and insulin treatment	Not reported	FPG and HOMA-IR

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostic criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Hajimoosayi et al., 2020</i>	Iran	70 (n=37 for I and n=33 for C)	Considering a 99% CI, power of 90%, and 30% dropout rate, a sample size of 38 per group was determined.	International Association of the Diabetes in Pregnancy Study Group guidelines	6 weeks	RCT double-blinded <i>Intervention:</i> received 126 tablets of ginger, <i>Control:</i> received 126 tablets of placebo	Age <i>Intervention:</i> 29.68 ± 5.05 <i>Control:</i> 31.15 ± 5.26 Wks of gestation at baseline <i>Intervention:</i> 27.72 ± 3.6 <i>Control:</i> 27.78 ± 3.6 BMI (at baseline) <i>Intervention:</i> 29.60 ± 3.6 <i>Control:</i> 29.50 ± 4.3	FPG, PPG and HOMA-IR
<i>Jamilian et al., 2018</i>	Iran	40 (n=20 for both groups)	Not reported	American Diabetes Association guidelines	6 weeks	RCT double-blind <i>Intervention:</i> 1000 mg fish oil capsules, containing 180 mg eicosapentaenoic acid and 120 mg docosahexaenoic acid twice a day <i>Control:</i> placebo	Age 30.8 ± 2.4 Wks of gestation at baseline 25.3 ± 1.1 BMI (at baseline) 27.0 ± 3.1	FPG and HOMA-IR

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostic criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Jamilian et al., 2019</i>	Iran	60 (n=30 for both groups)	Considering a type 1 error of 5%, power of 80%, and hs-CRP mean distinction of 3.2mg/L as outcome, a sample size of 25 per group was determined.	American Diabetes Association guidelines	6 weeks	RCT double-blind <i>Intervention:</i> magnesium-zinc-calcium-vitamin D supplements <i>Control:</i> placebo	Age <i>Intervention:</i> 27.7 ± 4.0 <i>Control:</i> 29.1 ± 4.1 BMI (at baseline) <i>Intervention:</i> 25.8 ± 3.7 <i>Control:</i> 25.3 ± 2.5	FPG
<i>Jamilian et al., 2020</i>	Iran	60 (n=26 for I and n=25 for C)	Considering a type 1 error of 5%, power of 80%, and PPAR-γ change of 0.20 as outcome, a sample size of 25 per group was determined.	American Diabetes Association guidelines	6 weeks	RCT double-blinded <i>Intervention:</i> 2 × 1000 mg/d n-3 fatty acids from flaxseed oil containing 400 mg α-linolenic acid in each capsule <i>Control:</i> placebo	Age <i>Intervention:</i> 29.5 ± 5 <i>Control:</i> 28.5 ± 4.1 BMI (at baseline) <i>Intervention:</i> 28.9 ± 4.8 <i>Control:</i> 27.3 ± 4.1	FPG and HOMA-IR

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostic criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
Lindsay et al., 2015	Ireland	100 (n=48 for I and n=52 for C)	Considering a type 1 error of 5%, power of 80%, and 0.4mmol/L reduction in fasting plasma glucose as outcome, a sample size of 50 per group was determined.	Based on a 100 g-oral glucose tolerance test (Carpenter and Coustan, 1982)	Diagnosis until delivery	RCT double-blinded <i>Intervention:</i> daily probiotic (Lactobacillus salivarius UCC118) from diagnosis until delivery <i>Control:</i> placebo capsule from diagnosis until delivery	Age <i>Intervention:</i> 33.5 ± 5.0 <i>Control:</i> 32.6 ± 4.5 Wks of gestation at baseline <i>Intervention:</i> 29.8 ± 2.5 <i>Control:</i> 29.5 ± 2.4 BMI (at baseline) <i>Intervention:</i> 29.06 ± 6.70 <i>Control:</i> 28.94 ± 5.79	FPG and HOMA-IR
Ostadmohammadi et al., 2019	Iran	54 (n=27 for both groups)	Not reported	American Diabetes Association guidelines	6 weeks	RCT double-blind <i>Intervention:</i> 233 mg/day Zinc Gluconate plus 400-IU/day vitamin E supplements <i>Control:</i> placebo	Age <i>Intervention:</i> 31.1 ± 5.1 <i>Control:</i> 30.5 ± 3.1 Wks of gestation at baseline <i>Intervention:</i> 25.7 ± 1.40 <i>Control:</i> 25.3 ± 1.3 BMI (at baseline) <i>Intervention:</i> 29.3 <i>Control:</i> 28.5	FPG, PPG and HOMA-IR

I, intervention; *C*, control; *CI*, confidence interval; *RCT*, randomised controlled trial; *wks*, weeks; *BMI*, body mass index; *FPG*, fasting plasma glucose; *PPG*; postprandial glucose; *HbA_{1c}*, glycated haemoglobin; *HOMA-IR*, Homeostatic Model of Assessment – Insulin Resistance.

Table 3.3: Summary of RCTs and crossover studies investigating effect of diet-based interventions on glycaemic indices in GDM

Author, year	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Asemi et al., 2013</i>	Iran	34 (n=17 for both groups)	Considering a type I error of 5 %, power of 80 % and serum HDL cholesterol levels as outcome, a sample size of 16 per group was determined.	American Diabetes Association guidelines	4 Weeks	RCT <i>Intervention:</i> DASH diet <i>Control:</i> control diet contained 45–55 % carbohydrates, 15–20 % protein and 25–30 % total fat	Age <i>Intervention:</i> 30.7 ± 6.7 <i>Control:</i> 29.4 ± 6.2 BMI (at baseline) <i>Intervention:</i> 29.0 ± 3.2 <i>Control:</i> 31.4 ± 5.7	FPG, PPG and HbA _{1c}
<i>Grant et al., 2011</i>	Canada	26 (n=10 for I and n=16 for C for GDM) (IGTP; n=12)	Considering 85% power and to detect a difference of 0.6 mmol/L in capillary glucose between groups, a sample size of 50 was determined.	Canadian Diabetes Association guidelines	~8 weeks	RCT <i>Intervention:</i> low glycaemic index dietary intervention as a supplement to the standard medical nutrition therapy (Canadian guidelines) <i>Control:</i> standard medical nutrition therapy (Canadian guidelines)	Age <i>Intervention:</i> 34 ± 0.1 <i>Control:</i> 34 ± 1.1 Wks of gestation at baseline <i>Intervention:</i> 29 ± 0.7 <i>Control:</i> 29 ± 0.5 BMI (pre-pregnancy) <i>Intervention:</i> 27 ± 1 <i>Control:</i> 26 ± 1	FPG, PPG and HbA _{1c}

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Hernandez et al., 2014</i>	USA	16	Considering a type 1 error of 5%, power of 80%, and AUC as outcome, a sample size of 16 was determined.	American College of Obstetricians and Gynaecologists guidelines	3 days	Randomized crossover <i>Intervention:</i> Higher complex CHO/ Lower fat diet <i>Control:</i> conventional low-carbohydrate/ higher-fat diet	Age 28.4 ± 1.0 Wks of gestation at baseline 31.2 ± 0.5 BMI (pre-pregnancy) 30.6 ± 1.3	FPG
<i>Hernandez et al., 2016</i>	USA	12 (n=6 for both groups)	Not reported	Based on a 100 g-oral glucose tolerance test (Carpenter and Coustan, 1982)	~7 weeks	RCT <i>Intervention:</i> a higher-complex carbohydrate/lower-fat diet (60% carbohydrate/ 25% fat/ 15% protein) <i>Control:</i> conventional low-carbohydrate/ higher-fat diet (40% carbohydrate/ 45% fat/ 15% protein)	Age <i>Intervention:</i> 30 ± 1.0 <i>Control:</i> 28 ± 2.0 Wks of gestation at enrolment <i>Intervention:</i> 31.7 ± 1.0 <i>Control:</i> 31.2 ± 0.4 BMI (at baseline) <i>Intervention:</i> 34.3 ± 1.6 <i>Control:</i> 33.4 ± 1.4	HOMA-IR

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Jamilian, 2015</i>	Iran	68 (n=34 for both groups)	Considering the type 1 error of 5% power of 80%, a sample size of 28 per group was determined.	American Diabetes Association guidelines	6 weeks	RCT <i>Intervention:</i> soy diet containing the same amount of protein with 35% animal protein, 35% soy protein, and 30% other plant proteins <i>Control:</i> control diet containing 0.8-g/kg protein (70% animal and 30% plant proteins)	Age <i>Intervention:</i> 28.2 ± 4.6 <i>Control:</i> 29.3 ± 4.2 Wks of gestation at baseline <i>Intervention:</i> 29 ± 0.7 <i>Control:</i> 29 ± 0.5 BMI (at baseline) <i>Intervention:</i> 28.9 ± 5.0 <i>Control:</i> 28.4 ± 3.4	FPG and HOMA-IR
<i>Louie et al., 2011</i>	Australia	77 (n=38 for I and n=39 for C)	Considering power of 80% and to detect a ~260 g difference in birth weight, a sample size of 60 per group was determined.	Australasian Diabetes in Pregnancy Society (ADIPS) guidelines	~6-7 weeks	RCT Both diets consisted of similar protein (15–25%), fat (25–30%), and carbohydrate (40–45%) content <i>Intervention:</i> an Low-glycaemic index (target GI ≤50) <i>Control:</i> a high-fibre content and moderate GI, similar to the Australian population average (target GI ~60)	Age <i>Intervention:</i> 34.0 ± 4.1 <i>Control:</i> 32.4 ± 4.5 Wks of gestation at baseline <i>Intervention:</i> 29.0 ± 4.0 <i>Control:</i> 29.7 ± 3.5 BMI (pre-pregnancy) <i>Intervention:</i> 23.9 ± 4.4 <i>Control:</i> 24.1 ± 5.7	HOMA-IR and HbA _{1c}

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Ma et al., 2015</i>	China	83 (n=41 for I and n=42 for C)	Not reported	Chinese Medical Association and the American Diabetes Association guidelines	Every 2 weeks from 24–26 weeks of gestation to delivery	RCT <i>Intervention:</i> intensive low-GL intervention <i>Control:</i> individualized general dietary intervention	Age <i>Intervention:</i> 30.1 ± 3.8 <i>Control:</i> 30.0 ± 3.5 Wks of gestation at baseline <i>Intervention:</i> 27.5 ± 1.1 <i>Control:</i> 27.9 ± 1.1 BMI (pre-pregnancy) <i>Intervention:</i> 21.90 ± 3.14 <i>Control:</i> 21.15 ± 2.75	FPG, PPG and HbA _{1c}
<i>Perichart- perera et al., 2012</i>	Mexico	107 (n=55 for I and n=42 for C)	Considering the type 1 error of 5% power of 80%, and 10 mg/dL difference in glucose, a sample size of 32 per group was determined.	American Diabetes Association guidelines	Not reported	RCT <i>Intervention:</i> Women received an individual food plan based on CHO restriction (only low glycaemic index (GI) carbohydrates (CHO)) <i>Control:</i> Women received an individual food plan based on CHO restriction (all types of CHO)	Age <i>Intervention:</i> 32.3 ± 4.8 <i>Control:</i> 31.8 ± 5.3 Wks of gestation at enrolment <i>Intervention:</i> 22.5 ± 4.9 <i>Control:</i> 20.7 ± 6.7 BMI at baseline <i>Intervention:</i> 30.5 ± 5.2 <i>Control:</i> 32.0 ± 6.3	FPG

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Rae et al., 2000</i>	Australia	124 (n=66 for I and n=58 for C)	Considering the type 1 error of 5% power of 80%, and frequency of insulin and macrosomia use as outcomes, a sample size of 60 per group was determined.	Not reported	Treatment until delivery (not further specified)	RCT <i>Intervention:</i> a moderately energy restricted diabetic diet providing between 1590-1776 kilocalories. Representing 70% of the RDI for pregnant women (National Health and Medical Research Council of Australia) <i>Control:</i> a diabetic diet which was not energy restricted	Age <i>Intervention:</i> 30.2 <i>Control:</i> 30.8 Wks of gestation at diagnosis <i>Intervention:</i> 28.1 ± 5.8 <i>Control:</i> 28.3 ± 4.6 BMI (at diagnosis) <i>Intervention:</i> 37.9 ± 0.7 <i>Control:</i> 38.0 ± 0.7	FPG and HbA _{1c}
<i>Rasmussen et al., 2020</i>	Denmark	12	Considering the power of 80%, and to detect 5% between groups based on Dalfrá (2013), a sample size of 12 was determined.	WHO diagnostic criteria	4 days	Randomised crossover Study Low carbohydrate morning intake vs high carbohydrate morning intake	Age 33.6 Gestational age 33.5 BMI (pre- pregnancy) 25.2	FPG

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Valentini et al., 2012</i>	Italy	20 (n=10 for both group s)	Pilot study	American Diabetes Association guidelines	Not reported	RCT <i>Intervention:</i> an ethnic meal plan (EMP), a food plan that included dishes typical of the foreign women's original countries <i>Control:</i> a standard meal plan (SMP) prepared according to the ADA guidelines	Age <i>Intervention:</i> 28.9 ± 3.3 <i>Control:</i> 30.2 ± 4.7 BMI (pre- pregnancy) <i>Intervention:</i> 25.7 ± 3.6 <i>Control:</i> 24.1 ± 4.7	FPG, PPG and HbA _{1c}
<i>Wang et al., 2015b</i>	China	84 (n=41 for I and n=43 for C)	Not reported	Based on a 75g-oral glucose tolerance test	~6-8 weeks	RCT <i>Intervention:</i> an oil- rich diet, with sunflower oil (45-50 g daily) used as cooking oil <i>Control:</i> a low-oil diet, with sunflower oil (20 g daily) used as cooking oil	Age <i>Intervention:</i> 30.29 ± 4.17 <i>Control:</i> 29.72 ± 4.64 Wks of gestation at baseline <i>Intervention:</i> 27.41 ± 1.52 <i>Control:</i> 27.34 ± 1.96 BMI (pre- pregnancy) <i>Intervention:</i> 21.36 ± 3.0 <i>Control:</i> 22.18 ± 3.6	FPG and PPG

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
Yao <i>et al.</i> , 2015	China	33 (n=17 for I and n=16 for C)	Considering a 75g birthweight difference between groups, a sample size of 21 per group was determined.	American Diabetes Association guidelines	4 weeks	RCT <i>Intervention:</i> DASH diet <i>Control:</i> control diet including 45-55% carbohydrates, 15- 20% protein and 25- 30% total fat.	Age <i>Intervention:</i> 30.7 ± 5.6 <i>Control:</i> 28.3 ± 5.1 Wks of gestation at baseline <i>Intervention:</i> 26.9 ± 1.4 <i>Control:</i> 25.7 ± 1.3 BMI (pre- pregnancy) <i>Intervention:</i> 29.6 ± 5.3 <i>Control:</i> 30.9 ± 4.3	FPG and HOMA-IR

I, intervention; *C*, control; *CI*, confidence interval; *RCT*, randomised controlled trial; *wks*, weeks; *BMI*, body mass index; *FPG*, fasting plasma glucose; *PPG*; postprandial glucose; *HbA_{1c}*, glycated haemoglobin; *HOMA-IR*, Homeostatic Model of Assessment – Insulin Resistance.

Table 3.4: Summary of RCTs investigating effect of exercise-based interventions on glycaemic indices in GDM

Author, year	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Bo et al., 2014</i>	Italy	200 (n=99 for I and n=101 for C)	Considering an effect size of 0.50, power of 95%, and a 10% reduction in fasting plasma glucose as outcome, a sample size of 200 was determined.	Based on a 75g-oral glucose tolerance test	~12-14 weeks	2x2 design single-blinded All women were given the same diet (carbohydrates 48-50%, proteins 18-20%, fats 30-35%, fiber 20-25 g/day, no alcohol <i>Intervention:</i> received dietary recommendations <i>Control:</i> instructed to briskly walk 20-min/day	Age <i>Intervention:</i> 35.9 ± 4.8 <i>Control:</i> 33.9 ± 5.3 BMI (pre-pregnancy) <i>Intervention:</i> 25.1 ± 4.6 <i>Control:</i> 24.8 ± 4.2	FPG, PPG and HOMA-IR
<i>Brankston et al., 2004</i>	Canada	24 (n=12 for both groups)	Considering a type 1 error of 5%, power of 80%, and insulin use reduced to 25% as outcome, a sample size of 32 per group was determined.	Canadian Diabetes Association guidelines	At least 4 weeks	RCT <i>Intervention:</i> circuit-type resistance training three times per week and same standard diet. <i>Control:</i> standard diabetic diet that consisted of 40% carbohydrate, 20% protein, and 40% fat.	Age <i>Intervention:</i> 30.5 ± 4.4 <i>Control:</i> 31.3 ± 5.0 Wks of gestation at baseline <i>Intervention:</i> 29.0 ± 2.0 <i>Control:</i> 29.6 ± 2.1 BMI (pre-pregnancy) <i>Intervention:</i> 26.4 ± 7.1 <i>Control:</i> 25.2 ± 6.7	FPG and PPG

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>De Barros et al., 2010</i>	Brasil	64 (n=32 for both groups)	Considering a type 1 error of 5%, power of 80%, and insulin use required up to 20%, a sample size of 30 per group was determined.	Based on a 2hr-75 g- or 3hr-100g-oral glucose tolerance test	~6 weeks	RCT <i>Intervention:</i> resistance exercise program <i>Control:</i> no resistance exercise program	Age <i>Intervention:</i> 31.81 ± 4.87 <i>Control:</i> 32.40 ± 5.40 Wks of gestation at baseline <i>Intervention:</i> 31.56 ± 2.29 <i>Control:</i> 31.06 ± 2.30 BMI (pre-gestational) <i>Intervention:</i> 25.34 ± 4.16 <i>Control:</i> 25.39 ± 3.81	FPG
<i>Halse et al., 2014</i>	Australia	40 (n=20 for both groups)	Considering a type 1 error of 5%, power of 80%, and to detect a minimum 0.3mM difference in fasting plasma glucose, a sample size of 20 per group was determined.	Based on a 75g-oral glucose tolerance test (Australian criteria)	~6 weeks (until week 34 of pregnancy)	RCT <i>Intervention:</i> home-based exercise training in combination with conventional management <i>Control:</i> conventional management alone	Age <i>Intervention:</i> 34 ± 5 <i>Control:</i> 32 ± 3.0 Wks of gestation at enrolment <i>Intervention:</i> 28.8 ± 0.8 <i>Control:</i> 28.8 ± 1.0 BMI (pre-pregnancy) <i>Intervention:</i> 26.4 ± 7.1 <i>Control:</i> 25.2 ± 6.7	FPG, PPG and HbA _{1c}

Author, year (Continued)	Country	N	Estimated sample Size	Definition of GDM (diagnostics criteria)	Intervention duration	Design intervention description	Participant characteristics	Outcomes measures
<i>Kokic et al., 2018</i>	Croatia	38 (n=18 for I and n=20 for C)	Not reported	International Association of the Diabetes and Pregnancy Study Groups guidelines	From the time of diagnosis of GDM until birth (minimum 6 weeks)	RCT single-blinded <i>Intervention:</i> standard antenatal care for GDM, and regular supervised exercise programme (two times per week 50–55 min; mixed exercises) plus daily brisk walks of at least 30 min. <i>Control:</i> only standard antenatal care for GDM.	Age <i>Intervention:</i> 32.78 ± 3.83 <i>Control:</i> 31.95 ± 4.91 Wks of gestation at baseline <i>Intervention:</i> 22.44 ± 6.55 <i>Control:</i> 20.80 ± 6.05 BMI (at baseline) <i>Intervention:</i> 24.39 ± 4.89 <i>Control:</i> 25.29 ± 4.65	FPG and PPG
<i>Qazi et al., 2020</i>	Pakistan	50 (n=25 for both groups)	Considering a CI of 95% and power of 80%, a sample size of 27 per group was determined.	Based on a 75 g-oral glucose tolerance test	5 weeks	RCT <i>Intervention:</i> combination of moderate intensity aerobics, stabilization and pelvic floor muscles exercises twice a week (40 min per session) along with dietary and medical interventions <i>Control:</i> only medical and dietary interventions with postural education	Age <i>Intervention:</i> 34.36 ± 5.21 <i>Control:</i> 35.92 ± 5.24	HbA _{1c}

I, intervention; C, control; CI, confidence interval; RCT, randomised controlled trial; wks, weeks; BMI, body mass index; FPG, fasting plasma glucose; PPG; postprandial glucose; HbA_{1c}, glycated haemoglobin; HOMA-IR, Homeostatic Model of Assessment – Insulin Resistance.

3.4.1 Nutritional Supplement-based Interventions

In total, 8 RCTs that reported on the effect of nutritional supplements on markers of dysglycaemia were identified, comprising a total of 541 participants. Of these, 8 studies reported fasting glucose, 1 study reported PPG and HbA_{1c}, and 6 studies reported HOMA-IR. The included studies encompassed geographic regions: Europe (i.e., Ireland [n=1]: 18% of participants), Western Asia (i.e., Iran [n=6]: 64%), and Eastern Asia (i.e., China [n=1]: 18%). The average maternal age was 30.5±3.8 years, gestational age was 27.1±1.8 weeks and BMI was 27.9±3.7 kg/m², the mean BMI value indicating that this group falls in the overweight category of ≥ 25 kg/m². The supplement-based interventions had an average duration of 7±1 weeks and focused on alpha-lipoic acid, probiotic, ginger, fish oil, or combination of zinc and vitamins intake versus a placebo. The nutritional supplement-based interventions significantly reduced FPG (8 RCTs, MD -0.30 mmol/L; 95% CI -0.55 , -0.06; p = 0.02; I² = 95%, **Figure 3.2**), albeit with high heterogeneity. Only 1 RCT reported PPGR and HbA_{1c}, so no meta-analysis was performed. Additionally, HOMA-IR was significantly reduced by supplement-based interventions (HOMA-IR; 6 RCTs, MD -0.40; 95% CI -0.58 , -0.22; p <0.0001; I² = 14%, **Figure 3.3**), with low heterogeneity. The funnel plots for FPG and HOMA-IR did not indicate asymmetry (**Appendix Figures A.1 and A.2**).

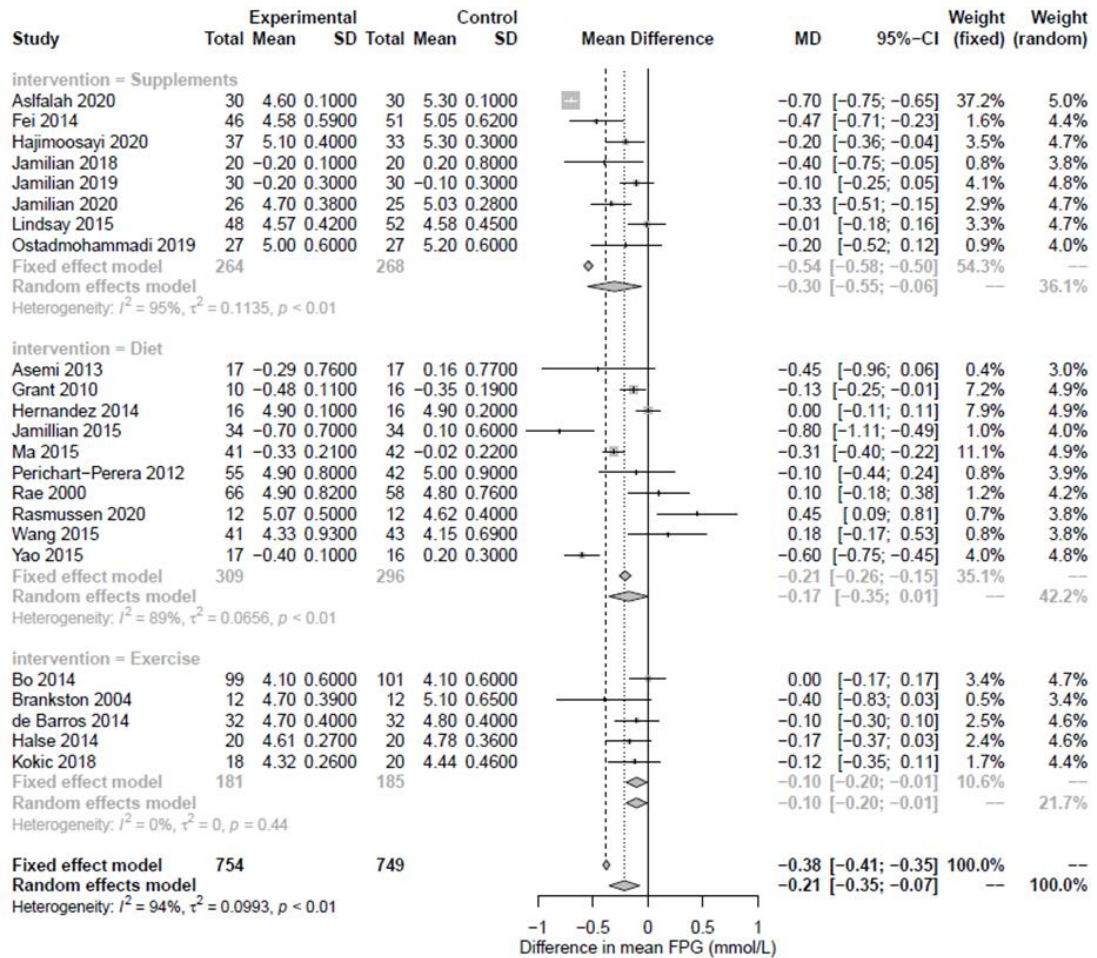


Figure 3.2: Forest plot of fasting plasma glucose (mmol/L). Fixed and random-effect meta-analysis of included studies. Overall test for effect of any lifestyle intervention (with all studies; $n = 23$) and subgroup analysis by intervention type – nutritional supplements ($n = 8$), diet ($n = 10$), and exercise ($n = 5$) – are presented. SD, standard deviation; CI, confidence interval.

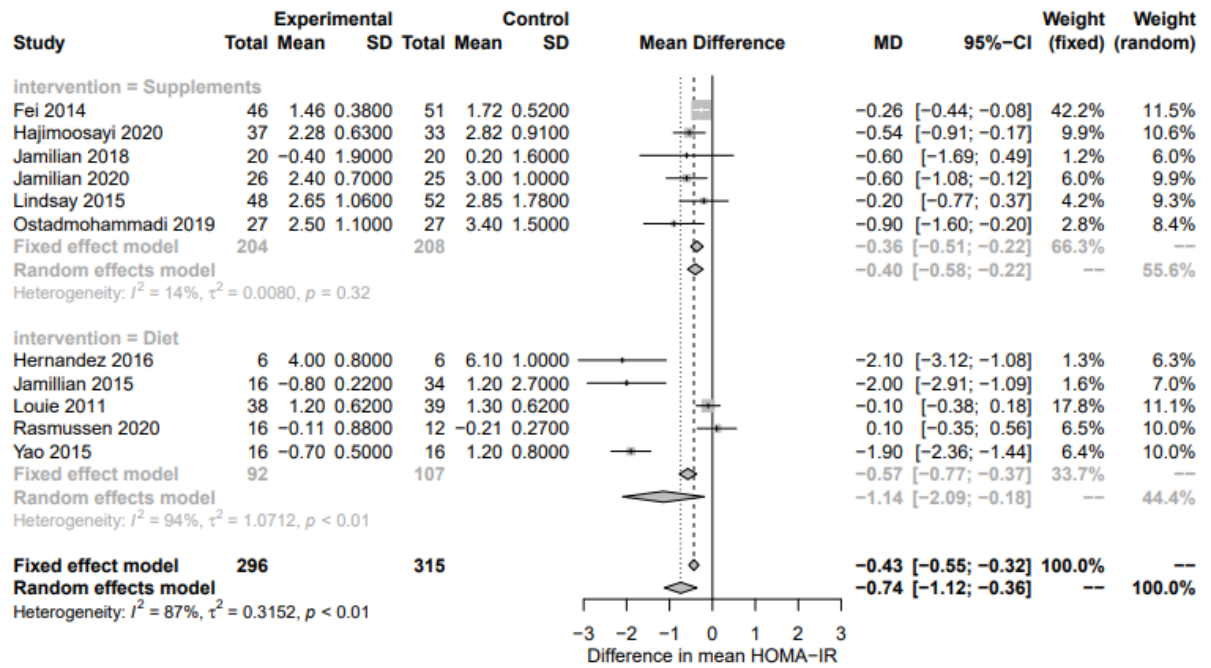


Figure 3.3: Forest plot of HOMA-IR. Fixed and random-effects meta-analysis of include studies. Overall test for effect of any lifestyle intervention (with all studies; $n=23$) and subgroup analysis by intervention type – nutritional-supplements ($n=8$), diet ($n=10$), and exercise ($n=5$) – are presented. SD, standard deviation; CI, confidence interval.

Subgroup analysis of nutritional supplement-based – including maternal age, gestational age, body weight, GDM diagnostic criteria, and geographic region – for FPG did not greatly change the effect size from the overall analysis but it did suggest that studies initiated at later maternal age (maternal age ≥ 30.5 years: 3 RCTs, MD -0.20 mmol/L; 95% CI -0.33, -0.07; $p = 0.002$; $I^2 = 45%$, **Table 3.5**) and in women with recommended weight (5 RCTs; MD -0.18 mmol/L; 95% CI -0.31, -0.05; $p = 0.005$; $I^2 = 55%$, **Table 3.5**) may be less effective. For interventions initiated at earlier gestational age and in non-Western countries, supplement-based interventions may be more effective, albeit with high heterogeneity (gestational age < 28 weeks: 4 RCT, MD -0.39 mmol/L, 95% CI -0.72, -0.05; $p = 0.02$; $I^2 = 93%$ and non-Western country: 5 RCT, MD -0.35 mmol/L, 95% CI -0.59, -0.10; $p = 0.005$; $I^2 = 94%$, **Table 3.5**). Taking HOMA-IR as outcome measure, the analysis suggests that supplement-based interventions initiated earlier in pregnancy, in younger women, and in non-Western countries are most likely to be effective (gestational age < 28 weeks: 3 RCTs; MD -0.62; 95% CI -0.69, -0.07; $p = 0.002$; $I^2 = 0%$, maternal age < 30.5 years 2 RCTs; MD -0.56; 95% CI -0.86, -0.27; $p = 0.002$; $I^2 = 0%$, and non-Western country: 5 RCTs;

MD -0.45; 95% CI -0.67 , -0.23; $p < 0.001$; $I^2 = 27\%$, **Table 3.5**). Furthermore, the analysis suggests that interventions are more effective in studies using the ADA guidelines and other diagnosis criteria less effective (3 RCTs; MD -0.68; 95% CI -1.05 , -0.31; $p < 0.001$; $I^2 = 0\%$ and 3 RCTs; -0.30; 95% CI -0.46 , -0.15; $p < 0.001$; $I^2 = 0\%$, respectively, **Table 3.5**). With only 1 RCT of the nutritional supplement intervention studies reporting PPG and HbA_{1c} (Hajimoosayi et al., 2020 and Aslfalah et al., 2020, respectively), subgroup analyses for these outcomes were not performed. Hajimoosayi et al. (2020) reported no change in PPG levels after intervention between nutritional supplement- (i.e., ginger tablets) and control group (i.e., placebo tablets) ($p = 0.54$). Comparing means within the study groups (before and after intervention) PPG was reduced significantly in the supplement group (7.1 vs 6.5 mmol/L; $p = 0.003$). The other study by Aslfalah et al. (2020) observed no significant changes in the values of HbA_{1c} ($p = 0.496$) in the alpha-lipoic acid supplementation compared to the placebo group. Comparing means within the study groups (before and after intervention) HbA_{1c} was reduced in the supplement group (HbA_{1c} in supplement group: $5.29\% \pm 0.13$ vs $4.94\% \pm 0.13$; $p = 0.059$, respectively) and no change was observed in the control group ($5.31\% \pm 0.12$ vs $5.09\% \pm 0.16$ 0.274, respectively).

Table 3.5: Subgroup analysis of nutritional supplement vs control interventions.

<u>Category</u>	<u>Outcome measure</u>	<u>RCTs (n)</u>	<u>MD</u>	<u>95% CI</u>	<u>p-value</u>	<u>I²</u>
<i>Fasting Plasma Glucose (FPG, mmol/L)</i>						
Main analysis	<i>Overall</i>	8	-0.30	(-0.55 , -0.06)	0.02	95
Maternal Age ¹	<i>< Mean age</i>	4	-0.33	(-0.76 , 0.10)	0.13	96
	<i>≥ Mean age</i>	3	-0.20	(-0.33 , -0.07)	0.002	45
Gestational Age ²	<i>< 28 weeks</i>	4	-0.39	(-0.72 , -0.05)	0.02	93
	<i>≥ 28 weeks</i>	1	-0.01	(-0.18 , 0.16)	0.905	NA
Weight (pre-pregnancy) ³ (kg/m ²)	<i>Recommended BMI</i>	5	-0.18	(-0.31 , -0.05)	0.005	55
	<i>Overweight</i>	1	-0.70	(-0.75 , -0.65)	<0.001	NA
Diagnostic Criteria for GDM	<i>ADA</i>	5	-0.35	(-0.66 , -0.04)	0.03	94
	<i>Other</i>	3	-0.30	(-0.39 , 0.02)	0.08	79
Geographic Region	<i>Western country</i>	1	-0.01	(-0.18 , 0.16)	0.905	NA
	<i>Non-western country</i>	7	-0.35	(-0.59 , -0.10)	0.005	94

<u>Category</u>	<u>Outcome measure</u>	<u>RCTs (n)</u>	<u>MD</u>	<u>95% CI</u>	<u>p-value</u>	<u>I²</u>
HOMA-IR						
Main analysis	<i>Overall</i>	6	-0.40	(-0.58 , -0.22)	<0.001	14
Maternal Age ¹	<i>< Mean age</i>	2	-0.56	(-0.86 , -0.27)	0.002	0
	<i>≥ Mean age</i>	3	-0.51	(-0.96 , -0.05)	0.03	15
Gestational Age ²	<i>< 28 weeks</i>	3	-0.62	(-0.93 , -0.30)	<0.001	0
	<i>≥ 28 weeks</i>	1	-0.2	(-0.77 , 0.37)	0.501	NA
Diagnostic Criteria for GDM	<i>ADA</i>	3	-0.68	(-1.05 , -0.31)	<0.001	0
	<i>Other</i>	3	-0.30	(-0.46 , -0.15)	<0.001	0
Geographic Region	<i>Western country</i>	1	-0.2	(-0.77 , 0.37)	0.501	NA
	<i>Non-western country</i>	5	-0.45	(-0.67 , -0.23)	<0.001	2

¹ Maternal age not reported in 5 study. ² Gestational age not reported in 4 studies for FPG and 2 for HOMA-IR. ³ Weight not reported in 6 studies for FPG and only 1 for HOMA-IR. Mean age for the supplement-based interventions was 30.5 yrs. Overweight and recommended weight pregnancies were defined as pre-pregnancy BMI of ≥ 25 or BMI < 25 , respectively. If pre-pregnancy weight was unavailable, overweight, and recommended weight pregnancies were defined as BMI of ≥ 30 or BMI < 30 , respectively.

3.4.2 Diet-based Interventions

In total, 10 RCTs and 2 crossover trials reported on the effect of diet on markers of dysglycaemia (n= 676 participants). Of these, 10 studies reported fasting glucose, 5 reported PPG, 4 reported HbA_{1c}, and 5 reported HOMA-IR. The included studies encompassed 5 geographic regions: Europe and Northern America (i.e., Canada, Denmark, and USA [n=4]: 9% of participants), Australia/New Zealand (i.e., Australia [n=2]: 30%), Latin America (i.e., Mexico [n = 1]: 16%), Western Asia (i.e., Iran [n=2]: 15%), and Eastern Asia (i.e., China [n=3]: 30%). The average maternal age was 30.7±3.8 years, gestational age was 28.3±2.2 weeks and BMI was 28.5±3.3 kg/m², the mean BMI value indicating that this group falls in the overweight category of ≥ 25 kg/m². The dietary-based interventions had an average duration of 6±1 weeks for longitudinal studies and 3.5±1 days for short-term studies, and primarily focused on higher complex CHO/

lower GI, restricted energy intake, and DASH versus a standard care diet. HOMA-IR was significantly reduced by diet interventions (HOMA-IR; $n = 5$ RCTs, MD -1.15; 95% CI -2.36, -1.44; $p = 0.02$; $I^2 = 94\%$, **Figure 3.3**), while FPG suggested some evidence of an effect, albeit not significant and with high heterogeneity (FPG; $n = 10$ RCTs, MD -0.17 mmol/L; 95% CI -0.35, 0.01; $p = 0.06$; $I^2 = 89\%$, **Figure 3.2**). The shape of the funnel plots for FPG and HOMA-IR did not suggest asymmetry (**Appendix Figures A.3 and A.4**). Postprandial glucose and HbA_{1c} were not significantly associated with diet-based interventions ($n = 5$ RCTs, MD -0.23 mmol/L; 95% CI -0.69, 0.32; $p = 0.34$; $I^2 = 95\%$ and $n = 4$ RCTs, MD -0.08%; 95% CI -0.23, 0.08; $p = 0.34$; $I^2 = 70\%$, respectively, **Figures 3.4 and 3.5**).

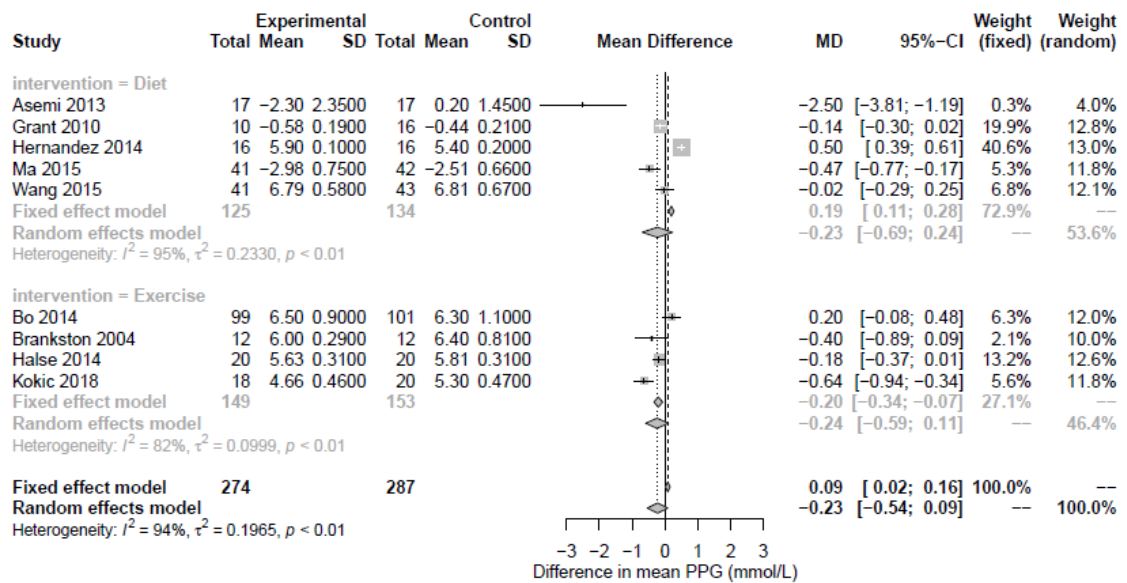


Figure 3.4: Forest plot of postprandial plasma glucose (mmol/L). Fixed and random-effects meta-analysis of include studies. Overall test for effect of any lifestyle intervention (with all studies; $n=23$) and subgroup analysis by intervention type – nutritional-supplements ($n=8$), diet ($n=10$), and exercise ($n=5$) – are presented. SD, standard deviation; CI, confidence interval.

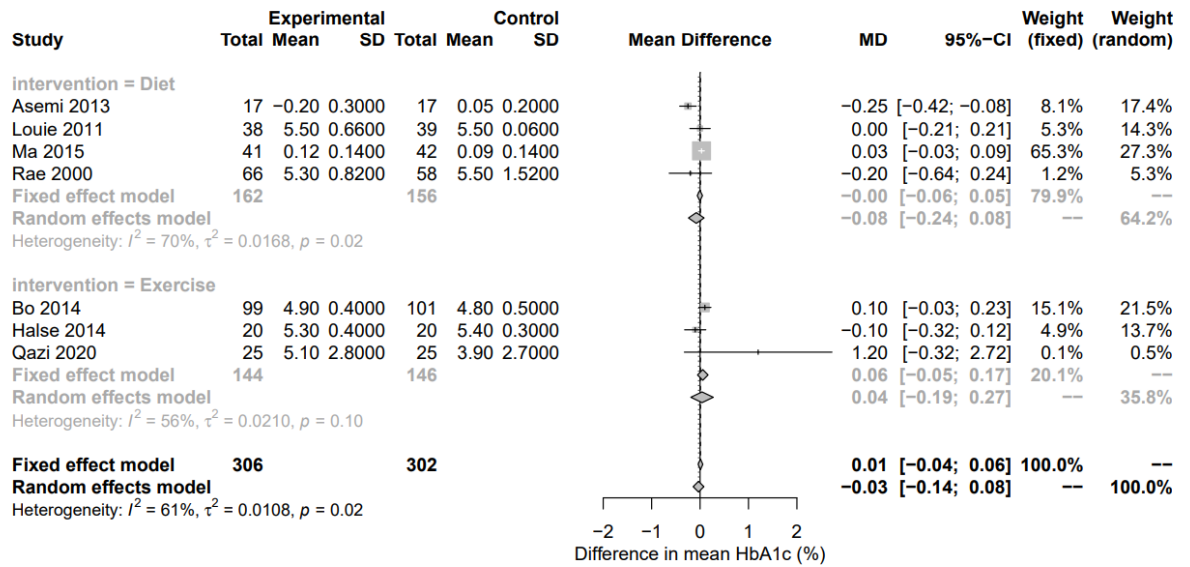


Figure 3.5: Forest plot of glycated haemoglobin (%). Fixed and random-effects meta-analysis of include studies. Overall test for effect of any lifestyle intervention (with all studies; $n=23$) and subgroup analysis by intervention type – nutritional-supplements ($n=8$), diet ($n=10$), and exercise ($n=5$) – are presented. SD, standard deviation; CI, confidence interval.

Subgroup analysis for FPG and PPG did not differ greatly from the main overall analysis and high heterogeneity remained (**Table 3.6**). However, for HbA_{1c}, subgroup analysis suggested that the effectiveness of diet interventions is primarily driven by its effect in overweight individuals (2 RCTs; MD -0.24%; 95% CI -0.40, -0.08; $p = 0.003$; $I^2 = 0\%$, **Table 3.6**). Additionally, subgroup analysis of diet on HOMA-IR suggested that diet is most effective in younger participants and in non-Western countries that use the ADA criteria (3 RCTs; MD -1.94; 95% CI -2.33, -1.56; $p < 0.001$; $I^2 = 0\%$ and 2 RCTs; MD -1.92; 95% CI -2.33, -1.51; $p < 0.001$; $I^2 = 0\%$, respectively, **Table 3.6**).

Table 3.6: Subgroup analysis of dietary vs control interventions.

<u>Category</u>	<u>Outcome measure</u>	<u>RCTs</u> <u>(n)</u>	<u>MD</u>	<u>95% CI</u>	<u>p-value</u>	<u>I²</u>
<i>Fasting Plasma Glucose (FPG, mmol/L)</i>						
Main analysis	<i>Overall</i>	10	-0.17	(-0.35 , 0.01)	0.06	89
Maternal Age	<i>< Mean age</i>	7	-0.26	(-0.50 , -0.03)	0.03	91
	<i>≥ Mean age</i>	3	0.05	(-0.29 , 0.81)	0.79	78
Gestational Age ¹	<i>< 28 weeks</i>	5	-0.25	(-0.51 , 0.01)	0.06	86
	<i>≥ 28 weeks</i>	4	-0.08	(-0.33 , 0.16)	0.51	88
Weight (pre-pregnancy) (kg/m ²)	<i>Recommended BMI</i>	3	-0.32	(-0.74 , 0.10)	0.14	88
	<i>Overweight</i>	7	-0.11	(-0.34 , 0.12)	0.35	89
Diagnostic Criteria for GDM ²	<i>ADA</i>	4	-0.51	(-0.78 , -0.24)	<0.001	69
	<i>Other</i>	5	-0.02	(-0.21 , 0.17)	0.83	88
Geographic Region	<i>Western country</i>	5	0.02	(-0.13 , 0.16)	0.83	63
	<i>Non-western country</i>	5	-0.41	(-0.66 , -0.15)	0.002	85
Study Duration ³	<i>Acute</i>	2	0.19	(-0.25 , 0.63)	0.39	82
	<i>Longitudinal</i>	7	-0.29	(-0.49 , -0.08)	0.006	88

<u>Category</u>	<u>Outcome measure</u>	<u>RCTs</u> <u>(n)</u>	<u>MD</u>	<u>95% CI</u>	<u>p-value</u>	<u>I²</u>
Continued						
Postprandial Glucose (PPG, mmol/L)						
Main analysis	<i>Overall</i>	5	-0.23	(-0.69 , 0.24)	0.34	95
Maternal Age	<i>< Mean age</i>	4	-0.32	(-0.97 , 0.32)	0.33	95
	<i>≥ Mean age</i>	1	-0.14	(-0.30 , 0.02)	0.10	NA
Gestational Age ¹	<i>< 28 weeks</i>	2	0.18	(-0.44 , 0.81)	0.57	98
	<i>≥ 28 weeks</i>	2	-0.24	(-0.68 , 0.20)	0.29	79
Weight (pre-pregnancy) (kg/m ²)	<i>Recommended BMI</i>	2	-0.24	(-0.68 , 0.20)	0.29	79
	<i>Overweight</i>	3	-0.25	(-0.92 , 0.42)	0.46	97
Diagnostic Criteria for GDM	<i>ADA</i>	1	-2.5	(-3.81 , -1.19)	<0.001	NA
	<i>Other</i>	4	-0.02	(-0.46 , 0.42)	0.93	96
Geographic Region	<i>Western country</i>	2	0.18	(-0.44 , 0.81)	0.57	98
	<i>Non-western country</i>	3	-0.63	(-1.33 , 0.06)	0.07	88
Study Duration	<i>Acute</i>	1	0.50	(0.39 , 0.61)	<0.001	NA
	<i>Longitudinal</i>	4	-0.36	(-0.73 , 0.02)	0.06	82

<u>Category</u>	<u>Outcome measure</u>	<u>RCTs</u> <u>(n)</u>	<u>MD</u>	<u>95% CI</u>	<u>p-value</u>	<u>I²</u>
Continued						
Glycated haemoglobin (HbA _{1c} , %)						
Main analysis	<i>Overall</i>	4	-0.08	(-0.23 , 0.08)	0.34	70
Maternal Age	< <i>Mean age</i>	3	-0.11	(-0.34 , 0.12)	0.33	80
	≥ <i>Mean age</i>	1	0.00	(-0.20 , 0.20)	1	NA
Gestational Age ¹	< <i>28 weeks</i>	1	-0.20	(-0.64 , 0.24)	0.356	NA
	≥ <i>28 weeks</i>	2	-0.03	(-0.21 , 0.15)	0.71	0
Weight (pre-pregnancy) (kg/m ²)	<i>Recommended BMI</i>	2	0.03	(-0.03 , 0.09)	0.35	0
	<i>Overweight</i>	2	-0.24	(-0.40 , -0.08)	0.003	0
Diagnostic Criteria for GDM ²	<i>ADA</i>	1	-0.25	(-0.42 , -0.07)	0.007	NA
	<i>Other</i>	2	0.03	(-0.03 , 0.09)	0.35	0
Geographic Region	Western country	2	-0.03	(-0.21 , 0.15)	0.71	0
	Non-western country	2	-0.10	(-0.37 , 0.18)	0.48	89

<u>Category</u>	<u>Outcome measure</u>	<u>RCTs</u> <u>(n)</u>	<u>MD</u>	<u>95% CI</u>	<u>p-value</u>	<u>I²</u>
Continued						
HOMA-IR						
Main analysis	<i>Overall</i>	5	-1.15	(-2.12 , -0.17)	0.02	94
Maternal Age	<i>< Mean age</i>	3	-1.94	(-2.33 , -1.56)	<0.001	0
	<i>≥ Mean age</i>	2	-0.06	(-0.30 , 0.19)	0.66	0
Gestational Age	<i>< 28 weeks</i>	1	-1.9	(-2.36 , -1.44)	<0.001	NA
	<i>≥ 28 weeks</i>	4	-0.91	(-1.84 , 0.02)	0.05	90
Weight (pre-pregnancy) (kg/m ²)	<i>Recommended BMI</i>	2	-1.00	(-2.86 , 0.86)	0.29	93
	<i>Overweight</i>	3	-1.27	(-2.77 , 0.22)	0.10	94
Diagnostic Criteria for GDM	<i>ADA</i>	2	-1.92	(-2.33 , -1.51)	<0.001	0
	<i>Other</i>	3	-0.54	(-1.39 , 0.31)	0.22	87
Geographic Region	<i>Western country</i>	3	-0.54	(-1.39 , 0.31)	0.22	87
	<i>Non-western country</i>	2	-1.92	(-2.33 , -1.51)	<0.001	0
Study Duration	<i>Acute</i>	1	0.10	(-0.42 , 0.62)	0.699	NA
	<i>Longitudinal</i>	4	-1.48	(-2.71 , -0.26)	0.02	95

¹ Gestational age not reported in 1 study for FPG, PPG and HbA_{1c}. ² Diagnostic criteria for GDM not reported in more than 1 study for FPG and HbA_{1c}. ³ Study duration not reported in more than 1 study for FPG. Mean age for the supplement-based interventions was 30.7 yrs. Overweight and recommended weight pregnancies were defined as pre-pregnancy BMI of ≥ 25 or BMI < 25 , respectively. If pre-pregnancy weight was unavailable, overweight, and recommended weight pregnancies were defined as BMI of ≥ 30 or BMI < 30 , respectively.

3.4.3 Exercise-based Interventions

In total, 5 RCTs and 1 crossover trial reported on the effect of exercise on markers of dysglycaemia (n=416 participants). Of these, 5 reported fasting glucose, 4 reported PPG, 1 reported HbA_{1c}, and none reported HOMA-IR. The included studies encompassed 4 geographic regions: Europe and Northern America (i.e., Italy, Croatia, and Canada [n=3]: 63% of participants), Latin America (i.e., Brazil [n = 1]: 15%), Australia/New Zealand ([n=1] 10%), and Southeastern Asia (i.e., Pakistan [n=1]: 12%). The average maternal age was 33.1±4.7 years, gestational age was 27.8±2.9 weeks and BMI was 25.4±5.4 kg/m², the mean BMI value indicating that this group falls in the overweight category of ≥ 25 kg/m². The exercise-based interventions had an average duration of 7±1 weeks, and focused on brisk walks, resistance exercise, home-based exercises, and moderate-intensity aerobics versus standard antenatal care. FPG was significantly reduced by exercise-based interventions (n = 5 RCTs, MD -0.10mmol/L; 95% CI -0.20 , -0.01; p = 0.04; I² = 0%, **Figure 3.2**). However, PPG and HbA_{1c} were not significantly affected by exercise-based interventions (n = 4 RCTs, MD -0.17mmol/L; 95% CI - 0.35 , 0.01; p = 0.17; I² = 82% and n = 3 RCTs, MD 0.04%; 95% CI - 0.19 , 0.27; p = 0.73; I² = 56%, respectively, **Figures 3.4 and 3.5**). Only 1 RCT reported HOMA-IR; therefore, no meta-analysis was performed. This study reported marginal change in HOMA-IR in exercise (i.e., brisk walks at least 20 minutes/day every day) compared to standard diet with no exercise (MD -0.02; 95% CI -0.14 , 0.11; p = 0.79) (Bo et al., 2014). The funnel plot for FPG did not indicate asymmetry (**Appendix Figure A.1**).

Subgroup analysis of exercise-based interventions by moderators of gestational dysglycaemia – maternal age, gestational age, and body weight – suggested that maternal age, gestational age, and pre-pregnancy weight may modify the effectiveness of exercise-based interventions, although not greatly, when taking FPG as outcome (maternal age < 33.1 years; n = 4 RCTs, MD -0.17mmol/L; 95% CI - 0.27 , -0.04; p = 0.01; I² = 0%, and recommended weight and gestational age > 28 weeks; n = 3 RCTs, MD -0.16mmol/L; 95% CI - 0.29 , - 0.03; p = 0.02; I² = 0%, **Table 3.7**). PPG and HbA_{1c} were not significantly affected by exercise and subgroup analysis did not change effect sizes or heterogeneity.

Table 3.7: Subgroup analysis of exercise vs control interventions.

<u>Category</u>	<u>Outcome measure</u>	<u>RCTs (n)</u>	<u>MD</u>	<u>95% CI</u>	<u>p-value</u>	<u>I²</u>
<i>Fasting Plasma Glucose (FPG, mmol/L)</i>						
Main analysis	<i>Overall</i>	5	-0.10	(-0.20 , -0.01)	0.04	0
Maternal Age	<i>< Mean age</i>	4	-0.15	(-0.27 , -0.04)	0.01	0
	<i>≥ Mean age</i>	1	0.00	(-0.17 , 0.17)	1.00	NA
Gestational Age ¹	<i>< 28 weeks</i>	1	-0.12	(-0.35 , 0.11)	0.336	NA
	<i>≥ 28 weeks</i>	3	-0.16	(-0.29 , -0.03)	0.02	0
Weight (pre-pregnancy) (kg/m ²)	<i>Recommended BMI</i>	3	-0.16	(-0.29 , -0.03)	0.02	0
	<i>Overweight</i>	2	-0.04	(-0.18 , 0.10)	0.56	0
Diagnostic Criteria for GDM	<i>75g OGTT</i>	2	-0.08	(-0.24 , 0.09)	0.37	40
	<i>Other</i>	3	-0.12	(-0.16 , -0.07)	0.17	36
<i>Postprandial Glucose (PPG, mmol/L)</i>						
Main analysis	<i>Overall</i>	4	-0.24	(-0.59 , 0.12)	0.17	82
Maternal Age	<i>< Mean age</i>	3	-0.39	(-0.71 , -0.07)	0.02	70
	<i>≥ Mean age</i>	1	0.20	(-0.08 , 0.48)	0.161	NA
Gestational Age ¹	<i>< 28 weeks</i>	1	-0.64	(-0.94 , -0.34)	0.0002	NA
	<i>≥ 28 weeks</i>	2	-0.21	(-0.39 , -0.03)	0.02	0
Weight (pre-pregnancy) (kg/m ²)	<i>Recommended BMI</i>	2	-0.21	(-0.39 , -0.03)	0.02	0
	<i>Overweight</i>	2	-0.22	(-1.04 , 0.60)	0.60	94
Diagnostic Criteria for GDM	<i>75g OGTT</i>	2	0.00	(-0.38 , 0.37)	0.98	79
	<i>Other</i>	2	-0.58	(-0.83 , -0.32)	<0.0001	0

<u>Category</u>	<u>Outcome measure</u>	<u>RCTs (n)</u>	<u>MD</u>	<u>95% CI</u>	<u>p-value</u>	<u>I²</u>
<i>Glycated haemoglobin (HbA_{1c}, %)</i>						
Main analysis	<i>Overall</i>	3	0.04	(-0.19 , 0.27)	0.73	56
Maternal Age	<i>< Mean age</i>	1	-0.10	(-0.32 , 0.12)	0.377	NA
	<i>≥ Mean age</i>	2	0.38	(-0.56 , 1.31)	0.43	50
Weight ² (pre-pregnancy) (kg/m ²)	<i>Recommended BMI</i>	1	0.1	(-0.03 , 0.23)	0.12	NA
	<i>Overweight</i>	1	-0.10	(-0.32 , 0.12)	0.377	NA
Geographic Region	<i>Western country</i>	2	0.02	(-0.17 , 0.21)	0.83	59
	<i>Non-western country</i>	1	1.2	(-0.32 , 2.72)	0.130	NA

¹ Gestational age not reported in 1 study for FPG and PPG. ² Weight not reported in 1 study for HbA_{1c}. Mean age for the supplement-based interventions was 33.1 yrs. Overweight and recommended weight pregnancies were defined as pre-pregnancy BMI of ≥ 25 or BMI < 25, respectively. If pre-pregnancy weight was unavailable, overweight, and recommended weight pregnancies were defined as BMI of ≥ 30 or BMI < 30, respectively.

3.4.4 Risk of Bias Assessment

Risk of bias assessment across the studies indicated low risk / some concerns for the majority of included studies (12 studies and 14 studies, respectively) due to lack of information on randomisation concealment and blinding of outcome assessors (**Appendix Tables A.2 and A.3**). Nutritional supplement-based studies had the lowest risk of bias and exercise-based interventions had the highest level concerns associated with bias risk (**Appendix Figure A.6**). One study of the diet-based interventions was considered 'high risk' due to concerns in three or more domains – i.e., lack of information on randomization concealment, blinding of outcome assessors and p-values / standard deviations. This study, Valentini et al. (2012), was removed for these reasons and lack of data on p-values for the meta-analysis. Visual inspection of the funnel plots did

not indicate asymmetry (**Appendix Figures A.1-A.5**), which is indicative of an absence of publication bias.

Grading the Evidence

The GRADE assessments for all outcome measures were conducted, which is summarized in supplemental material (**Appendix Tables A.4-A.6**). Evidence on nutritional supplement-based interventions was graded as 'moderate' for HbA_{1c} and HOMA-IR, and 'low' and 'very low' for FPG and PPG, mainly due to low ratings for consistency, directness, and precision. The assessment for dietary-based interventions revealed 'moderate' grade for HOMA-IR, and 'low' and 'very low' grades for fasting glucose, PPG, and HbA_{1c} in GDM, which were most commonly downgraded due to inconsistency and imprecision of the outcome reporting. Furthermore, assessment for exercise-based interventions revealed 'moderate' grade for FPG, and 'very low' and 'low' grades for PPG, HbA_{1c} and HOMA-IR, due to inconsistency, indirectness, and imprecision of these outcomes.

3.5 Discussion

To the best of our knowledge, this is the first SRMA with a comprehensive analysis on the impact of these types of lifestyle intervention (i.e., nutritional supplement, diet and exercise) in GDM on measures of glucose control. In total, 5304 records were identified; however, these studies were only conducted in women with GDM. No RCTs or crossover trials in pregnant women with pre-gestational T1DM or T2DM that reported on maternal glucose and lifestyle interventions were identified. In the end, a total of 24 RCTs and 3 randomized crossover trials were identified to investigate the magnitude and generalizability of the effects of lifestyle on glycaemic control (i.e., FPG, PPG, HbA_{1c}, and HOMA-IR) in women with GDM. The included studies reported on the effects of nutritional supplement- (n=8), diet- (whole foods, n=13), or exercise-based (n= 6) interventions on measures of maternal glucose control. Comparing with previous systematic reviews in women with GDM published before 2019 (Allehdan et al., 2019; Yamamoto et al., 2018) this review included 5 more RCTs, and conducted several subgroups analyses to control for heterogeneity – including maternal age, maternal BMI, gestational age, ethnicity, diagnosis guidelines used, intervention duration, and intervention types. These subgroups were defined to

better characterize and present the effects of lifestyle modifications on dysglycaemia in diverse populations. The results suggest that nutritional supplement-based interventions improved both FPG and HOMA-IR, while diet- and exercise-based interventions only improved one glycaemic measure (HOMA-IR or FPG, respectively).

3.5.1 Nutritional Supplement-based Interventions

In total, 8 RCTs (n=541 participants) reported on the effects of nutritional supplements on markers of dysglycaemia. Supplement interventions focused on alpha-lipoic acid, probiotic, ginger, fish oil, or zinc and vitamins supplements versus a placebo. Overall, the nutritional supplement-based interventions significantly improved FPG and HOMA-IR (-0.30 mmol/L; $p = 0.02$ and -0.40; $p < 0.001$, respectively). Clinical significance may be reached when target levels of ≤ 5.6 mmol/L for FPG and <2.89 for HOMA-IR, as this is the cut-off for insulin resistance and insulin therapy (Webber et al., 2015; Sokup et al., 2013). Given the magnitude of effect reported in this SRMA (FPG: -0.30 mmol/L and HOMA-IR: -0.40), clinical significance can be reached in women with mild dysglycaemia, but more studies are needed to draw conclusions for women with more severe dysglycaemia.

Subgroup analysis suggested that common moderators of GDM risk do not modify the effectiveness of nutritional supplements on dysglycaemia, except for maternal age, gestational age, body weight and ethnicity, which could be important when considering nutritional supplement-based interventions. Later maternal age and recommended body weight reduced the effectiveness of the intervention on FPG. In addition, nutritional supplements were more effective at earlier gestational age and in non-Western women, albeit with high heterogeneity. Taking HOMA-IR as outcome, nutritional supplements were more effective in younger, non-Western women at earlier pregnancy, reducing heterogeneity. Therefore, maternal age, gestational age and body weight could be considered as moderators. Unfortunately, the effect of nutritional supplement-based interventions on PPG and HbA_{1c} was reported in only 1 RCT and could not be generalised. Furthermore, given the magnitude of effect these studies reported the clinical significance of nutritional supplements on PPG and HbA_{1c} needs to be further examined.

Meta-analysis of RCTs on the effects of probiotics on glycaemia in pregnancy by Pan et al. (2021) indicated that probiotic supplements improved FPG level (14 RCTs) and insulin resistance (i.e., HOMA-IR, 13 RCTs), specifically in GDM and overweight pregnant women, which is in trend with the results of this current SRMA regarding nutritional supplements and improved levels of FPG and HOMA-IR (Pan et al., 2021). Maternal age is a known confounder of glucose status with dysglycaemia individuals typically older (Raviv et al., 2022). The results suggest that nutritional supplements are less effective in reducing fasting glucose and insulin resistance in the higher maternal age subgroup, as this group might have more severe dysglycaemia, which could be less modifiable. The exact mechanisms of probiotics on glycaemic control remain unknown. Another meta-analysis (5 RCTs) by Ojo et al. (2019), concluded that vitamin D supplementation decreased FPG (Ojo et al., 2019). A review by Qu et al. (2022) on magnesium supplementation found significant improvement in glucose metabolism and insulin sensitivity (i.e., FPG and insulin concentration) in addition to specific markers of oxidative stress (i.e., total antioxidant capacity) (Qu et al., 2022). While the mechanisms of vitamin D and magnesium on dysglycaemia are not certain, potential mechanisms could include: 1) direct action on β -cell function; 2) regulation of intracellular calcium and glucose transport, and 3) reduction of systemic inflammation associated with insulin resistance (Qu et al., 2022; Poel et al., 2012).

These results confirm that nutritional supplements can reduce fasting glucose and insulin resistance, though underlines the difficulty of generalisability due to the magnitude of effect, heterogeneity and variety of nutritional supplements and the limited evidence regarding their effect on postprandial and long-term estimates of dysglycaemia (i.e., PPG and HbA_{1c}). Based on the findings, future studies with a more uniform nutritional supplementation approach are warranted to make informed recommendations to care guidelines for management, regarding which supplements should be included and for how long.

3.5.2 Diet-based Interventions

In total, 10 RCTs and 2 randomized crossover trials reported on the effect of diet on markers of dysglycaemia (n= 676 participants). The dietary interventions primarily focused on higher complex CHO / lower GI, restricted energy intake, and Dietary Approaches to Stop Hypertension (DASH) diets versus a standard

care diet. The trial by Valentini et al. (2012) was excluded from the meta-analysis due to serious bias concerns (Valentini et al., 2012). The meta-analysis concluded that dietary interventions are advantageous for improving HOMA-IR (MD -1.15; 95% CI -2.12, -0.17; $p = 0.02$) during pregnancy in women with GDM. Clinical significance may be reached when target levels <2.89 for HOMA-IR are achieved (Webber et al., 2015; Sokup et al., 2013). Given the magnitude of effect reported in this SRMA (HOMA-IR: -1.15), clinical significance can be reached in women with mild dysglycaemia and in women with moderate dysglycaemia. Also, potential improvements in FPG were reported, however the clinical significance is small (given the mean difference of -0.17 mmol/L) and subgroup analysis did not improve the effect size or heterogeneity. Furthermore, subgroup analysis suggested that most common moderators of GDM risk do not modify the effectiveness of dietary interventions on dysglycaemia, except for younger maternal age, ADA diagnostic criteria and non-western country. Younger pregnant women are less likely to suffer from severe dysglycaemia, thus interventions might be more effective and insulin resistance might be easier to improve in this subgroup (Raviv et al., 2022). All non-western country studies used ADA guidelines as diagnostic criteria, suggesting a disagreement of diagnosis criteria as a previous study found IADPSG (i.e., ADA) criteria more favorable than NICE for identification of adverse pregnancy outcomes among Asian and Hispanic women, while they are comparable to NICE among White women (He et al., 2022). Furthermore, studies with lower glucose thresholds for GDM selection may see less of an impact.

Prescribing a low/reduced carbohydrate diet for pregnant women with GDM, which is the first-line treatment, has been linked with reduced FPG, decreased risk of postprandial glucose excursions, and reduced risk of requiring insulin to manage dysglycaemia (Webber et al., 2015; Major et al., 1998; American Diabetes Association, 2017). A previous review on a variety of modified dietary interventions and maternal glycaemia by Yamamoto et al. (2018) pooled results from 18 RCTs (including women with GDM, impaired glucose tolerance or hyperglycaemia) and found a moderate effect of dietary interventions on maternal glycaemic outcomes, including changes in FPG (13 RCTs), PPG (9 RCTs) and need for medication treatment, and nearly significant effect on HOMA-IR (4 RCTs) (Yamamoto et al., 2018). This current SRMA found a potential advantageous effect of dietary interventions on FPG (10 RCTs), but was unable

to find an effect on PPG (5 RCTs); this is possibly due to only studies published after 2000, where actual diets were prescribed to the participants, were included. Hence, fewer studies were available. However, this current meta-analysis included 1 more RCT (4 vs 5 RCTs) and did demonstrate a significant effect on HOMA-IR. Both Yamamoto et al., (2018) and this current meta-analysis demonstrated a high heterogeneity, which could be explained by differences in baseline FPG, or PPG levels having influenced the glucose-related outcomes. These improvements in glycaemic markers could be the result of dietary intervention's ability to reduce spikes in PPGR (Louie et al., 2010). To conclude, this meta-analysis supports current recommendations that prescribe dietary interventions to manage dysglycaemia during pregnancy. However, future work that accounts for adherence to the diet may allow for better clarity of the effectiveness, and feasibility of distinct diets.

3.5.3 Exercise-based Interventions

In addition to dietary modifications, exercise is a vital component in GDM management. The ADA, and NICE guidelines recommend that pregnant women with GDM, who have no medical contraindications, should undertake brisk walks 20min/day or moderate exercise consisting of 30 min most days of the week as part of GDM treatment (American Diabetes Association, 2020b; Webber et al., 2015). In total, this current meta-analysis included 5 RCTs and 1 randomized crossover trial reporting on the effect of exercise on markers of dysglycaemia in a total of 416 participants. These exercise interventions focused on brisk walks, resistance exercise, home-based exercises, and moderate intensity aerobics versus standard antenatal care. Pooled analysis demonstrated that exercise interventions statistically significantly improve FPG in women with GDM. However, the effect size was small (MD -0.10; $p = 0.04$), thus more studies are needed to draw conclusions regarding its clinical significance. Even though, subgroup analysis for this type of intervention was limited due to fewer included studies (meaning that not all categories could be used for subgroups analysis). Maternal age, gestational age, and body weight were suggested to modify the effectiveness of exercise interventions and could be considered as moderators.

Previous published SRMAs by Brown et al. (2017) (11 RCTs) and Cremona et al. (2018) (12 RCTs) on aerobic/resistance exercise or combination for women with GDM reported that exercise interventions were associated with

reduced FPG, and PPG concentrations compared with conventional interventions (Cremona et al., 2018; Brown et al., 2017). Another systematic review by Allehdan et al. (2019) (8 RCTs) showed evidence that dietary management plus aerobic or resistance exercise interventions improved glycaemic outcomes and lowered FPG and PPG levels for women with GDM compared with dietary management alone (Allehdan et al., 2019). Both aerobic and resistance exercise are beneficial for improving glycaemic control, and it is optimal to do both types of exercise (Bird and Hawley, 2017). Previous research has established that exercise increases the rate of glucose uptake into the skeletal muscle, this occurs during the exercise and for several hours post-exercise. The increased uptake is a result of translocation of glucose transporter proteins (e.g., GLUT4), thereby increasing the sites where glucose can diffuse into the muscle cells (Bird et al., 2017; Ryder et al., 2001). Exercise also stimulates glucose uptake by (i) promoting insulin action via increasing use of intracellular fatty acids, (ii) improving insulin sensitivity, and (iii) stimulating glucose uptake independently from insulin sensitivity (Turcotte and Fisher, 2008). These confirmed effects and associations of exercise with improved insulin sensitivity may explain the improvement in FPG levels shown in the reported results.

This meta-analysis shows an advantageous effect of exercise on FPG, which is in agreement with previous conducted studies, but did not report a significant effect on PPG or HbA_{1c}. As such, future studies are needed to determine the effect of exercise interventions on PPG, HbA_{1c}, and HOMA-IR. Overall, larger effect sizes, higher graded evidence and less heterogeneity was reported in the nutritional supplement-based interventions compared to diet- and exercise interventions. This is likely due to ease of adherence and standardisation of supplements compared to diet and exercise, which are likely more susceptible to changes in routine and circumstance (e.g., extended work hours, family commitments, sickness, etc.). As such, diet- and exercise-based interventions may require greater personalisation and prescribed flexibility to accommodate patient needs.

3.5.4 Strengths and Limitations

This review included six studies that were pilot studies or underpowered to determine significant differences for the primary outcomes of this review (Grant et al., 2011; Hernandez et al., 2016; Valentini et al., 2012; Yao et al., 2015;

Brankston et al., 2004; Qazi et al., 2020). Furthermore, subgroup analysis based on common moderators of GDM risk could not be performed for some of the outcomes. Moreover, the short duration of some of the interventions and late gestational age at which the interventions were started may have limited their impact on glycaemic outcomes. Finally, a very- or low-GRADE quality score for most outcomes (nutritional supplements: FPG and PPG; diet: FPG, PPG, and HbA_{1c}; exercise: PPG, HbA_{1c}, and HOMA-IR) due to limitations in the design of included studies (e.g., allocation concealment, lack of blinding of either outcome assessors or participants, reporting of adherence to the intervention). This could also explain the lack of difference between reported outcomes for intervention and control. Noteworthy, caution is warranted when interpreting the findings and exploring their wider application, due to the individual nature of the studies (i.e., different intervention strategies) within each of the lifestyle categories. Differences in intervention strategies could influence the physiological mechanisms underlying glycaemic control.

The strengths of this review should be noted, as far as we know, this is the first SRMA that shows benefits of nutritional supplement-, dietary-, and exercise interventions on measures of glucose control in GDM, including more recent studies not included by the preceding SRMAs (Aslfalah et al., 2020; Hajimoosayi et al., 2020; Jamilian et al., 2020; Rasmussen et al., 2020; Qazi et al., 2020). Moreover, this SRMA primarily included RCTs, which are the 'gold standard' of study designs. When recruitment and randomisation are carefully considered in the design of a study, causality can be inferred to an extent that is not possible in other designs, which are more susceptible to confounding and biases. Regardless of the strengths, this study design has its limitations: (i) RCTs are costly, restricting study duration and sample size; (ii) RCTs are often not generalizable due to limited sample size and restricted recruitment strategies; (iii) it is not always possible to assess the long-term effect of a nutritional supplement, diet, or exercise on a health outcome. Overall, this SRMA included substantial number of participants with varied backgrounds and examines the effectiveness of lifestyle interventions on maternal glycaemic control; ultimately, reducing the risk of adverse perinatal outcomes.

3.5.5 Conclusion

This meta-analysis highlights the key role of nutritional supplements, diet, and exercise for the management of GDM and shows promising advantageous effects on measures of maternal glucose control – i.e., FPG, PPG and HOMA-IR. HOMA-IR had largest significant effect sizes, least heterogeneity and best GRADE. Future RCTs should consider incorporating HOMA-IR as an outcome in the study design and perhaps should combine the different intervention types. Furthermore, no RCTs in women with pre-gestational T1DM or T2DM were identified. Demonstrating there is a need for large well-designed RCTs that clarify the most effective lifestyle intervention or combination across a range of outcomes in women with all diabetes types during pregnancy and ideally incorporate longer term outcomes in mothers and offspring, to eventually develop more suitable lifestyle recommendations for women with maternal diabetes.

3.6 Summary

- This meta-analysis included 26 studies and builds on a previous SRMA with 21 RCTs. However, all identified studies were conducted in women with GDM, demonstrating the lack of lifestyle intervention studies in pregnant women with pre-gestational T1DM or T2DM.
- This meta-analysis highlights the key role of nutritional supplements, diet, and exercise in the management of GDM and shows promising advantageous effects on measures of dysglycaemia; (i) nutritional supplements can reduce FPG and IR, (ii) diet can reduce IR and has potential for reducing FPG, and (iii) exercise can reduce FPG. Maternal age and gestational age may be effect modifiers of lifestyle-interventions on maternal glycaemia, suggesting that interventions might be more effective in earlier in pregnancy and in younger women. Given the magnitude of effect and the individual nature of the studies within each of the lifestyle categories, caution is warranted when interpreting the findings and exploring their wider application.
- Future studies should focus on larger well-designed RCTs that clarify the most effective lifestyle intervention or combination across a range of outcomes in women with pre-gestational T1DM, T2DM, and GDM during pregnancy, ideally incorporating longer term outcomes in mothers and

offspring. Thereby, more suitable lifestyle recommendations for diabetes in pregnancy can be developed.

Chapter 4

Relationship of Dietary and Pharmacological Treatment on 24-hr Continuous Measures of Glycaemia in Gestational Diabetes Mellitus: an Observational Study

Authors' original report of the study submitted as Dingena, C. F., Holmes, M. J., Campbell, M. D., Cade, J. E., Scott, E. M. and Zulyniak, M. A. 2023b. Observational assessments of the relationship of dietary and pharmacological treatment on continuous measures of dysglycemia over 24 hours in women with gestational diabetes. *Frontiers in Endocrinology*, **14**. <https://doi.org/10.3389/fendo.2023.1065985>

What do we know? *Chapter 1* discussed the importance of CGM as a 'new' tool in the management of glucose control in DIP. Furthermore, in *Chapter 3*, the key role of lifestyle interventions in the management of GDM was highlighted and showed promising advantageous effects on measures of glycaemia.

Key issues: While previous studies have uncovered associations between CGM-defined dysglycaemia and maternal, and infant offspring health, they were not yet able to uncover mealtime periods of a day when dysglycaemia is most likely to be observed and the relationship between clinical care (i.e., diet/exercise vs diet/exercise with pharmacological treatment) to glucose control over a 24-hour period.

Aims: To characterise glycaemic control over 24-hrs, using CGM metrics, and evaluate the effect of clinical care – diet (i.e., macronutrient intake) and treatment (i.e., diet with or without metformin) – on dysglycaemia in a diverse population of women with gestational diabetes.

Thesis implications: The planned main study of this PhD, which will be discussed in *Chapter 5*, aimed to assess the role of diet as a mediator of dysglycaemia in early, mid, and late pregnancy by providing standardised meals in a nested randomized cross-over study. Results of this *Chapter 4* informed the dietary composition (i.e., which macronutrient to focus on) and timing of the standardized meals.

4.1 Abstract

Recent studies using CGM in GDM pregnancies highlight the importance of managing dysglycaemia over a 24-hr period. However, the effect of current treatment methods on dysglycaemia and timing of disrupted glucose control over 24-hrs are currently unknown. This study aimed to i) characterise CGM metrics over 24-hrs and ii) examine the moderating effect of treatment strategy (i.e., diet alone or diet+metformin) in women with GDM.

CGM data from 128 women with GDM in antenatal diabetes clinics were retrospectively analysed. CGM was measured for 7-days between 30-32 weeks gestation. Non-parametric tests were performed to evaluate differences of CGM between periods of day (6-hr periods; morning, afternoon, evening, and overnight) and between treatment methods (i.e., diet with or without metformin). In addition, an exploratory analysis in a subgroup of 34 of participants was performed to investigate the association between self-reported macronutrient intake and glycaemic control.

Glycaemic control significantly differed throughout a 24-hr period, glucose levels during the day (i.e., morning to evening; $P < 0.001$) were significantly higher (i.e., mean blood glucose and AUC) and more variable (i.e., SD and CV) than overnight glucose levels. Morning showed the highest amount of glycaemic variability (CV; 8.4% vs 6.5%, $p < 0.001$ and SD; 0.49 mmol/L vs 0.38 mmol/L, $p < 0.001$). When comparing treatment methods, mean glucose (6.09 vs 5.65 mmol/L; $p < 0.001$) and AUC_{glucose} (8760.8 vs 8115.1 mmol/L.min⁻¹, $p < 0.001$) were significantly higher in diet+metformin compared to diet alone. Finally, the exploratory analysis demonstrated a favourable association between higher protein intake (+1SD or +92 kcal/day) and lower mean glucose (-0.91 mmol/L, $p = 0.020$) and total AUC_{glucose} (1209.6 mmol/L.min⁻¹, $p = 0.021$).

To conclude, glycaemia varies considerably across a day, with morning glycaemia demonstrating greatest level of variability. Additionally, this study confirms that individuals assigned to diet with metformin have greater difficulty managing their glucose control and the results suggest that increased dietary protein may assist in improving glucose control. However, future work is needed to investigate the benefit of increased protein intake on management of dysglycaemia.

4.2 Background

Pregnancy induces a natural state of IR to shuttle a greater proportion of maternal nutrients to the infant for growth and development (Powe et al., 2019). However, in up to 18% of UK pregnancies (Law et al., 2019; Webber et al., 2015) this metabolic shift leads to unhealthy increases in blood glucose levels, known as GDM, risking the health of the mother and growing foetus (Powe et al., 2019; Kampmann et al., 2019b; Salzer et al., 2015a; Filardi et al., 2019). Treatment aims to control maternal glucose levels and mitigate adverse pregnancy outcomes, resulting in improved long-term maternal and offspring health (Feig et al., 2002). First-line treatment for GDM typically consists of dietary and lifestyle education, with pharmacological therapy incorporated if glycaemic control remains unimproved (Powe et al., 2019; Schaefer-Graf et al., 2018).

Most common nutritional management focusses on diets consisting of low GI foods and reduced overall carbohydrate intake (Powe et al., 2019; Schaefer-Graf et al., 2018) but no consensus on the best nutritional approach has been agreed (Feig et al., 2020; McCance, 2015). Clinical recommendations in the UK focus on improving carbohydrate quality and reducing overall carbohydrate intake (Webber et al., 2015). While replacing processed carbohydrates with higher-quality carbohydrates, and lower overall carbohydrate intake can help to control glucose levels, its effectiveness on managing dysglycaemia is not consistent between populations, this has been demonstrated in a recent meta-analysis (Xu et al., 2020). The study showed high levels of heterogeneity (>60%) of low GI diets on fasting and postprandial glucose levels (Xu et al., 2020). This inconsistency may be present because trials often prescribe specific low-GI nutrients to be consumed at defined times over a 24-hour period, while real-life meals are often mixtures of foods consumed at various points throughout the day (Vega-López et al., 2007; Zeevi et al., 2015b; Matthan et al., 2016). Furthermore, previous research has demonstrated that dietary protein and preload attenuate the subsequent rise in the postprandial glucose response (Meng et al., 2017b; Meng et al., 2017a). However, real meals consist of mixed macronutrients consumed at different times of the day, suggesting that a single measure of PPGR may be inadequate to characterise the full effect of diet on dysglycaemia in a diverse population.

Randomised controlled trials suggest that approximately 80% of women with GDM achieve euglycaemia through diet and lifestyle modification alone (Bashir et al., 2020). However, in cases where management of dysglycaemia is more difficult, pharmacological therapy will be initiated. Metformin, an oral anti-hyperglycaemic drug, is used as first-line pharmacological treatment for glycemic control in T2DM for decades (Zhang et al., 2021; Joseph, 2021). The UK clinical guidelines recommend metformin also as first-line treatment in the pharmacological management of dysglycaemia in women with GDM (Webber et al., 2015). Metformin has additional benefits linked to reduced gestational weight gain, maternal hypertensive disorders, macrosomia, neonatal hypoglycaemia, and intensive care admissions (Webber et al., 2015). Current evidence suggests no difference in standard measures of glycaemia in the mother or neonatal outcomes after delivery in women treated with diet or metformin (Simeonova-Krstevska et al., 2018).

Maternal glucose is dynamic, with glucose tolerance and insulin sensitivity varying over a 24-hour period (Scott et al., 2020a; Law et al., 2015; Tan and Scott, 2014), and emerging evidence suggests that glycaemic peaks, troughs and patterns rather than single measures of glycaemia may be more predictive of disrupted glucose control and provide novel information regarding maternal and offspring health risks (Scott et al., 2020a; Law et al., 2015). These details are captured by CGM, which repeatedly record glucose measures in close succession (every 5 minutes) over a specific period of time (days or weeks), and offer detailed records of glucose levels and fluctuations (Danne et al., 2017). A recent study demonstrated that CGM was capable to uncover novel associations between CGM-defined markers of dysglycaemia at (i) 12-weeks' gestation with infant health outcomes (i.e., preterm birth: OR = 1.52 [1.08 , 2.13]; large-for-gestational age: OR = 1.49 [1.06 , 2.08]) and (ii) 24 -week gestation with maternal outcomes (pre-eclampsia: OR = 1.98 [1.17 , 3.37]) (Meek et al., 2021). Suggesting that CGM can (i) offer new information regarding associations between disrupted glycaemic control and maternal and offspring health, and (ii) be used to inform and direct care more accurately at an earlier point of pregnancy.

Interestingly, the relationship between lifestyle treatment with or without metformin to glucose spikes and variability over a 24-hour period in a diverse population of women with GDM is still unclear. Furthermore, examining which

periods of the day are associated with dysglycaemia would be of interest for improving mealtime glycaemia in GDM. Evaluation of these associations could offer novel insights regarding treatment strategies (i.e., diet with or without metformin) as mediators of dysglycaemia in GDM pregnancies. Therefore, this study aimed to identify key time points during the day of disrupted glucose control, and the relationship of treatment and dietary mediators to this disrupted glucose control in a diverse population of pregnant women with GDM.

4.3 Methods

4.3.1 Study Participants

Participants between 18 and 45 years of age with GDM and a singleton pregnancy, were recruited from antenatal diabetes clinics in Leeds Teaching Hospitals Trust (LTHT). GDM was diagnosed according to the NICE guideline criteria — i.e., fasting glucose ≥ 5.6 mmol/L (≤ 100.8 mg/dL) and/or 2-hr glucose ≥ 7.8 mmol/L (≥ 140.4 mg/dL) after a 75-g oral glucose tolerance test at ~26 weeks of gestation (Webber et al., 2015). All women were advised to aim for self-monitored blood glucose targets of fasting glucose ≤ 5.3 mmol/L and 1-hr post meal ≤ 7.8 mmol/L (Webber et al., 2015; Law et al., 2019), as per clinical guidelines. Diet and lifestyle modifications were implemented as first-line therapy and with metformin and/or insulin as second-line therapy, if glucose targets are not achieved. NICE guidelines state that if blood glucose targets are not achieved with diet and lifestyle changes within 1 to 2 weeks, metformin will be offered (Webber et al., 2015). All women with GDM attending the antenatal diabetes clinic at LTHT were invited to participate. Women were excluded if they were diagnosed with a physical disease (e.g. overt diabetes complications, cancer, gut mobility or digestion disorder) or psychological disease/mental disorder (e.g. eating disorders) likely to interfere with the conduct of the study, and did not speak English. More detail on study participants and recruitment can be found in the original article by Law et al. (2019).

4.3.2 Study Design

Secondary retrospective analysis of an observational cohort of 162 pregnant women with GDM (Law et al., 2019). The original aim of this study was to examine the role of temporal glucose variation on the development of LGA infants in

women with treated GDM. However, some individuals were excluded due to incomplete participant data and/or >30% missing CGM data across the 7 days, resulting in a total number of 128 participants included for analysis (**Figure 4.1**). CGM data were collected between 16/01/2014 and 23/08/2016 at the earliest convenient time point following GDM testing and diagnosis between 26-28 weeks' gestation (typically at 30-32 weeks' gestation). All women provided written informed consent. The study was approved by the Yorkshire and Humber Regional Ethics Committee (13/YH/0268) and NHS Health Research Authority (NRES) Committee South Central–Oxford (14/SC/1267).

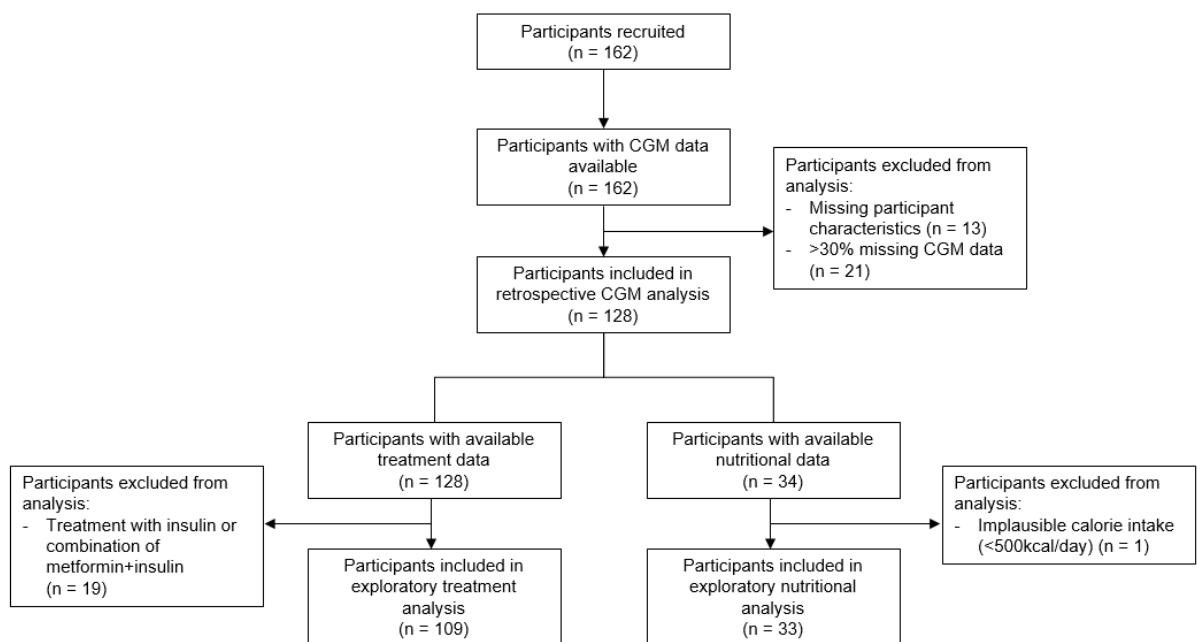


Figure 4.1: Participant flowchart.

4.3.3 Continuous Glucose Monitoring

The iPro2 (Medtronic) CGM device was used for monitoring of glucose levels. The CGM data were calibrated by simultaneous self-monitoring of blood glucose, using approved and standardised blood glucose meters and test strips (Contour XT; Bayer) (Law et al., 2019). Data were anonymised using a unique identification number for each participant and was downloaded via CareLink (Medtronic) for analysis. Glucose levels were captured every 5 minutes for 24 hours and 7 days by the CGM device, providing 288 measures every day for 7 days. Due to missing measurements across the 7-day period and unequal number of total measurements between participants, the individual time-point measurements

were averaged across 7 days to be able to analyse mean glycaemic control over a 24-hr period. This provided 288 average measures of glucose levels over a 24-hr period.

To analyse temporal differences and key time points across the 24-hr day, the CGM glucose data were analysed by splitting the data into four equal periods of six hours (e.g., morning 06:00-11:55, afternoon 12:00-17:55, evening 18:00-23.55, and overnight 00:00-05.55). These windows were chosen so that the all day time-periods (e.g., morning, afternoon and evening) include pre- and postprandial glucose levels, and the overnight time-period monitors a sleep cycle and a continuous fasted state. To evaluate dysglycaemia, the primary outcome of interest was the coefficient of variation (CV), the most commonly used CGM marker for glycaemic variation.

$$CV = \frac{\sigma}{\mu} * 100, \quad \sigma = \text{standard deviation and } \mu = \text{mean}$$

Although, additional indices were examined for the full 24 hour day and for each period, including mean glucose levels, standard deviation (SD), area under the curve (AUC_{glucose}) and incremental area under the curve (iAUC_{glucose}), which quantifies the deviation of glucose levels from baseline over given length of time, and the percentage of time spent within the pregnancy glucose target range (TIR; 3.5–7.8 mmol/L [70.2– 140.4 mg/dL]), time spent above (TAR; >7.8 mmol/L [\geq 140.4 mg/dL]) and below (TBR; <3.5 mmol/L [\leq 70.2 mg/dL]) target range (Danne et al., 2017).

4.3.4 Nutritional Data

For exploratory analysis, complete nutritional information was available in a subgroup of 34 of the 128 women with ‘matching’ CGM data (**Figure 4.1**). Average daily dietary intake data were collected using an online food diary (myfood24) (Gianfrancesco et al., 2018). Participants’ instructions stated to complete the online dietary record for 5 days. Dietary intake was recorded as mean total grams (g) and/or kilocalories (Kcal) per day. After removal of 1 participant with an implausible total kilocalorie intake <500 kcal/day (NutriGen Alliance et al., 2016), the nutrient residual model was utilised to perform tests for linear association between individual macronutrients and glycaemic measures in

33 participants (Willett et al., 1997), after adjustment for maternal age, ethnicity, parity, maternal BMI, and weeks of gestation (Zhang et al., 2006; Van Leeuwen et al., 2010). Briefly, to explain the nutrient residual model, this model reduces confounding by using the residuals of total energy intake, which represent the difference between each individual's actual intake and the intake predicted by their total energy intake, thereby removing the variation caused by total energy intake rather than absolute intake (Willett et al., 1997). Total Kcal intake per day for each participant was standardised to the average energy intake per day within our study (1500 Kcal/day).

To assess the association of macronutrients and glycemic control, a multiple variable regression models for each CGM metric (i.e., mean glucose, SD, CV, AUC, iAUC, TIR, TAR or TBR) was constructed. Each model CGM model included all macronutrients – i.e., total carbohydrate intake (Kcal) + total fat intake (Kcal) + total energy intake (Kcal) – and covariates (i.e., maternal age, ethnicity, parity, maternal BMI, and weeks of gestation). This model permits the assessment of substituting carbohydrates, fats, or proteins (reflected by total energy intake) with an isocaloric equivalent quantity of the other macronutrients. Specifically, these models examine the association of each macronutrient independently with CGM metrics, when all other variables (i.e., other macronutrients, energy, and covariates) are held constant. For example, with three macronutrient sources of energy, when 'carbohydrates' and 'fats' are held constant, the increase in the 'calorie' variable represents an increase for 'protein' (Willett et al., 1997). Only macronutrients were assessed, due to limited and incomplete dietary intake data.

4.3.5 Statistical Analysis

Friedman's test and pairwise Wilcoxon signed rank test with Bonferroni corrections for multiple comparisons between periods of the day were performed. Because of visually apparent asymmetric data, non-parametric tests were applied. Twenty-one or more participants between comparison groups were required, to achieve 80% power. Statistical power was calculated using recent evidence suggesting a difference in effect size of 0.924 (Cohen's d) on mean glucose between diet alone and diet+metformin (Afandi et al., 2017). To assess the association between dietary macronutrients and glycaemic control, multiple variable linear regression analyses were performed and adjusted for maternal

age, ethnicity, parity, maternal BMI, and gestational week. Furthermore, to evaluate the association between adverse pregnancy outcome (e.g., birthweight) and maternal glycaemic control over a 24-hr period, exploratory post-hoc analysis was conducted. Using 90th and 10th percentile adjusted for sex and gestational age, offspring was categorised in low (small for gestational age – SGA), normal (normal for gestational age – NGA), and high (large for gestational age – LGA) birthweight groups. The Cook's Distance was used for influential outlier assessment. Statistical significance was set at $p < 0.05$. All statistical analyses were conducted in RStudio (version 4.0.3), and all figures were created in GraphPad Prism 9.

4.4 Results

Glucose measures were collected every 5 minutes, over a 24-hour period, yielding a total of 288 glucose measurements per individual, resulted in a total of 36,864 glucose measurements for 128 women. The average age and BMI of participants was 33 years and 30.6 kg/m². The majority of participants self-identified as White European (61%) and their dysglycaemia was managed with diet alone (n=58), diet with metformin (n=51), diet with insulin (n=2), or diet with metformin and insulin (n=17). Analysis on treatment effect was limited to diet alone and diet with metformin groups (i.e., diet or diet+metformin), due to small numbers of individuals and inadequate power of insulin and metformin+insulin treatment groups (i.e., <21 participants). Approximately 30% of women, 34 out of 128 with available CGM data, used myfood24 to record their dietary intake. Participant characteristics are further summarised in **Table 4.1: Participant Characteristics**.

Table 4.1: Participant characteristics

	Total group (n=128)	Nutritional data subgroup (n=34)	Diet subgroup (n=58)	Diet+metformin subgroup (n=51)
Characteristics				
<i>Age (yrs)</i>	33.0 ± 4.5	32.2 ± 5.0	32.8 ± 4.8	33.4 ± 5.1
<i>BMI at start of pregnancy(kg/m²)</i>	30.5 ± 6.1	29.7 ± 5.9	28.9 ± 5.7	31.1 ± 6.4
<i>Gestational week</i>	31.1 ± 1.2	31.5 ± 1.2	31.1 ± 1.3	31.1 ± 1.1
<i>Parity</i>	1.0 ± 1.1	1.0 ± 0.6	1 ± 1.3	1 ± 0.9
Treatment				
<i>Diet</i>	58 (53%)	18 (53%)	58 (100%)	NA
<i>Diet+metformin</i>	51 (47%)	16 (47%)	NA	51 (100%)
Ethnicity				
<i>White European</i>	78 (61%)	25 (74%)	34 (59%)	27 (53%)
<i>Ethnic minority (Black or Asian)</i>	50 (39%)	9 (26%)	24 (41%)	24 (47%)

For characteristics, data reported as mean ± standard deviation (SD) per day of each nutrient and total energy intake. For treatment and ethnicity, number of participants (n) is reported and proportion of total participants is reported in parentheses. Yrs, years; NA, Not applicable.

4.4.1 Continuous Glucose Monitoring Data

A significant effect of ‘time of day’ was identified for the majority of CGM metrics – including, mean glucose, SD, CV, AUC, iAUC, and TAR (**Figure and Table 4.2**). Therefore, pairwise analyses were performed on all CGM metrics. For measures of glycaemic variability (i.e., CV and SD), glucose levels were relatively stable during the day but decreased ‘overnight’ (**Figure and Table 4.2**). Contrarily, absolute glucose and total AUC increased steadily from morning to evening (for both mean glucose and AUC_{glucose}; each time comparisons $p > 0.001$). Returning to measures of glycemic variability, SD and CV of glucose were greatest in the morning and steadily decreased towards the lowest levels overnight (SD; 0.49mmol/L vs 0.30mmol/L and CV; 8.41% vs 4.99%, $p < 0.001$). iAUC_{glucose} fluctuated over the 24-hour period, with the highest levels recorded in the morning and evening (1244.5 vs 1311.6 mmol/L.min⁻¹, $p = 0.87$), reductions in

the afternoon (1106.0 mmol/L.min⁻¹, $p < 0.001$) and recording the lowest levels overnight (604.9 mmol/L.min⁻¹, $p < 0.001$) (**Table 4.2**). When orienting on the time-in-ranges, the Friedman test reported no significant differences when glucose levels were within (TIR), or below (TBR) a specific range, and no differences were confirmed between time-of-day either (**Table 4.2**). However, TAR significantly differs across the day and was highest during the evening (TAR evening; 4.41%, $p = 0.018$).

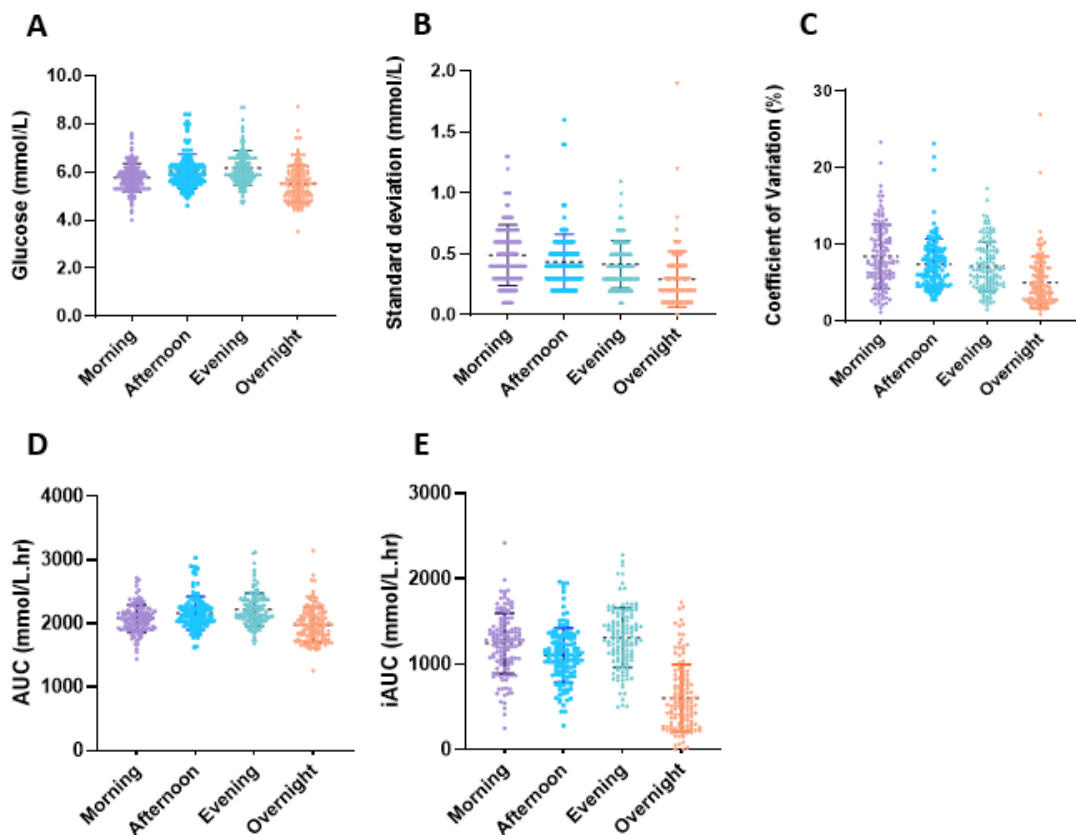


Figure 4.2: Scatter plots of the CGM metrics and time-of-day. A panel of 5 metrics are depicted for visual aid: A, mean glucose; B, standard deviation (SD) of glucose; C, coefficient of variation (CV) of glucose; D, area under the curve ($AUC_{glucose}$) of glucose; E, incremental AUC of glucose ($iAUC_{glucose}$). Time in ranges are not depicted as these were non-significant.

Table 4.2: Summary of measures of continuous glucose monitoring over a 24-hour period.

	Daily Average	Morning (6:00-11:55)	Afternoon (12:00-17:55)	Evening (18:00-23:55)	Overnight (24:00-5:55)
Glucose (mmol/L)					
<i>Mean±SD</i>	5.86±0.64	5.76±0.60 ^a	6.02±0.72 ^b	6.17±0.71 ^c	5.51±0.64 ^d
<i>95% CI</i>	[5.75 , 5.97]	[5.66 , 5.87]	[5.89 , 6.14]	[6.04 , 6.29]	[5.38 , 5.64]
Standard deviation of Glucose (mmol/L)					
<i>Mean±SD</i>	0.57±0.21	0.49±0.45 ^a	0.43±0.22 ^b	0.41±0.20 ^{b,c}	0.30±0.22 ^d
<i>95% CI</i>	[0.54 , 0.61]	[0.45, 0.53]	[0.40 , 0.47]	[0.38 , 0.45]	[0.26 , 0.33]
Coefficient of variation of Glucose (%)					
<i>Mean±SD</i>	9.76±3.36	8.41±4.17 ^a	7.35±3.32 ^b	7.08±3.22 ^{b,c}	4.99±3.38 ^d
<i>95% CI</i>	[9.18 , 10.35]	[7.69 , 9.14]	[6.78 , 7.93]	[6.52 , 7.64]	[4.40 , 5.58]
Area Under the Curve of Glucose (AUC; mmol/L.min⁻¹)					
<i>Mean±SD</i>	8433.8±913.9	2073.7±216.8 ^a	2160.5±260.8 ^b	2218.6±255.8 ^c	1980.9±276.9 ^d
<i>95% CI</i>	[8275.4, 8592.1]	[2036.2, 2111.3]	[2115.4, 2205.7]	[2174.3, 2262.9]	[1932.9 , 2028.8]
Incremental Area Under the Curve of Glucose (iAUC; mmol/L.min⁻¹)					
<i>Mean±SD</i>	3606.4±1034.5	1244.5±354.3 ^a	1106.0±318.1 ^b	1311.6±349.0 ^{a,c}	604.9±393.1 ^d
<i>95% CI</i>	[3427.2, 3785.6]	[1183.1, 1305.9]	[1050.8, 1161.1]	[1251.1, 1372.0]	[536.8 , 673.0]
Time in Range Metrics					
<i>TIR (% of day)</i>	96.91 ±9.35	98.46±5.70 ^a	96.03±14.55 ^a	95.59±15.17 ^a	97.57±11.92 ^a
<i>TAR (% of day)</i>	2.90 ±9.16	1.5±5.69 ^a	3.97±14.55 ^a	4.41±15.17 ^a	1.71±8.88 ^a
<i>TBR (% of day)</i>	0.19 ±2.15	0.04±0.49 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.72±8.10 ^a

All time metrics are mean measures of 7-days over a 24-hour period: TIR, time with glucose level measured within 3.5-7.8 mmol/L; TAR, time with glucose level measured above 7.8mmol/L; TBR, time with glucose level measured below

3.5 mmol/L. Significant differences between times of day ($P<0.05$) for individual metrics are denoted by different superscripts (*a, b, c, d*).

4.4.2 Exploratory Analysis

4.4.2.1 Treatment

The post-hoc analysis of treatment included 109 women (subgroups; n=58 in diet alone and n=51 in diet+metformin). Exploratory analysis revealed significant associations between treatment and glucose metrics adjusted for covariates (i.e., maternal age, BMI, gestational week, parity and ethnicity) (mean glucose; $F [3,1] = 27.3$, $p<0.001$ and AUC_{glucose} ; $F[3,1] = 28.9$, $p<0.001$, respectively), BMI and gestational week were found to be significant confounders. Both mean glucose (5.65 vs 5.97mmol/L) and total AUC_{glucose} (8115.1 vs 8586.1 mmol/L.min⁻¹) were significantly higher in metformin subgroup. However, no interactions between time-of-day and treatment on CGM metrics were found.

4.4.2.2 Nutrients

The exploratory analysis of nutritional data included a total of 33 women (**Figure 4.1**). Of the 8 assessed CGM metrics (i.e., mean glucose, SD, CV, AUC, iAUC, TIR, TAR and TBR), mean glucose and AUC_{glucose} were the only metrics showing significant associations with dietary mediators (**Tables 4.3 and 4.4**). To clarify, the nutrient residual models applied assesses the association of each macronutrient with glycaemic metrics, when the other macronutrients are held at a constant level – e.g., carbohydrates when intake of dietary fat and protein are held constant. There are three macronutrient sources of energy (i.e., carbohydrates, fats, and protein), when ‘carbohydrates’ and ‘fats’ are held constant, any increase in the ‘calorie’ variable represents an increase in ‘protein’ (Willett et al., 1997). After adjusting for known confounders (i.e., maternal age, BMI, gestational age at CGM measurement, parity, ethnicity, and treatment), an increase (+1 SD) of fats or carbohydrates associated with higher mean glucose and AUC_{glucose} , while dietary protein (+1SD) associated with reduced mean glucose and AUC_{glucose} (-0.91mmol/L; $p=0.02$ and -1296 mmol/L.min⁻¹; $p=0.021$) (**Table 4.4**). These results postulate that increasing dietary protein intake reduces mean 24-hr glucose levels. A post-hoc analysis suggested the multiple variable model was well powered to minimise the risk of for type II errors (i.e., false

negatives) for protein as a covariate (power >80%) but was not adequately powered (< 50%) to minimize the risk for fats and carbohydrates.

Table 4.3: Average values of self-reported macronutrients intake in a subsample of 34 participants (with available dietary records).

	Daily intake (kcal/day) (% total kcal/day)	Daily intake (gram/day)
<i>Protein</i>	246 ± 92 (16%)	61 ± 26
<i>Fats</i>	577 ± 290 (38%)	64 ± 33
<i>Carbohydrates</i>	716 ± 311 (47%)	176 ± 74
<i>Non-sugar</i>	474 ± 208	117 ± 50
<i>Sugar</i>	242 ± 179	59 ± 43
<i>Total intake</i>	1513 ± 517	N/A

Data reported as mean intake ± standard deviation (SD) per day of each nutrient and total energy intake. Mean proportion of nutrients of total caloric intake reported in parentheses.

Table 4.4: Multivariable regression of dietary mediators (carbohydrates, fats, and protein) and glycaemia stratified by outcome metric of 33 participants (with dietary records and CGM metrics available).

Variables	Mean glucose (mmol/L)		AUC (mmol/L.min ⁻¹)	
	β (95% CI)	p-value	β (95% CI)	p-value
Age	-0.015 (-0.05, 0.02)	0.38	-22.1 (-70.2, 25.9)	0.38
Maternal BMI	0.022 (-0.005, 0.05)	0.12	31.8 (-7.1, 70.7)	0.12
Gestational week	0.009 (-0.12, 0.14)	0.89	12.5 (-173.3, 198.3)	0.90
Parity	0.093 (-0.24, 0.28)	0.49	132.5 (-240.4, 505.3)	0.50
Ethnicity	0.22 (-0.36, 0.4)	0.93	23.2 (-526.2, 572.6)	0.93
Treatment type	0.17 (-0.08, 0.52)	0.17	315.5 (-121.5, 752.5)	0.17
Adjusted carbohydrates	0.63 (0.13, 1.1)	0.021	887.9 (173.6, 1602.2)	0.023
Adjusted fats	0.49 (0.04, 0.93)	0.043	694.7 (48.5, 1340.8)	0.046
Adjusted protein	-0.91 (-0.2, -1.6)	0.02	-1296.0 (-265.0, -2327.0)	0.021

Mean glucose $R^2 = 0.32$, AUC $R^2 = 0.18$. Treatment was coded as follows: 0=diet, 1=diet+metformin. Parity was reported as having 0, 1, 2, 3, 4, 5 or 6 children. Ethnicity was coded as: 0=White and 1=Ethnic minority (e.g., Asian, Black African). CI = confidence interval. Significant associations ($p < 0.05$) in bold.

4.4.2.3 Pregnancy Outcome

To examine the associations between adverse pregnancy outcomes and dysglycaemia at different times-of-day, exploratory post-hoc analysis was conducted assessing birthweight of 126 infants, measures of glycaemic control of their mothers and time-of-day. Using previously described methods, infants were categorised as small-, normal- and large for gestational age (SGA: 15, NGA: 95 and LGA: 16 infants). Of the 8 CGM metrics examined as outcome, no significant interactions were found between the birthweight groups (i.e., SGA, NGA and LGA) and time-of-day (i.e., morning, afternoon, evening and overnight) on measures on glycaemia (BG: $F[6,492] = 0.346$, $p = 0.88$; SD: $F[6,492] = 0.354$, $p = 0.91$; CV: $F[6,492] = 0.765$, $p = 0.59$; AUC_{glucose}: $F[6,492] = 0.394$, $p = 0.88$; iAUC_{glucose}: $F[6,492] = 0.275$, $p = 0.95$; time out of range: $F[6,492] = 0.636$, $p = 0.70$). As an example results of BG as outcome are depicted in a box plot (**Figure**

4.3). The general trend shows that SGA infants are exposed to lowest level of mean glucose and LGA infants are exposed to highest level of mean glucose.

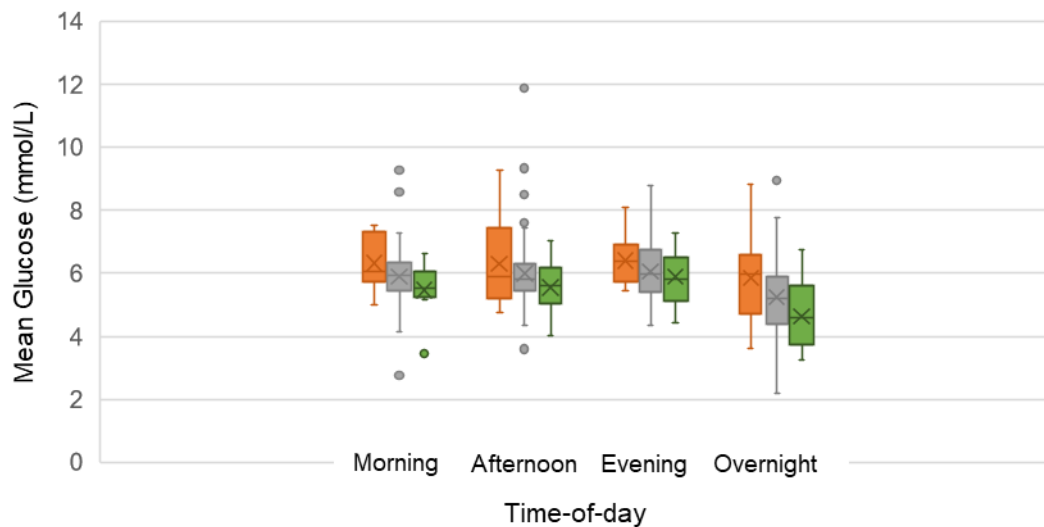


Figure 4.3: Boxplots of mean glucose categorised by birthweight and time-of-day. Birthweight categories are LGA (orange), NGA (grey), and SGA (green).

4.5 Discussion

In an observational cohort of 162 women diagnosed with GDM, this study has demonstrated that (i) CGM offers different methods of assessing glycaemic health; (ii) measures of dysglycaemia vary considerably over a 24-hr period; and (iii) distinct periods of day are prone to lower or higher absolute glucose levels as well as glucose variability. Depending on the CGM metric used, 'morning' and 'overnight' showed to have highest and lowest glucose levels, thus times of greatest dysglycaemia. More specifically, glucose levels were most variable during the day (i.e., morning to evening) but were spent in an acceptable range most of the time ($\approx 95\%$ of the time), while 'overnight' showed extended periods of lower glucose levels with relatively less glucose variability. Additionally, exploratory analysis of the association between treatment type (i.e., diet vs diet+metformin), time-of-day and maternal glucose control demonstrated no significant interaction between treatment type and time-of-day on maternal glycaemia over a mean 24hr period. However, individuals assigned to diet with metformin appeared to have higher levels of dysglycaemia, as reflected by elevated mean glucose and total AUC_{glucose} . Furthermore, no significant

associations or interactions were found between the birthweight groups (i.e., SGA, NGA and LGA), measures of maternal glycaemic control and time-of-day (i.e., morning, afternoon, evening and overnight). Therefore, this study could not formulate conclusions regarding adverse pregnancy outcomes – expressed as birthweight (i.e., SGA and LGA) – and maternal glucose control.

4.5.1 Time-of-day and Glycaemic Variability

Current glycaemic variables commonly used are; fasting glucose levels, mean glucose levels, or coefficient of variation to evaluate glycaemic control or dysglycaemia. This study reports that mean morning, afternoon, and evening glucose levels are significantly higher compared to mean glucose levels overnight. This is in agreement with existing understanding of overnight glycaemic control, detailing that glucose levels typically drop overnight (Zaharieva et al., 2020). Meanwhile, recent work have stipulated that glucose excursions expose a health risk that is independent of mean glucose levels (Zaccardi and Khunti, 2018; Monnier et al., 2008). The proposed standard metric for glycaemic variability is the CV of glucose (Monnier et al., 2008; Danne et al., 2017), which quantifies the magnitude of glycemic variability standardised to mean glucose levels. Despite seeing no difference in mean glucose levels between afternoon and evening, this study shows that CV steadily declines during the day reaching lowest values ‘overnight’. In addition, morning CV was reportedly higher compared to other times-of-day. This agrees with trends observed in non-diabetic men and women (n=60) showing significantly higher ‘daytime’ CV (i.e., 06:00-21:59) compared to ‘overnight’ CV (i.e., 22:00-05:59) (Barua et al., 2021). Although, disagrees with evidence from adolescent boys and girls (n=107; 13.1 ±2.6 years) that suggests CV increases from early morning (i.e., 06:00) and peaks from midday to late-night (i.e., 12:00-23:00) (Zhu et al., 2020). However, the significance in temporal CV patterns was not formally assessed for adolescents, so its importance is uncertain. Other studies suggest that diabetes CV is related with offspring growth in the second trimester in women with T1DM (Kristensen et al., 2019), and may be an indicator of risk of future health complications associated with T2DM (Scott et al., 2020b) – including cardiovascular disease, coronary events, non-cardiovascular mortality, and total mortality (Scott et al., 2020b; Kampmann et al., 2019b). Increased morning GV may be due to higher cortisol levels, as result of the dawn-effect – getting the

body ready for the start of day (Edwards et al., 2001). Cortisol levels are also increased when insulin resistance is present and during pregnancy (de Guia et al., 2014; Vleugels et al., 1986). Circadian/diurnal rhythm in normal glucose tolerance results in i) a higher glucose excursion at dinner than at breakfast after identical meals (Saad et al., 2012; Takahashi et al., 2018); ii) higher insulin sensitivity at waking and reaches the trough during sleep (Schulz et al., 1983); iii) same effect is seen glucose tolerance (la Fleur et al., 2001). Increased morning CV in this study's group of women might also be the result of a lack in regular routine, these women may need to get their other children ready for school and/or get ready for work and may not have time for breakfast. An explanation of why and how glucose levels and GV changes across the day is given; however, this does not take away the fact that morning-time control of glucose levels may be a key point of interest for managing maternal and offspring health.

4.5.2 Treatment, Times-of-day, and Glycaemic Control

Exploratory post-hoc analysis of treatment effect adjusted for confounders (i.e., maternal age, BMI, gestational week, parity and ethnicity) demonstrated a statistically significant relationship of women who respond to first-line therapy and those requiring second-line therapy, showing persistent higher mean glucose levels and total AUC_{glucose} in women treated with diet+metformin compared to women treated with diet alone. Although, BMI and gestational age were found to be significant confounders, mean gestational age did not differ between treatment groups. Higher BMI and later pregnancy have been previously associated with decreased glucose control (Bashir et al., 2020; Salzer et al., 2015a; Martínez et al., 2017). It is important to note that blood glucose levels vary significantly day by day (Martínez et al., 2017); therefore, a lack of significant relationship between the metformin treatment group and other CGM metrics may be observed in this study.

Glycaemic control and variability depend on a variety of different exogenous and endogenous determinants such as (i) elevated IR, hepatic glucose production and insulin antagonistic hormones production; (ii) increased sedentary lifestyle and unhealthy dietary behaviour, and (iii) age related metabolic deterioration (Martínez et al., 2017). Although metformin is the most commonly prescribed anti-hyperglycemic medication for diabetes in the UK, its effectiveness on glycemic control in DIP is only recently being documented.

Noteworthy, metformin is only prescribed when women are failing to achieve glucose targets with diet alone. Therefore, glucose levels in this group are initially higher. Estimates from recent trials suggest that higher doses of metformin can reduce HbA_{1c} by 1–2% (11– 22 mmol/mol) (Hirst et al., 2012). This effect is promising as previous research reported that a 1% reduction in HbA_{1c} is associated with improved maternal and offspring outcomes in women with GDM (Kiefer et al., 2022). Furthermore, a recent study by Bashir et al (Bashir et al., 2020) found that women with GDM on pharmacological treatment were diagnosed earlier than women on dietary treatment, and it is likely that early treatment intensification with diet and metformin has led to reduced foetal glucose levels, hyperinsulinemia and macrosomia.

4.5.3 Dietary Mediators, Times-of-day, and Glycaemic Control

An exploratory subgroup analysis was conducted in 33 of 34 participants with dietary intake available (records for 3 days using myfood24) (Gianfrancesco et al., 2018). According to the recommended daily intakes (RDI) set by the Diabetes Care Programmes (Kapur et al., 2020), carbohydrate and protein intake are both low (i.e., carbohydrates; 47.3% vs up-to 60% and protein; 16.3% vs 15-25%), and fat intake (i.e., 38.1% vs 30-35%) is above recommendations. Of the 8 CGM metrics assessed, mean glucose and total AUC_{glucose} showed significant associations with dietary mediators. The exploratory analysis shows an increase in glucose levels and AUC_{glucose} associated with carbohydrate and fat intake. Various dietary carbohydrates – e.g., glucose, sucrose, cooked starches found in pastas and white bread – are readily digested and absorbed in the small intestines, this contributes to a rapid increase in blood glucose levels (Mustad et al., 2020). Other studies have established that maternal glucose responses can be considerably influenced by the total amount of carbohydrates consumed (Mustad et al., 2020). Increased dietary fat intake – that is high in saturated fat – has been associated with increased PPG levels and circulating fatty acids (FA) (Lichtenstein and Schwab, 2000). Chronic increased level of circulating FAs have been linked to increased IR and inflammation, which both are associated with risk of preeclampsia and preterm delivery (Lichtenstein et al., 2000; Chen et al., 2010). Additionally, previous studies have demonstrated that elevated PPGR contributes to an increased glucose transport to the foetus correlating with infant size and/or adiposity (Mustad et al., 2020).

Furthermore, this study shows that increasing protein intake by 1 standard deviation – while holding dietary carbohydrates and fats quantities constant – is associated with lower mean glucose and total AUC_{glucose} . While current positions and recommendations of major health bodies (National Health Services [UK], Canadian Diabetes Association, the American Diabetes Association, and the European Association for the Study of Diabetes) focus on replacing low-quality processed (i.e., high glycaemic-index) carbohydrates with high-quality (i.e., low glycaemic-index) carbohydrates for diabetic patients, this study positions protein as an additional dietary marker to manage gestational dysglycaemia. The influence of protein on glycaemia is likely to be explained by its more efficacious effect stimulating a rise in glucagon levels than glucose is in suppressing it – meaning that based on weight, protein is 10 times more efficacious than glucose in affecting the glucagon response in normal individuals (Meng et al., 2017a). Previously has been demonstrated that substituting some of the fruit content with slowly digestible starch sources (e.g. legumes and al dente pasta, etc.), and increasing the protein content may result in a diet that is more acceptable for management of T2DM (Gannon et al., 1998).

One limitation taking in account the nature nutritional intake data should be noted – underreporting of the energy intake is present. Underreporting can result in systematic error that can affect the association between dietary pattern and outcome measure; therefore, the method of obtaining energy-adjusted amounts of nutrients is necessary in studies looking for diet-disease associations (Markussen et al., 2016; Mirmiran et al., 2006). Previous studies have recommended that researchers use energy-adjusted amounts of nutrients by residual method in assessing this diet-disease relationship, because these amounts are independent of total energy intake. Controlling for the effect of energy intake, using these energy-adjusted amounts, should account for under- and over-reporters with regard to nutrient intakes (Mirmiran et al., 2006; Poslusna et al. 2009). The residual method of energy adjustment is an adequate tool to decrease the influence of misreporting when interpreting results of studies based on food records and 24-hour recalls (Poslusna et al. 2009). The nutrient residual model describes the actual overall relation of diet to disease, with energy intake having its standard biological meaning and the nutrient residual representing the composition of the diet (Willet et al., 1997). In most populations investigated,

variation in total energy intake will occur due to physical activity, differences in body size, and differences in energy efficiency (Willet et al., 1997). Thus, total energy intake can confound associations with specific nutrients if any of these factors are associated with disease risk (Willet et al., 1997). Analyses to evaluate the relation between dietary composition and risk of disease should be based on an isoenergetic principle, the outcomes of this method can provide focus for nutritional intervention studies and eventually informing real-life implications (Willet et al., 1997). However, future studies should aim to identify the best method of dealing with misreporting when interpreting results. Although this study was not designed to investigate interactions between carbohydrates quality consumed and time of day, it shows that future studies need to be appropriately designed to investigate such an interaction, and report on the importance of timing and nutritional quality of meals to manage dysglycaemia.

4.5.4 Pregnancy Outcomes, Times-of-day, and Glycaemic Control

Although this study was not able to show any associations between adverse pregnancy outcome (i.e., birthweight) and maternal glucose control over a 24-hr period, other studies have linked temporal dysglycaemia and adverse pregnancy outcomes. Recently, a study examined the associations between women with GDM who gave birth to LGA offspring and maternal CGM glucose levels (Law et al., 2019). Mean glucose was significantly higher in women who delivered an LGA infant (6.2 ± 0.6 mmol/L vs. 5.8 ± 0.6 mmol/L, $p = 0.025$). Also, mean nocturnal glucose (i.e., 00:00–06:00h) was significantly higher in mothers of LGA infants (6.0 ± 1.0 mmol/L vs. 5.5 ± 0.8 mmol/L, $p = 0.005$). Mean ‘daytime’ glucose (i.e., 06:00–24:00h) was slightly higher in mothers of LGA infants, but not statistically significant (6.3 ± 0.6 mmol/L vs. 6.0 ± 0.6 mmol/L, $p = 0.058$), which was consistent with the results from the current study. During the night from 00:30–06:30h, mothers who delivered LGA infants presented significantly higher glucose levels compared with those displayed by mothers who did not deliver LGA infants (Law et al., 2019). Possible sources of difference between this study and the study by Law et al. (2019) are i) our aim was to characterise CGM metrics over a 24-hr period related to mealtimes and examine the moderating effect of treatment strategy, and Law et al. aimed to examine the role of temporal glucose variation on the development of LGA; ii) analysing the temporal glucose levels – 6hr-widows around mealtimes vs functional data analysis; and iii) total number of

participants included in final analysis, perhaps due to missing data handling. Demonstrating there is further research needed to make final recommendations regarding timing of glycaemic control and adverse pregnancy outcomes.

4.5.5 Strengths and Limitations

This study has offered insights into temporal changes of dysglycaemia and demonstrated the value of commonly reported CGM metrics, offering unique information to characterise dysglycaemia in women with GDM. However, this study is not without limitations. First, although the study population was ethnically diverse ($\approx 40\%$ non-European ancestry), there was inadequate power to test for ethnic-specific associations. Additionally, all women were diagnosed with GDM according to the NICE criteria (Webber et al., 2015); therefore, the study population may not be representative of women diagnosed with GDM by alternative criteria (e.g., IADPSG criteria) (Coustan, 2013; Behboudi-Gandevani et al., 2019). The CGM data were obtained at one specific time-period of gestation, which may not be representative of glycaemia at other times during the pregnancy. Also, as participants were diagnosed for GDM and recruited at the similar times; therefore, treatment duration did not vary greatly. However, duration of treatment may modify dysglycaemia – this may be evident in a larger sample size. In addition, due to unequal number of total measurements between days and participants, an average of the 7-days data into a 24-hr period was chosen to analyse. While this prevented from assessing glucose shifts over multiple days or comparing weekdays and weekends, it did allow for characterising of time-points in a 24-hour period where glucose excursions were common. Furthermore, no physical activity data were available, thus its influence on the results could not be evaluated. Dietary logs were available only for a subgroup of participants and their mealtimes were not recorded. One of the biggest limitations is the small sample size, this influences the critical discussion and statistical significance of the results. Noteworthy, for the times-of-day and treatment analysis, power of 80% was achieved (21 participants or more per comparison group; $n = 128$ and $n = 109$, respectively). Nonetheless these limitations, the results suggest that timing and the macronutrient content (e.g., increasing dietary protein) of meals are of importance and future investigations of the role of dietary protein and carbohydrate quality on dysglycaemia are warranted.

4.5.6 Conclusion

In summary, these results confirm that CGM is a rich source of information that could detect and quantify periods of dysglycaemia. Additionally, the analysis demonstrated that each of the CGM metrics, offers unique information to identify individual glucose profiles and variability. Thereby, demonstrating the complexity of maternal dysglycaemia, which is not easily summarised by a single glycaemic metric. Highlighting the importance of additional research examining dysglycaemia using multiple and more detailed analysis techniques (including CGM). Moreover, individuals assigned to diet with metformin appeared to have the greatest difficulty managing their glycaemia, suggesting the need for more directed care, and follow-up may benefit this group of individuals. Finally, the exploratory analysis suggests that increased dietary protein intake may assist with dysglycaemia management, and that consideration of both protein and carbohydrate quality may provide optimal support for managing glycaemia in pregnancy.

4.6 Summary

- GDM management typically consists of dietary and lifestyle education, with pharmacological therapy – initially metformin – incorporated if glycaemic control remains unimproved.
- Maternal glucose is dynamic, glucose tolerance and insulin sensitivity vary over a 24-hr period, and evidence suggests that glycaemic peaks, troughs and patterns may be more predictive of disrupted glucose control. However, it remains unclear which periods of the day are prone to dysglycaemia and the relationship between lifestyle treatment with or without metformin to glucose spikes and variability over a 24-hr period in women with GDM.
- Secondary retrospective analysis found that (i) glycaemic control significantly differed throughout a 24-hr period, with morning glucose levels demonstrating greatest level of variability, (ii) results confirmed that individuals assigned to diet+metformin have greater difficulty managing their glucose control, and (iii) exploratory nutritional analysis demonstrated a favourable association between higher protein intake and lower mean glucose and total AUC_{glucose}.

- Future work is needed to investigate the benefit of timing and increased intake of dietary protein on management of dysglycaemia in women with GDM treated with diet alone or diet with pharmacological treatment.

Chapter 5

Individualised Patient Care and Treatment for Maternal Diabetes (INFORMED): Evaluation of an Observational and Randomised Crossover Trial Embedded within Routine Care

Authors' original report of the study submitted as Dingena, C. F., Mahendra, A., Holmes, M. J., Clement, N. S., Scott, E. M., & Zulyniak, M. A. 2023. Protocol for the INFORMED (Individualised Patient Care and Treatment for Maternal Diabetes) Study: a randomised controlled trial embedded within routine care. *BMJ open*, **13**(2), e065388. <http://dx.doi.org/10.1136/bmjopen-2022-065388>

What do we know? Diabetes in pregnancy presents a unique physiological challenge to manage glycaemia. Previous *Chapters 3 and 4* concluded; (i) nutritional lifestyle interventions were linked to improved glucose control but is limited in pre-gestational diabetes, (ii) morning time showed highest level of glycaemic variability and (iii) dietary protein may improve mealtime glycaemia.

Key issues: Pregnant women with diabetes are at greater risk of adverse pregnancy outcomes. (Postprandial) glycaemic control is key to reduce this risk but it is not yet clear (i) how diet and lifestyle moderate the shift in dysglycaemia across the duration of pregnancy or (ii) what aspects of maternal and offspring health are associated.

Aims: to assess the role of diet as mediator of dysglycaemia throughout pregnancy in pre-gestational T1DM and T2DM and the moderating effects of personal, physiological and environmental parameters using CGM and experimental breakfast meals.

Thesis implications: This study was designed to provide the main results and form the basis of the experimental chapters within this thesis. However, due to the COVID-19 pandemic, delays in ethical approval and other sing-offs within the NHS, recruitment was significantly delayed and results have not yet been obtained. Therefore, a fourth study was undertaken (*Chapter 6*).

5.1 Abstract

Evidence suggests that control of (postprandial) glycaemia is key to manage maternal and offspring health in DIP but it is limited regarding (i) the role of diet and lifestyle as mediators of glucose variability and control throughout the course of pregnancy, and (ii) what aspects of maternal and offspring health are associated. Therefore, this study aims to investigate these points more closely in a clinical setting with pregnant women with pre-gestational diabetes (i.e., T1DM and T2DM) using routine clinical data obtained from CGM and experimental breakfast meals.

To investigate these gaps, a cross-over randomised clinical trial has been embedded within routine clinical care. Seventy-six pregnant women with T1DM or T2DM (with or without medication) in their 1st trimester attending their routine antenatal appointments at Diabetes in Pregnancy Clinics of NHS Leeds Teaching Hospitals Trust (LTHT) will be recruited. Following informed consent, data on women's health, glycaemia, pregnancy, and delivery will be shared. At each visit in the 1st (~10-12 weeks), 2nd (~18-20 weeks), and 3rd (~28-34 weeks) trimester (i) data on lifestyle and diet via questionnaires, (ii) blood samples, and (iii) urine samples will be obtained. Additionally, participants will be asked to consume two blinded meal replacements in duplicate at second and 3rd trimester. Glycaemia will be assessed by CGM which is part of routine care. The primary outcome is the effect of experimental meals (i.e., high versus low protein) on postprandial glycaemia. Secondary outcomes include (i) the association between dysglycaemia and maternal and newborn health, and (ii) the association between maternal metabolic profiles in early pregnancy with dysglycaemia in later pregnancy.

This study was reviewed and approved by the Leeds East Research Ethics Committee (REC: 21/NE/0196) and NHS. In addition, this study was registered at clinicaltrials.gov (ISRCTN 57579163 – Protocol CPMS 50813). Results will be published in peer-reviewed journals and disseminated to participants and the wider public. Recruitment has started, but was significantly delayed due to the COVID-19 pandemic, and delays in ethics approval and other NHS sign-offs. Unfortunately, no participants have been yet recruited and thus no results have been obtained.

5.2 Background

Globally, the prevalence of DIP is rising, affecting ~17% of all pregnancies (Chivese et al., 2021). Pregnancy naturally induces a unique state of glucose metabolism resulting in decreased insulin sensitivity and increased insulin resistance, to shuttle more nutrients to the growing foetus; however, in women with DIP, disrupted glucose metabolism (i.e., excessive IR and persistent hyperglycaemia) elevates the risk of adverse pregnancy outcomes for both mother and her offspring (Symonds and Ramsay, 2010; Lapolla et al., 2019; Murphy et al., 2021). Compared to women without diabetes, women with DIP are at increased risk of pre-eclampsia, preterm delivery, and mortality, while their offspring are at increased risk of unhealthy birthweight (i.e., small for gestational age: <2.5 kg or large for gestational age: >4.5 kg), dysglycaemia, birth injuries, and elevated risk of obesity/overweight, T2DM and CVD in later life (International Diabetes Federation, 2019; Tennant et al., 2014; Macintosh et al., 2006).

Mealtime glucose represents a key target for improving glycaemic control, as uncontrolled postprandial glucose responses have been linked to adverse pregnancy outcomes (Tennant et al., 2014; Macintosh et al., 2006). The NICE dietary guidelines in the UK primarily focus on carbohydrate content and quality as part of a balanced diet including wholegrains, fruits and vegetables to manage maternal glucose control (Webber et al., 2015). Previous research supports a healthy diet and lifestyle – including wholegrains, vegetables and fruits, and regular physical activity – as the foundation for managing DIP, which is effective in 70-85% of women with DIP (National Institute for Clinical Excellence, 2015; Smith et al., 2021; American Diabetes Association, 2017). Although low GI diets are known to reduce mean glucose levels, their effect on reducing hypo- and hyperglycaemic events and ability to reduce risk of pregnancy complications, and why guidelines are not effective in the remaining 30-15% is not clearly established (Yu et al., 2019b; Dingena et al., 2023b). A possible reason for the reduced effectiveness outside a clinical setting is that real-life meals consist of mixed macronutrients and foods, consumed at different times of the day, and glucose responses are influenced by our daily activities and physical attributes (Matthan et al., 2016; Vega-López et al., 2007; Mendes-Soares et al., 2019a).

Alternatively, recent preclinical and human studies have suggested that the amount of maternal protein intake can improve management of dysglycaemia

in GDM but the effect on metabolism and 24-hr dysglycaemia in DIP remains unknown (Yu et al., 2019b; Dingena et al., 2023b). Moreover, some women may find it challenging to routinely follow a balanced diet, as a result of barriers such as availability, accessibility and affordability of healthy foods, lack of time and cooking skills (Webber et al., 2015; Smith et al., 2021). Thus, a nutritious and cost-effective meal replacement may be useful for supporting healthy eating patterns. The results described in *Chapter 4* show that the greatest level of glycaemic variability is observed in the 'morning', suggesting that breakfast may particularly be a point of interest in the timing of meals to support management glycaemic control in pregnancy (Dingena et al., 2023b).

CGM is becoming a more routinely used method for assessing glucose control within the NHS and in perinatal clinical settings for women with DIP (Webber et al., 2015). These CGM monitors are unobtrusive patches and record an individual's glucose every 5 minutes for up to 14 days. These monitors can offer quantitative information to identify interstitial glucose variations over a 24-hr period. By measuring glucose continuously for a longer period of time (i.e., hours or days), a more detailed and accurate representation of dysglycaemia can be obtained and offer novel insight regarding the parameters that drive and associate with dysglycaemia, and their relationship with maternal and offspring health (Yu et al., 2019b).

Previous studies using CGM data have uncovered new associations and identified novel points of interest for managing dysglycaemia in maternal diabetes and improving health risks (Perichart-Perera et al., 2012; Moses et al., 2006). However, the development of new strategies to manage these areas of concern is limited by the current understanding of the contribution of biological, lifestyle, and environment exposures on dysglycaemia throughout the course of pregnancy (e.g., early, mid, and late pregnancy) and the moderating effect of these exposures on maternal and offspring health. To address these gaps, the Individualised patieNt care and treatment FOR MatErnal Diabetes (INFORMED) study was designed. This study aims to investigate (postprandial) CGM glucose profiles in early, mid and late pregnancy and how they are associated with personal (lifestyle) characteristics and physiological parameters. Please take note; no results will be presented in this chapter.

5.3 Research Objectives

The overall aim of the INFORMED study was to assess postprandial glucose profile over the course of pregnancy how this associates with personal (lifestyle) characteristics and physiological parameters by using CGM data and providing experimental breakfast meals.

Primary objective:

to assess the effect of easy-to-prepare experimental breakfast meals and dietary protein on dysglycaemia throughout pregnancy.

Secondary objectives:

- i. To assess the association between dysglycaemia and maternal and newborn health, and
- ii. To assess the association between maternal diet and metabolic profiles in early pregnancy with dysglycaemia in later pregnancy

5.4 Methods

The INFORMED clinical trial, a longitudinal observational study with a nested single-blind randomised crossover trial, was reviewed and approved by the Leeds East Research Ethics Committee at the University of Leeds (21/NE/0196).

5.4.1 Study Participants and Recruitment

Pregnant women in their 1st trimester with T1DM or T2DM will be recruited from the Diabetes in Pregnancy Clinics at the LTHT. Women were initially approached by their direct clinical care team and given a study infographic (**Appendix C.1**). If interested to participate or wished to discuss the study in more detail, they were invited to contact the research team (via phone or email). Women expressing interest were emailed a participant information sheet (PIS) and a web link to a secure electronic informed consent (**Appendices C.2 and C.3**). Once the potential participant read the PIS and agreed to take part in the study, a secure electronic signature was provided, the potential participant's eligibility were assessed according to study inclusion and exclusion criteria (**Appendices C.4 and C.5**).

5.4.2 Inclusion- and Exclusion Criteria

All pregnant women from the Diabetes in Pregnancy Clinics at LTHT over the age of 18, with pre-gestational T1DM or T2DM, in their 1st trimester and having a singleton pregnancy, were considered for the study (**Table 5.1**). Women that develop GDM and without T1DM or T2DM were excluded from the study because they are not diagnosed until 26-28 weeks gestation and are currently not offered CGM. Other exclusion criteria include: (i) inability to understand English sufficiently to understand the PIS and provide consent; (ii) mental and/or psychological disorders that undermine informed consent; (iii) comorbidities (e.g., cancer and digestive tract disorders); (iv) lack of internet access to a computer or tablet at home (**Table 5.1**).

Table 5.1: Study participant inclusion and exclusion criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
Women aged 18-45 years	Women under 18 or above 45 years of age
Singleton pregnancy	Multiple pregnancy
Women in the 1 st trimester of pregnancy	Foetal congenital abnormality
Previously diagnosed with T1DM or T2DM	No diagnosis of diabetes
	Diagnosis of gestational diabetes
	Significant co-existent medical condition (e.g. overt diabetes complications, cancer, gut mobility or digestion disorder)
	Significant psychological (e.g. anorexia, Bulimia) and/or mental disorders which undermines informed consent
	Dietary allergies or intolerance for the experimental meals
	Lack of internet access on a computer or tablet at home
	Unable to understand written English and provide informed consent

5.4.3 Data Collection and Procedures

The study took place at the St James's hospital (LTHT) with the dietary intervention and interviews conducted remotely (e.g., participant's home) (**Figure 5.1**). Every pregnant women with pre-gestational T1DM or T2DM is scheduled for regular clinical visits (every two weeks throughout pregnancy) at LTHT.

Furthermore, each of these women have an assigned diabetes midwife, who caseloads their pregnancy, and liaises with the rest of the clinical care team. Due to the COVID-19 pandemic and intermittent lockdowns, pregnant women only attended face-to-face meetings at the clinic when due for a scan (a *dating scan* at 10-12 weeks', an *anomaly scan* at 18-20 weeks' and *growth scans* at 26-28, 32-34, and 36 weeks' gestation), unless deviated due to early delivery or other complications. CGM is currently offered to all women with T1DM or T2DM, as part of their clinical care. The CGM data automatically uploads to a secure clinical database. From this database, the CGM data can be securely, remotely accessed, and reports and raw data of the glucose levels could be downloaded by the clinical care team and authorised researchers.

The participants consented to (i) authorised researchers securely accessing routinely collected clinical details regarding maternal and offspring health at each clinical visit and delivery (i.e., height, weight, blood pressure, HbA_{1c}, lipids), CGM data, and delivery outcomes; (ii) researchers using the residual urine from routine clinically collected samples for metabolite analysis; (iii) researchers conducting online and interview questionnaires to assess diet and lifestyle during each trimester at ~10-12 weeks', ~18-20 weeks', and ~28-34 weeks' gestation; and (iv) providing a 10ml blood sample which would be taken with routine clinical phlebotomy at the ~10-12 week, ~18-20 week, and ~28-34 week visits for subsequent metabolomic and genetic analysis. Each participant would be contacted three times for a phone or video chat – participants can express their preference – for less than 30 minutes for each call within 2 weeks of the clinical appointment (i.e., at ~10-12 week, ~18-20 week, and ~28-34 week visits).

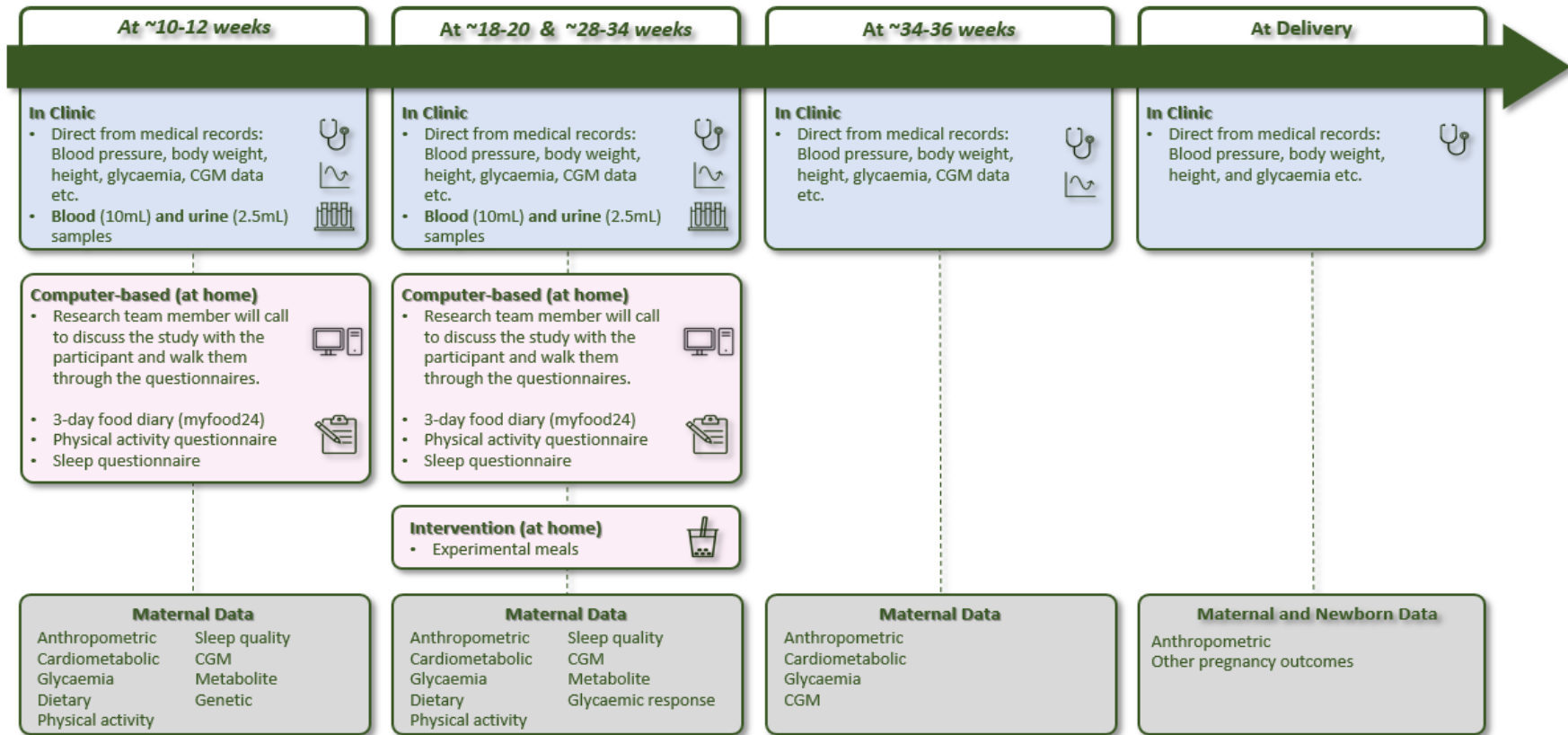


Figure 5.1: Study timeline. At each clinical visit, routine data will be collected from each participant as standard of care (e.g., anthropometrics, blood samples and CGM). This information will be supplemented with lifestyle information (e.g., diet and sleep) collected directly from the participant via electronic questionnaires. The maternal and offspring data available for analysis at each time point are listed below the timeline. The interventions will be delivered at two time points (18–20 and 34–36 weeks). CGM, continuous glucose monitoring; NHS, National Health Service.

5.4.3.1 Patient and Public Involvement (PPI)

Patients or the public have not been involved in the design of this study. However, parts of the design of this study were based on the Maternal Glucose in Pregnancy (MaGiC) study, which did involve patient focus groups for the design of the study. Further, upon completion of the study, participants will be invited to provide insight and comments regarding the study itself, the burden of enrolment and intervention, and identifying other research priorities relevant to DIP, this can be integrated by the researchers into future studies. In addition, these women will be asked to consent to follow-up discussions and would be keen to be updated on study results and publication materials. These points will be invaluable for guiding future work in this area of research.

5.4.3.2 First Call (~10-12 weeks' gestation)

A member of the research team provides details and explains how each stage of the study looks like to the participant and answers any remaining questions. Following, the participants are instructed how to use the myFood24 app and to record their dietary intake for three days in the app – two weekdays and one weekend day. *MyFood24 is a validated online food diary system created to analyse nutritional intake*, which has previously been used for research in pregnancies complicated by diabetes (Morris et al., 2019; Gianfrancesco et al., 2018). MyFood24 can also collect patterns and habitual mealtimes. Via interviews details on participant's recent physical activity levels (Pregnancy Physical Activity Questionnaire [PPAQ]; **Appendix C.6**) (Sattler et al., 2018) and sleep quality (Leeds Sleep Evaluation Questionnaire [LSEQ]; **Appendix C.7**) (Parrott and Hindmarch, 1980) are recorded (Gianfrancesco et al., 2018; Morris et al., 2019). Finally, the dietary meal intervention regarding the meal contents, preparation, purpose and timings of the meals (see '5.4.4 Nested Randomised Crossover Dietary Meal Intervention') and potential risks is discussed.

5.4.3.3 Second Call (~18-20 weeks' gestation)

During this call the participant's compliance to the study and details on physical activity and sleep is re-recorded and reminder and summary of when and how to use the myFood24 app is given. Furthermore, the participant is instructed about how and when the first set of experimental meals should be consumed.

5.4.3.4 Third call (between 28-34 weeks' gestation)

The participant's compliance and information transfer as in the second call is repeated. Furthermore, the participant is instructed about how and when the first set of experimental meals should be consumed. Subsequently, the participant is thanked for their participation in the study, their active role in the study will end and they will not be contacted again after this point, unless interest in follow-up is expressed (see '5.4.3.1 Patient and Public Involvement').

5.4.4 Nested Randomised Crossover Dietary Meal Intervention

Shortly after their ~18-20 week and ~28-34 week clinical visit, the participants are asked to consume the standardised breakfast meal replacements at home under free-living conditions (at breakfast time for 4 days). The meal replacement intervention consist of two standardised experimental breakfast meals (meal A and B; matched for 400 kcal and 13 g of fat). These experimental meals appear and taste similar but differ in protein quantity which alters the rate of glucose absorption into the blood (Meng et al., 2017a). One of the experimental meals has 20g of vegan and gluten-free protein powder added, this should slow gastric emptying and glucose absorption into the blood to a rate that is comparable to commonly consumed whole grain breakfast cereals (e.g., steel-cut or rolled oats; GI \approx 40) (Meng et al., 2017a; Meng et al. 2017b). The other experimental meal has no protein added. Each participant is assigned to one of six random orders to consume the experimental meals (i.e., AABB, ABAB, BBAA, BABA, ABBA, BAAB) over 4 days at 2nd and 3rd trimester (at ~18-20 and ~28-34 weeks' gestation) using an online randomiser (<https://www.random.org/integer-sets/>). Randomisation is done by block randomisation, which assigns 12-13 participants to each of the six possible orders of meal consumption. The participant follows this order for both sets of experimental meals (i.e., at ~18-20 and ~28-34 weeks' gestation).

The experimental meals are prepared and packaged in a COVID-safe lab, and send to the participant's home by a member of the research team. Each package contains (i) eight pre-weight bags (four with added protein and four without, labelled A or B) containing the experimental meals for consumption after their ~18-20 week and ~28-34 week clinical visit; (ii) a drink shaker; and (iii) instructions on how to prepare, when and how to consume the experimental

meals. To prepare the 'meal', the participant only needs to pour the powder into the shaker, add cold water to the line marked on the cup (i.e., 500 mL), and consume the meal replacement within 5-10 minutes. Following, the experimental meal consumption participants are asked to avoid consuming other foods and drinks (aside from water) for two hours, after which they may consume food and drinks per usual. However, they are freely permitted to measure their blood glucose levels at any point and are advised to manage any hyperglycaemic or hypoglycaemic events, even if this means eating or drinking within 2 hours of the meal. If any hyper-, hypoglycaemic or other adverse events occur, the participants instructed to inform the research team as soon as possible by email.

The meal replacement powder used, is a nutritionally complete meal replacement in drink form. Each 'meal' contains a balance of carbohydrates, protein, essential fats, fibre, and phytonutrients, plus all 26 essential vitamins and minerals (Huel - <https://uk.huel.com/pages/the-huel-black-edition-formula-explained>). Moreover, the powder is low in sugar, lactose-free, contains no nuts or palm oil, and has a long shelf-life. For the added protein meal replacement, 20g of unflavoured soy protein isolate is added to the meal replacement powder (MYPROTEIN - <https://www.myprotein.com/sports-nutrition/soy-protein-isolate/10529701.html>). Both products are commercially available and produced in high-quality standard facilities. These products were chosen to minimise time-burden for participants and it is free from many commonly avoided food items (i.e., lactose, animal protein, nuts, and gluten).

Table 5.2: Nutritional information - List of ingredients

Huel Chocolate	Huel Vanilla	Protein powder
Pea Protein, Ground Flaxseed, Brown Rice Protein, Tapioca Flour, Cocoa Powder (6.5%), Sunflower Oil Powder, Organic Coconut Sugar, Natural Flavourings, Micronutrient Blend*, Medium-Chain Triglyceride Powder (from Coconut), Stabiliser: Xanthan Gum, Sea Salt, Sweetener: Steviol Glycosides, Green Tea Powder, Kombucha Tea Powder, Bacillus Coagulans	Pea Protein, Ground Flaxseed, Brown Rice Protein, Tapioca Flour, Sunflower Oil Powder, Natural Flavourings, Organic Coconut Sugar, Micronutrient Blend*, Medium-Chain Triglyceride Powder (from Coconut), Stabiliser: Xanthan Gum, Sweetener: Steviol Glycosides, Green Tea Extract Powder, Kombucha Powder, Bacillus Coagulans.	100% Soy protein isolate
* Potassium Citrate, Potassium Chloride, Corn Starch, Calcium Carbonate, Vitamin C, Niacin (as Niacinamide), Lutein, Pantothenic Acid (as Calcium-D-Pantothenate), Lycopene, Vitamin B6 (as Pyridoxine Hydrochloride), Riboflavin, Vitamin A (as Retinyl Acetate), Thiamin Mononitrate, Zeaxanthin, Vitamin K2 (as Menaquinone-7), L- Methylfolate, Potassium Iodide, Vitamin D2, Plant- Derived Vitamin D3, Vitamin B12 (as Cyanocobalamin)	* Potassium Citrate, Potassium Chloride, Corn Starch, Calcium Carbonate, Vitamin C, Niacin (as Niacinamide), Lutein, Pantothenic Acid (as Calcium- D-Pantothenate), Lycopene, Vitamin B6 (as Pyridoxine Hydrochloride), Riboflavin, Vitamin A (as Retinyl Acetate), Thiamin Mononitrate, Zeaxanthin, Vitamin K2 (as Menaquinone-7), L- Methylfolate, Potassium Iodide, Vitamin D2, Plant- Derived Vitamin D3, Vitamin B12 (as Cyanocobalamin).	

Participants can choose either chocolate or vanilla flavour for their meal replacement shakes; however, all 4 meals should have the same flavour. The meal replacement with added protein will contain 20g additional soy protein powder.

Table 5.3: Nutritional information – Macronutrient composition

	Huel Chocolate (Per serving)	Huel Vanilla (Per serving)	Protein powder (Per serving)
Energy	1680kJ	1680kJ	334kJ
	400Kcal	400Kcal	80Kcal
Fat	17g	17g	0.37g
<i>Of which saturates</i>	2.9g	2.5g	0.15g
<i>Of which MUFA</i>	2.5	2.4g	NA
<i>Of which PUFA</i>	11g	12g	NA
Carbohydrates	17g	18g	0.37g
<i>Of which sugars</i>	4.6g	4.5g	0.15g
Fibre	8.2g	6.3g	NA
Protein	40g	40g	18g
Salt	1.1g	0.84g	0.15g

Participants can choose either chocolate or vanilla flavour for their meal replacement shakes; however, all 4 meals should have the same flavour. The meal replacement with added protein will contain 20g additional soy protein powder.

5.4.5 Data Management

At the recruitment phase, each participant is assigned with a unique random study ID. The study ID is used to anonymise and to harmonise the data accessed via the NHS clinical database (i.e., clinical records and CGM data) with the data collected by the research team at the University of Leeds (i.e., questionnaires, metabolite, and genetic data). Only authorised members of the research team and the clinical team are able to link the study ID to the participant. All personally identifiable information is stored in a password protected and encrypted database in a secure area.

5.4.5.1 Clinical Data

Standard clinical care measures and collects maternal anthropometric, glycaemic, medication, lipid levels, and blood pressure information during routine antenatal care visits, during and after labour. Additionally, part of routine care,

offspring anthropometry measures are taken by the clinical care team. To obtain access to this data consent is given to the research team by each participant.

5.4.5.2 Continuous Glucose Monitoring (CGM) Data

CGM is part of standard clinical care in T1DM and T2DM pregnancies, and consent is obtained to access this data. Numerous metrics are provided by CGM – i.e., mean glucose, SD, CV, MAGE, AUC, iAUC, and TIR (time in-, above-, and below range) – AUC_{glucose} is the primary outcome metric of interest for the study.

5.4.5.3 Urine Samples

For standard clinical care, urine samples are obtained throughout pregnancy, and for this study consent can be given to the research team for gaining access to save up to 2.5 mL of any urine not required for clinical analysis. These samples are for research purposes and stored for subsequent metabolic analysis. All samples will be stored at the University of Leeds in designated Human Tissue Act approved and compliant facilities.

5.4.5.4 Blood Samples

Standard clinical care requires blood samples for analysis. Participants can give consent to an additional 10 ml blood being collected, at the time of routine collection at each clinical visit, for purposes of this study. These blood samples will be stored for subsequent metabolic and genomic analysis – i.e., relevant to nutrition, diabetes, pregnancy and foetal growth. All samples will be stored at the University of Leeds in designated Human Tissue Act approved and compliant facilities.

5.4.5.5 Lifestyle Questionnaires

Questionnaires on participant's physical activity, sleep quality/patterns, and habitual mealtimes are conducted at three time points during pregnancy (~10-12, ~18-20, and ~28-34 weeks' gestation) via a call over the phone or video chat by member of the research team (**Appendices C.6 and C.7**).

5.4.5.6 Dietary Records

At two time-points during pregnancy (i.e., ~18-20 and ~28-32 weeks' gestation), each participant records their diet for three days (i.e., two week days and one weekend day) using a validated online food diary (myFood24), which estimates dietary intake (i.e., macronutrients, micronutrients and vitamins for up to 220

nutrients). Dietary intake data are obtained according to McCance and Widdowson (7th edition) and branded items that offer nutritional data (Wark et al., 2018). Briefly, the data are provided to researchers as a spreadsheet with anonymised identifiers (i.e., study IDs) for each participant, which can be directly imported into R for analysis. The performance of myFood24 and telephone-based 24 hour dietary recall are in agreement (interclass correlation 0.4-0.5) (Wark et al., 2018).

5.4.5.7 Outcomes of Interest

The *primary outcome* of interest is the CGM data, with AUC_{glucose} as the primary glucose metric of interest. *Secondary outcomes* of interest are (i) the other CGM glucose metrics (e.g., mean glucose, CV, TIR etc.), (ii) associations between CGM glucose metrics of dysglycaemia with maternal (e.g., gestational weight gain, pre-eclampsia, hypertension, mode of delivery, birth trauma, and pre-term delivery) and infant outcomes (e.g. birth weight, height, pre-term delivery, mortality, birth trauma, hypoglycaemia, congenital malformation, head and abdominal circumference), and (iii) the moderating effects of genetics, metabolism, and diet/lifestyle. All secondary analyses are considered exploratory.

5.4.6 Sample Size Calculation

There is no current evidence regarding the effect of diet composition and GL on glucose metrics of CGM in DIP in early, mid and late pregnancy. For this study, AUC_{glucose} was elected as the primary metric of interest, as it is commonly used to quantify postprandial glycaemia and easily-interpretable. Evidence from Fabricatore et al (2011) (Fabricatore et al., 2011) demonstrated a significant association between self-reported GI and measures of CGM (including AUC_{glucose} , mean glucose, and TAR%, $p < 0.05$) in a clinical trial of 21 women and 5 men with T2DM. To detect a significant pairwise effect of GI on these parameters with sufficient power (i.e., power = 0.90) and assuming similar effect sizes between GI (per unit) and AUC_{glucose} ($\beta = 0.36 \text{ mg/dl.min}^{-1}$; $R^2 = 0.38$), mean glucose ($\beta = 0.02 \text{ mmol/L}$; $R^2 = 0.38$), and time spent $> 10 \text{ mmol/L}$ blood glucose ($\beta = 0.41\%$; $R^2 = 0.36$), 63 participants are required. Law et al (2019) (Law et al., 2019) suggested that this number of participants provides sufficient power (power = 0.90) to compare subgroups of participants – stratified by BMI, age, type of

diabetes – and test for significant differences of minimum effect size (AUC_{glucose} ; $\pm 61 \text{ mmol/L}\cdot\text{min}^{-1}$, mean glucose; $\pm 0.5 \text{ mmol/L}$, and percentage time above range; $\pm 3.7 \%$). Furthermore, it is anticipated to have adequate power to compare these confounders of glycaemic response given the comparable proportions of women reported to be of White European vs non White European ethnicity (i.e., 57% vs 43%) or diet vs diet + pharmacological treatment (i.e., 46% vs 54%). To account for dropouts or loss-to-follow-up, the recruitment target is increased by 20% of the previously calculated sample size, suggesting a target sample size of 76 women. These power analyses were performed using G*Power (v3.1) (Faul et al., 2009).

5.4.7 Statistical Analysis

5.4.7.1 Primary Outcomes

The primary research objective is to assess the effect of dietary protein on AUC_{glucose} for the 24-hrs immediately after consumption of each experimental meal. For analysis, all standard CGM metrics will be calculated (Danne et al., 2017), with AUC_{glucose} (mean \pm SD) as the primary glucose measure of interest. The analysis will be constructed as pairwise linear model with study meal (0 = no added protein, 1 = added protein) as independent variable and 24-hr mean AUC_{glucose} as dependent variable. The model will be adjusted for study parameters (e.g., the randomised meal order) and participant covariates (e.g., maternal age, ethnicity, parity, BMI, gestational age, physical activity, and sleep quality). Statistical significance is set at $p < 0.05$, this suggests a significant effect of dietary protein on 24-hr postprandial AUC_{glucose} for each study meal ($p < 0.05$). The direction and significance of covariates is investigated to identify study and participant mediators of 24-hr postprandial glucose control. Statistical analysis will be conducted in R studio and SPSS.

5.4.7.2 Secondary Outcomes

Secondary research objectives include (i) the association between dysglycaemia and maternal and newborn health, and (ii) the association between maternal diet and metabolic profiles in early pregnancy with dysglycaemia in later pregnancy. To assess these objectives, all analyses will be performed using regression models adjusted for covariates, including the assessment of early pregnancy diet on longitudinal changes in dysglycaemia adjusted for time-points

of AUC_{glucose} (i.e., mixed-models). This association between early pregnancy diet and AUC_{glucose} will be performed using three different dietary metrics – all calculated from myfood24:

- 1) *Overall Diet Quality*: associations between diet quality and dysglycaemia will be assessed using an overall diet quality score (Dehghan et al., 2012). This method for scoring diet quality – i.e., the modified Alternative Healthy Eating Index (mAHEI) – has been modified and previously used to assess maternal diet quality in a multi-ethnic prospective birth cohort (NutriGen Alliance et al., 2016). This mAHEI score is calculated using the following method; per food category an individual will receive 10 points when they consume above or below a threshold of: (i) ≥ 5 servings vegetables, (ii) ≥ 4 servings fruit, (iii) ≥ 1 serving nuts or soy proteins, (iv) ≥ 3 servings whole grains, (v) a ratio of ≥ 4 servings fish to 1 serving meat and eggs, and (vi) ≤ 0.5 servings of ‘unhealthy’ foods (i.e., fried foods and processed meats). Intermediate intakes are scored proportionally between 0 and 10. The maximum mAHEI score an individual can get is 60; the higher the score, the ‘healthier’ the participant’s diet. Incorporating this mAHEI score as predictor and CGM measures of glycaemia as outcome, regression models will be employed.
- 2) *Macronutrients*: Daily macronutrient consumption (i.e., total carbohydrates, proteins, and fats) and markers of GI quality (e.g., fibre and sugars) will be adjusted for energy and regressed against CGM measures of dysglycaemia (Willett et al., 1997), similar to analyses conducted in *Chapter 4*. This allows for the contribution of each individual macronutrient on glycaemic measures to be evaluated.
- 3) *Cardinal Foods*: Partial-least Squares (PLS) models will be employed to identify foods that are more commonly observed in participants with favourable or unfavourable glycaemic control. Favourable glycaemic control is identified as below median for glycaemic measures and unfavourable glycaemic control is identified as above the median. Subsequently, these foods identified by PLS will be investigated for their association with measures of glycaemia using regression models.

5.4.8 Quality Control

All participants receive standard clinical care as per NICE guidance (Webber et al., 2015), which will minimise researcher bias. The researchers will not be able to influence the primary outcome, as these are based on laboratory measurements and predetermined cut-off values. Antenatal and perinatal outcome data are collected by clinical staff, who are independent from the study and not involved with the study outcome, or from the participants' medical records. Therefore, researcher bias in collecting the data is minimised. Furthermore, researcher bias in collecting lifestyle records is not foreseen, as standardised and validated questionnaires are used to obtain this information. Additionally, the order of the standardised experimental meals is randomised. Also, no conflict of interests are reported. No study data will be used to inform clinical care decisions, all the women will continue with their clinical care per usual for the duration of their pregnancy. Moreover, all women are free to terminate their participation in the study at any time with no effect on their quality of care.

5.5 Results

Due to the COVID-19 pandemic, the protocol of the INFORMED study had to be re-designed and encountered several delays in approval and final sign-off. First, a COVID-19 safe protocol had to be conceived, minimising face-to-face contact and staff burden. Secondly, Health Research Authority (HRA) / National Health Service (NHS) ethics application for all non-essential studies was suspended for several months, which delayed the process of acquiring ethical approval to conduct the study. Thirdly, once ethical approval was granted by the Research Ethics Committee (HRA/NHS ethics), NHS research passport and Capacity & Capability from the LTHT research site needed to be obtained. However, there were considerable backlogs, due to the COVID-19 pandemic, and a new process for acquiring these approvals was being piloted. This eventually resulted in another seven months delay, and consequently in an extensive delay of commencing the participant recruitment.

For patient and staff safety, face-to-face contact for external researchers was restricted. Therefore, the researchers were not to allow to directly recruit the participants and a research nurse was assigned for initial recruitment of the Diabetes in Pregnancy Clinic patients. Although, to minimise the staff's burden,

they only had to provide an information leaflet (**Appendix C.1**) and inform the participants to contact a member of the research team if they were interested in participating (contact details are provided on the information leaflet). Following, the research team would take consent and check for eligibility. Several months into the recruitment phase, this indirect process have proven to make recruitment difficult with loss to follow-up of several potential participants. To simplify the recruitment process, ethics amendments were obtained, so that the research nurse could take consent and then refer the potential participant to the research team for eligibility checks. Even though these amendments to the protocol, no participants have yet been recruited. In total 4 women expressed interest in the study; however, two of them were lost to follow-up (member of research team made several attempts to contact the potential participant without results) and two were past the recruitment time window (>14 weeks gestation).

5.5.1 Dissemination

There is no formal interim analysis planned, other than the continuing evaluation of the recruitment numbers. Eventually, results will be disseminated in peer reviewed scientific journals, conference presentations, and publication on (social) media and in newsletters, to inform the participants and wider public.

5.6 Discussion

The INFORMED clinical trial, a longitudinal observational study with a nested single-blind randomised crossover trial, to evaluate the effect of dietary protein from the maternal diet and within experimental meals on dysglycaemia in women with pre-existing T1DM or T2DM. In addition, this study will explore (i) the association between dysglycaemia and maternal and newborn health, and (ii) the association between maternal diet and metabolic profiles in early pregnancy with dysglycaemia in later pregnancy, which may provide insights into novel precision interventions and therapies for women with DIP (Schaefer-Graf et al., 2018). The identification of dietary mediators of glucose variability and dysglycaemia will aid in the development of more efficacious and appropriate management strategies to control glucose levels and minimise maternal and offspring health risks in women with DIP (Schaefer-Graf et al., 2018; Perichart-Perera et al., 2012; Moses et al., 2006).

However, to date, no results have yet been obtained, due to significant delays in commencement of the study and recruitment, this has resulted in no participants being recruited. The first and foremost reason for the lack of recruitment was the COVID-19 pandemic. Mitigating through the pandemic important lessons were learnt – i) quick pace thinking and learning to adapt the design and protocol of a study; ii) how the process of ethic approval works and changes during a pandemic; and iii) how to work with and communicate with third party institutions (NHS in this case). However, even after commencement of the study, the recruitment process remained slow.

As discussed in the results section, ethics amendments have been obtained to simplify the recruitment process, this should improve the recruitment rate. Although, recruitment rate has not improved at this point. A possible explanation could be that participants continue to be recruited indirectly by NHS staff. Participants may feel less inclined to participate if they are not being recruited by a member of the research team directly. Additionally, the NHS staff have an extensive case- and further workload, which may reduce the time they are actively recruiting participants. For the future, it may be beneficial to apply for other ethics amendments, which would allow a member of the research team to attend the DIP antenatal clinic visits to recruit the participants directly. Also, adding some form of incentive (e.g., small payment, gift box with useful supplies or a gift card) for completion of participation should be considered – this could make the potential participant feel more validated and more likely to participate. Another limitation to recruitment has resulted due to the COVID-19 pandemic, as patients are less likely to visit the DIP Clinics in person. Thus, even after above mentioned amendments recruitment may still continue to be an issue. During this study it was evident that recruitment is one of the most challenging, if not most challenging aspects of conducting a study. This requires a lot of flexibility and creative thinking, an essential skills for a researcher to have.

5.6.1 Strengths and Limitations

The trial is embedded within standard clinical care and uses routine data and biological samples collected by health services to minimise (i) burden of the participants, and (ii) non-essential and face-to-face participant contact, which will enable the study to continue even with the re-introduction of COVID-like restrictions and reduces risk of bias. Furthermore, analyses of (postprandial)

response to the experimental meals contiguous to repeated measures of blood and urine metabolite profiles, will offer new insights into shifts in maternal metabolism in T1DM and T2DM pregnancies, and their association with maternal and newborn health during pregnancy and at delivery.

Some limitations should be noted, as with all observational data, participant recall data (e.g., diet, physical activity, and sleep) are subject to social desirability bias. However, repeated and complementary measures (i.e., metabolite) inclusion may reduce this risk of bias, which could be evaluated in future analysis. The INFORMED study is being conducted in collaboration with the National Health Service in the UK; therefore, its results may not be directly generalizable to other government health services or nations. Regardless of the limitations of this chapter, this chapter has provided a novel study design and describes a ready-to-go study that can be implemented again in future studies.

5.7 Summary

- Evidence suggests that control of (postprandial) glycaemia is key to manage maternal and offspring health in DIP but it is limited regarding (i) the role of diet and lifestyle as mediators of glucose variability and control throughout the course of pregnancy, and (ii) what aspects of maternal and offspring health are associated.
- The overall aim of the INFORMED study is to assess (postprandial) glucose profile over the course of pregnancy how they are associated with personal (lifestyle) characteristics and physiological parameters by assessing CGM data and experimental breakfast meal glucose responses.
- Recruitment has started, but was significantly delayed due to the COVID-19 pandemic, and delays in ethics approval and other NHS sign-offs. Unfortunately, no participants have yet been recruited. Thus, for this thesis no results have been obtained. However, a novel study design has been provided and this chapter describes a ready-to-go study that can be implemented again in future studies.

Chapter 6

Association of Dietary Protein Intake and Glycaemic Control in Pregnancy: an Observational Analysis of the Born in Bradford cohort Study

What do we know? To counter hyperglycaemia and adverse pregnancy outcomes, nutritional management strategies have been prescribed. *Chapter 4* identified dietary protein as potential mediator of (postprandial) glycaemia in pregnancy – protein was associated with lower mean glucose levels and AUC. Recently, previous research has demonstrated how metabolomic profiling could aid in our understanding of maternal health during pregnancy and subsequent offspring health.

Key issues: To date, however, the relationship between dietary protein intake and protein source (i.e., meat- vs plant-based diet) and glycaemic control in pregnancy remains unclear.

Aims: This study aimed to assess the association of animal-based protein intake and glucose control in pregnancy, by (i) developing a metabolic meat intake score (MMS) using metabolites that associate with dietary intake of meat, and (ii) assessing whether this MMS associates with measures of glycaemic control.

Thesis implications: This study was undertaken to evaluate the association between components of meat, a major source of dietary protein, and glycaemia, and its potential as a dietary mediator of glycaemia in a diverse population of pregnant women.

6.1 Abstract

The association between dietary protein and gestational dysglycaemia has been demonstrated. However, the relationship between animal protein source, namely meat intake, and glycaemic control in pregnancy remains unclear. Current research utilises metabolomics to examine underlying mechanisms in diet-disease relationships, these metabolites offer a novel approach to untangle this association and better understand the role of animal protein metabolism on glucose metabolism in pregnancy. Therefore, this study aimed to (i) develop metabolic meat intake score (MMS) by identifying metabolites that associate with dietary patterns of meat intake, and (ii) whether this MMS associates with measures of glycaemic control in pregnancy.

A subgroup of 2,655 pregnant women (mean gestational age 26.3 weeks) in the Born in Bradford (BiB) cohort with available food frequency data were stratified as meat consumers or non-meat consumers. Partial least squares was then applied to identify cardinal metabolites that characterise each group based on quantitative NMR data for 227 metabolites from fasting serum. Subsequently, a weighted metabolite meat-intake score (MMS) was then calculated for each participant. In the total (n=7,004) and the independent group of women (n=4,349), this MMS was regressed against fasting and postprandial plasma glucose after adjustment for covariates.

38 metabolites were important (VIP score ≥ 1) distinguishing meat eaters from non-meat eaters; extremely and very large VLDL triglycerides and ratio of saturated fatty acids/total fatty acids were most important metabolites. Multiple linear regression adjusted for covariates (i.e., ethnicity, age, gestational age, BMI, parity, and IMD) revealed that MMS was associated with lower postprandial glucose (β : -0.003 mmol/L, CI: -0.006, -0.005, and $p = 0.02$). No association was observed between MMS and fasting plasma glucose.

To conclude, this study demonstrates that meat consumers can be characterised by a distinct metabolite profile, namely VLDL triglycerides and saturated fatty acids metabolites, and that this profile is associated with improved postprandial glucose response in pregnancy. Future work is needed to evaluate more distinct meat-intake profiles, how timing of meat intake and protein source (e.g., animal vs plant; whole foods vs protein powder) modify the association and its efficacy as a strategy for minimising risk of dysglycaemia in pregnancy.

6.2 Background

Pregnancy induces a unique state of glucose metabolism, to provide adequate energy to the developing foetus (Schaefer-Graf et al., 2018; Taylor et al., 2019). These changes in maternal metabolism are driven by maternal hormones (e.g., hPL, placental growth hormone, progesterone, estradiol, leptin, cortisol, prolactin, TNF- α , and other inflammatory mediators), starting as a mild reduction in insulin sensitivity and progresses to controlled insulin resistance in second and third trimesters (Powe et al., 2019; Taylor et al., 2019; Mao et al., 2017; Chen et al., 2018). When IR exceeds normal levels, a state of recurring hyperglycaemia arises (Mao et al., 2017; Chen et al., 2018). Prolonged hyperglycaemia in pregnancy has been linked to increased adverse pregnancy outcomes for both mother and infant – these include preterm delivery, LGA, birth injury, perinatal mortality, and in later-life obesity and T2DM (Johns et al., 2018; Tam et al., 2017; Burlina et al., 2019).

To counter hyperglycaemia and these adverse pregnancy outcomes, nutritional management strategies have been proposed (Reader et al., 2006; Monro and Williams, 2000). Subsequently, dietary guidelines have formed the cornerstone of hyperglycaemia management strategies in pregnancy (Webber et al., 2015; Dingena et al., 2023a). However, current literature continues to report that i) women with GDM struggle to maintain healthy glucose levels using current lifestyle recommendations (Zheng et al., 2019; Kampmann et al., 2019b), and ii) despite perceived reduced time in hyperglycaemia, women continue to give birth to large for gestational age offspring (Law et al., 2019). Furthermore, maternal diet itself influences (postprandial) glycaemia and may alter the risk of GDM (Schoenaker et al., 2016; Brand-Miller, 2003). A systematic review on the associations of nutrients, foods, and dietary patterns with GDM concluded that the current research is scarce and predominantly focused on intake of single foods and nutrients rather than on overall dietary patterns (Schoenaker et al., 2016). Notably, this review demonstrated that (i) certain nutrients (i.e., higher intake of total fat, cholesterol, and haem iron) were associated with higher GDM risk; (ii) certain foods (i.e., higher consumption of egg, red meat, and processed meat) was associated with a higher risk of GDM, and (iii) that a dietary pattern rich in fruit, vegetables, legumes, nuts, whole grains, and fish and low in red and

processed meat, refined products, eggs, and dairy may reduce GDM risk (Schoenaker et al., 2016).

Other studies identified dietary protein as potential mediator of (postprandial) glycaemia – protein preload attenuated the subsequent rise in the PPGR, resulted in lower $iAUC_{\text{glucose}}$ compared to carbohydrate or fat preload, and protein was associated with lower mean glucose levels and AUC_{glucose} (Meng et al., 2017b; Dingena et al., 2023b). This beneficial effect is possibly the result of protein reducing the rate of gastric emptying and its more efficacious effect stimulating a rise in glucagon levels than glucose is in suppressing it, thereby enhancing the insulin response (Meng et al., 2017b; Meng et al., 2017a). In addition to increased dietary protein intake, evidence has emerged for the beneficial effects of plant-based diets on glycaemic control (Kahleova et al., 2017). These plant-based diets, characterized by a reduction or elimination of animal product consumption, have been increasing in popularity for a number of ethical, environmental and health reasons (Leitzmann, 2014). Previous studies show improved glucose control as result of partial replacement of meat with soy products increasing insulin sensitivity and improved gastrointestinal hormone response (e.g., incretin), which plays an important role in postprandial increase of insulin (van Nielen et al., 2014; Nauck et al., 1986). Furthermore, Zulyniak et al. (2017) found that maternal consumption of a plant-based diet in pregnancy was associated with reduced birth weight and odds for LGA, in White Europeans, and whereas a plant-based diet was associated with increased birthweight among South Asians (Zulyniak et al., 2017). Further, prospective epidemiological evidence suggests that individuals following a plant-based diet may have reduced risks of some non-communicable diseases – including coronary heart disease, some cancers and diabetes – compared to meat-eaters (Leitzmann, 2014). Therefore, this study focussed on protein intake and grouping the participants into meat consumers vs non-meat consumers, to assess the effects of protein from animal protein/meat intake on measures of glycaemia. Crude consumer categories (i.e., meat vs non-meat consumers) were chosen, because this study was designed to explore the possibility of identifying specific dietary metabolite profiles and to assess association between dietary protein and glycaemia.

Metabolomics – the quantification of molecules resulting from metabolic processes – may reveal underlying mechanisms in such diet-disease

relationships, for metabolite concentrations reflect genetic, dietary, lifestyle, and environmental factors as well as disease conditions (Schmidt et al., 2021; Taylor et al., 2021). Developments high-throughput technologies for quantifying a large number of metabolites, as well as lipids and lipoprotein particles, has facilitated detailed investigation of human metabolism and disease risk prediction in large-scale epidemiological studies (Taylor et al., 2021). More recently, studies have demonstrated how metabolic profiling could aid us in our understanding of maternal metabolism and health during pregnancy and subsequent offspring health (Taylor et al., 2021). Moreover, the International Diabetes and Pregnancy Study Group called for increased research into the role of the metabolome on GDM (Schaefer-Graf et al., 2018). A better understanding of how metabolite profiles differ between diet groups in a diverse population will offer mechanistic insight into the role of protein on gestational glycaemia.

To date, however, the relationship between dietary protein intake and glycaemic control in pregnancy remains unclear. In light of this, the present study aims to assess the association of animal protein intake and glucose control in pregnancy. To answer the research objective this present study aims to i) develop metabolic meat-intake score (MMS) by identifying metabolites that associate with dietary patterns of meat intake, and ii) whether this MMS associates with measures of glycaemic control in pregnancy.

6.3 Methods

6.3.1 Study Population

Born in Bradford (BiB) is a large multi-ethnic prospective longitudinal birth cohort conceived in 2007 (Wright et al., 2013; Raynor, 2008). This cohort is based in Bradford, a city in the Northeast of England, was created in the response to high rates of childhood morbidity and mortality in this area. Bradford is the sixth largest city in the UK and is among the most deprived cities in the UK, with 60% of the babies being in the 20% poorest population of the UK. Almost half of the babies born in Bradford are from Pakistani origin (i.e., 50% White European (WE); 44% Pakistani; 4% Bangladeshi; and 2% other), with a high degree of variability in pregnancy health driven by socioeconomic, ethnic, and cultural diversity, making this an interesting cohort to study diabetes risks. The BiB cohort was established to examine the impact of genetic, nutritional, environmental,

behavioural and social factors on health and development during pregnancy, childhood, and subsequently adult life in a multi-ethnic, deprived population (Wright et al., 2013; Raynor, 2008). Ethical approval for the study was granted by the Bradford National Health Service Research Ethics Committee (reference number: 06/Q1202/48 and 08/H1302/21), and written informed consent was gained from all participants.

Between March 2007 and November 2011, mothers giving birth at the Bradford Royal Infirmary were recruited, when they attended for a routine oral glucose tolerance test at 26-28 weeks' gestation (Wright et al., 2013; Raynor, 2008). All women planning to give birth at the Bradford Royal Infirmary were eligible to be recruited. The only exclusion criterion for recruitment was if she planned to move away from Bradford before the birth. In total, 12,453 women with 13,776 pregnancies (including stillbirths and multiple pregnancies) were recruited and provided detailed information on socio-economic characteristics, ethnicity, lifestyle, environmental risk factors and physical/mental health. Mothers were asked to self-report their ethnicity, measured and weighed at recruitment, and detailed anthropometric assessment of infants was conducted at birth. Furthermore, results of an OGTT (11,442 women) and lipid profiles were obtained from the mothers during pregnancy at ~26-28 weeks gestation. This has resulted in a biobank of over 250,000 samples of maternal blood, DNA and urine. The baseline questionnaire developed for this study was designed to be completed as part of a semi-structured interview and administered by a trained project worker at recruitment. In addition, a food frequency questionnaire (FFQ) was given at recruitment to the mothers to complete in their own time without the assistance of the project workers (Wright et al., 2013; Raynor, 2008). Individuals who reported being of a Southeast Asian origin other than Pakistani (SA) were excluded due to the small sample size (therefore limited power) of these ancestry groups. Resulting in 7,004 women retained for the main analysis (**Figure 6.1**).

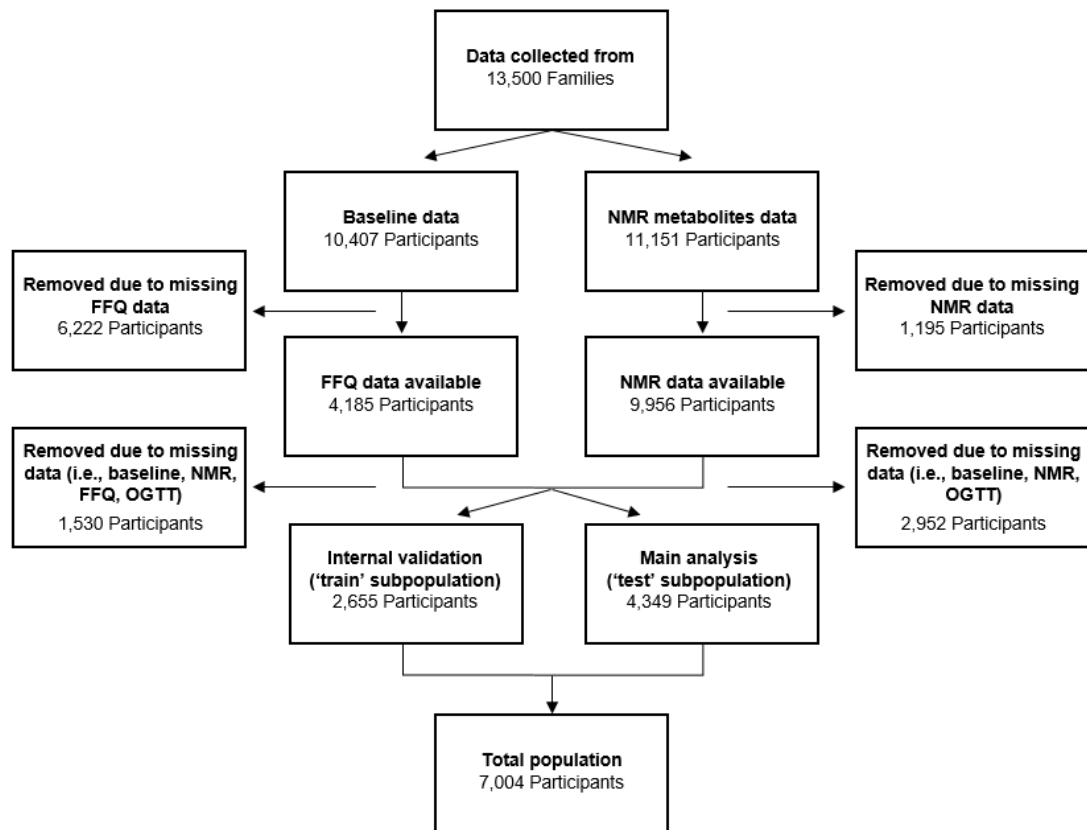


Figure 6.1: Participant flowchart.

6.3.2 Dietary Assessment

Dietary information during pregnancy was collected by using a validated, self-administered FFQ (**Appendix Table D.1**). Data were available about their habitual frequency of certain food groups on a weekly basis, including red meat, poultry, fish, eggs, and dairy products. Possible answers were “never/rarely”, “less than once a week”, “once a week”, “2-3 times a week”, “4-6 times a week”, “1-2 times a day”, “3-4 times a day”, and “5+ times a day”. If a food group was consumed “never/rarely” then that item was scored ‘0’, and if a food group was consumed “less than once a week” or more than that item was scored ‘1’. Subsequently, each individual was then categorised into ‘meat-eater’ (‘1’ on one or more items of these food groups) or ‘no meat-eater’ (‘0’ on all items of these food groups).

6.3.3 Metabolite Analysis

Comprehensive details of venous blood sample collection/processing/preparation, and metabolite quantification/validation have been previously described (Taylor et al., 2021). In brief, maternal overnight-fasted blood samples were taken during the OGTT by trained phlebotomists working in the antenatal clinic of the Bradford Royal Infirmary and sent immediately (i.e., within 2.5 hours) to the hospital laboratory for processing. Subsequently, the samples were stored at -80°C for further research and analyses. There were no freeze-thaw events of the samples prior to their use for metabolic profiling.

A total of 227 metabolite measures (i.e., molar concentrations, percentages or ratios) were obtained from serum samples using a targeted high-throughput nuclear magnetic resonance (NMR) spectroscopy platform (Nightingale Health Ltd., Helsinki, Finland) (Schmidt et al., 2021). NMR spectra were analysed for metabolite quantification of common lipids, lipoprotein subclass profiling (within 14 subclasses), fatty acid composition, and various low-molecular weight metabolites (including amino acids, ketone bodies and markers of glycolysis, fluid balance and inflammation) (Schmidt et al., 2021; Taylor et al., 2021). The NMR platform quality control of the data were undertaken by Nightingale Health Ltd., these procedures check various issues related to the sample integrity and the biomarker quantification (Taylor et al., 2021). Also, validation of some of the NMR measures was undertaken by comparing concentrations from the NMR platform to the same measures from the same samples assessed by clinical chemistry measurements (Taylor et al., 2021).

6.3.4 Diet Pattern Analysis – Metabolic Meat Scores (MMS)

Metabolite data were combined with the BiB reported descriptive, including participant's ethnicity, age (in years), gestational age at OGTT (in weeks), BMI (in kg/m^2), parity, and socio-economic status (i.e., index of multiple deprivation [IMD] in quintiles). A total of 2,655 women were stratified as meat consumers or non-meat consumers based on food frequency data, as FFQ data were only available in a subgroup of the participants. Partial least squares (PLS) was then applied to identify cardinal metabolites that characterise each group based on quantification of NMR metabolites as predictor and meat consumer Y/N as outcome. PLS is a method which reduces the predictor variables to a smaller set

of predictors, these are then used to perform a regression, yielding metabolites associated with meat consumption (Eriksson et al., 2013). The included predictor variables are ranked by the degree to which they explain the variance between groups (i.e., meat consumers compared with non-meat consumers) in the PLS model, known as VIPs (Variable Importance in Projection) (Eriksson et al., 2013). When a VIP score is ≥ 1 , the variable was denoted with good discriminatory quality and predictive ability (Perreault et al., 2014; Eriksson et al., 2013). Subsequently, MMS was calculated only incorporating metabolites with a VIP ≥ 1 , each metabolite's NMR value was multiplied by its PLS VIP score and the sum would yield the MMS of an individual.

6.3.5 Regression Analysis for OGTT and MMS

To assess the relationship between dietary protein intake and measures of glycaemic control in pregnancy, linear regression models investigating the association between MMS and OGTT (i.e., fasting glucose and 2-hr post-oral glucose) were performed. Known covariates of glycaemic control in pregnancy – including ethnicity, age, gestational age, BMI, parity, and IMD – were initially included in the models. First, logistic regression models were employed in a smaller 'training' subpopulation (i.e., with available FFQ data) to test the validity of the MMS; OGTT (fasting or postprandial plasma glucose) ~ MMS + meat-eater ('1' or '0') + ethnicity + age + gestational age + BMI + parity + IMD. Subsequently, linear regression models were employed in the total population, also assessing for interaction between dietary protein intake and ethnicity (i.e., with available NMR data); OGTT (fasting or postprandial plasma glucose) ~ MMS + ethnicity + MMS*ethnicity + age + gestational age + BMI + parity + IMD.

6.3.6 Statistical Analysis

Differences between 'training' and 'test' population were tested by analysis of variance (ANOVA; continuous variables) or χ^2 test (categorical variables). To explore patterns in metabolite profiles best differentiating diet groups, a PLS regression based on self-reported dietary protein intake within a subgroup with FFQ data available ($n = 2,655$) was employed (see '6.3.4 Diet Pattern Analysis'). To assess the validity of the MMS, internal validation was conducted by testing the association between MMS and self-categorised meat-eater ('yes' or 'no') in an unadjusted logistic regression (no covariates; model 1) and adjusted logistic

regression model (all covariates; model 2) in a 'training' subgroup (n = 2,655). Finally, to assess the association between the identified metabolite profiles and measures of glycaemia (i.e., FPG and PPG), linear regression models adjusting for known covariates were performed in the remaining participants (i.e., 'test' group of n = 4,349). Sensitivity analyses were also performed to examine the robustness of associations between MMS and OGTT, therefore the linear regression models were run (i) including all covariates (model 1), and (ii) excluding non-significant covariates (model 2). All analyses were performed excluding participants with missing metabolic markers. Based on previous analyses done by Pan et al. (2022) in 4,778 individuals that assessed similar metabolites and found an association between metabolic markers and disease risk, the assumption was made that this study is sufficiently powered. Furthermore, the BiB cohort is the largest cohort in pregnancy incorporating extensive metabolite, dietary, and OGTT and sociodemographic data. The significance levels were set $p < 0.05$ and all statistical analyses were conducted in RStudio (version 4.0.3 - Foundation for Statistical Computing, Vienna, Austria. URL: [https:// www.R-project.org/](https://www.R-project.org/)) ('plsVarSel' package for PLS regression analysis).

6.4 Results

6.4.1 Participant Characteristics

In total, 7,004 women were retained for final analysis; average age was 27.2 ± 5.7 years and BMI was 28.4 ± 5.5 kg/m² (**Table 6.1**). Of these women, 50.2% self-identified as White European and 49.5% as Pakistani, showing an equal distribution of ethnicity.

Table 6.1: Baseline participant characteristics from BiB cohort by total, training and test population.

Participant Characteristics	Overall (n=7,004)	'Training' population (n=2,655)	'Test' population (n=4,349)	'test' vs 'training' p-value
Maternal Age (yrs)	27.2 ± 5.7	27.0 ± 5.7	27.4 ± 5.7	0.002
BMI (kg/m ²)	28.4 ± 5.5	28.7 ± 5.5	28.3 ± 5.4	0.001
Gestational Age (wks)	26.3 ± 1.9	26.3 ± 1.8	26.3 ± 1.9	0.36
Ethnicity				
<i>White</i>	3,514 (50.2)	1581 (59.5)	1888 (43.4)	
<i>Pakistani</i>	3,469 (49.5)	1069 (40.3)	2445 (56.2)	<.001
<i>Missing</i>	21 (0.3)	5 (0.2)	16 (0.4)	
Parity				
<i>Nulliparous</i>	2,870 (41.0)	1124 (42.3)	1746 (40.1)	
<i>Multiparous</i>	3,706 (53.0)	1479 (55.7)	2227 (51.2)	<.001
<i>Missing</i>	428 (6.0)	52 (2.0)	376 (8.7)	
IMD				
<i>Quintile 1 (most deprived)</i>	2,488 (35.6)	840 (31.6)	1647 (37.9)	
<i>Quintile 2</i>	1,870 (26.7)	665 (25.0)	1205 (27.7)	
<i>Quintile 3</i>	1,346 (19.2)	545 (20.5)	801 (18.4)	<.001
<i>Quintile 4</i>	943 (13.5)	440 (16.6)	503 (11.6)	
<i>Quintile 5 (least deprived)</i>	260 (3.7)	121 (4.6)	139 (3.2)	
<i>Missing</i>	97 (1.4)	44 (1.7)	53 (1.2)	
MMS	338.6 ± 4.8	337.3 ± 20.9	337.5 ± 19.7	0.67
OGTT				
<i>FPG</i>	4.51 ± 0.5	4.45 ± 0.5	4.56 ± 0.6	>.001
<i>PPG</i>	5.70 ± 1.5	5.56 ± 1.4	5.78 ± 1.6	>.001

Summary table of population characteristics, expressed as mean \pm SD for continuous variables and counts (%) for categorical variables (significance $p < 0.05$). Differences between women with MMS above and below median for continuous variables were compared using analysis of variance, and for categorical variables were compared using χ^2 . MMS, metabolic meat score; yrs, years; BMI, body mass index, wks, weeks; IMD, index of multiple deprivation; OGTT, oral glucose tolerance test.

6.4.2 Metabolites and Metabolite Meat-intake Scores (MMS)

The PLS regression analysis reported 38 metabolite values with a VIP score ≥ 1 (**Appendix Table D.2**). This metabolite profile of 38 metabolites, assessed using a targeted high-throughput NMR, was important for distinguishing meat consumption (i.e., non-meat consumers above MMS mean = 340.4 ± 4.5 and meat consumer below MMS mean = 337.3 ± 2.9 ; MMS median = 338.67); extremely and very large VLDL (very low-density lipoprotein) triglycerides, and ratio of saturated fatty acids/total fatty acids were most important metabolites (**Appendix Table D.2**).

6.4.3 Validity of the Metabolic Meat-Intake Score

To assess the validity of the MMS, internal validation was conducted by testing the association between MMS and self-categorised meat-eater ('yes' or 'no') in an unadjusted (Model 1: meat-eater 'yes' or 'no' \sim MMS) and adjusted logistic regression model (Model 2: meat-eater 'yes' or 'no' \sim MMS + covariates) (**Table 6.2**). Both models showed significant associations (model 1: $p = 0.017$ and model 2: $p = 0.008$). After adjustment for covariates the significance level increased, albeit none of the covariates were significant. These results suggest that the developed MMS could be utilised as a new marker for source of protein intake, with a unit increase of MMS there 12% less likelihood of being a meat consumer.

Table 6.2: Internal validation of metabolite meat intake score (MMS) in ‘training’ set (n=2,655).

Variables	Model 1		Model 2	
	Odds (95% CI)	p-value	Odds (95% CI)	p-value
MMS	0.92 (0.85, 0.98)	0.017	0.88 (0.75, 0.97)	0.008
Age	NA	NA	1.00 (0.93, 1.08)	0.92
Gestational age	NA	NA	1.11 (0.87, 1.40)	0.41
IMD20	NA	NA	0.76 (0.55, 1.03)	<i>0.082</i>
Maternal BMI	NA	NA	1.01 (0.94, 1.08)	0.84
Parity	NA	NA	1.21 (0.79, 1.84)	0.38
Ethnicity	NA	NA	-2.333 (0.00, 1*10 ²⁹)	0.95
MMS * Ethnicity	NA	NA	1.01 (0.82, 1.2)	0.93

Logistic regression analysis of the association of calculated dietary meat intake (i.e., MMS) with reported dietary meat intake (i.e., FFQ) and interaction of dietary meat intake and ethnicity. Model 1 only included calculated dietary meat intake and reported meat intake as outcome (meat – yes ‘1’ or no ‘2’). Model 2 included all covariates and also reported meat intake as outcome. Parity was reported as having 0,1,2,3,4,5, or 6 children. IMD was reported in quintiles as 1,2,3,4,5. Ethnicity was self-reported and only included White Europeans (1) and Pakistani (2). Marginal significance denoted in italic with (·). CI, confidence interval; MMS, metabolic meat-intake score; NA, not applicable; IMD20, index of multiple deprivation in quintiles; BMI, body mass index.

6.4.4 Effect of Metabolic Meat Intake Score on Measures of Glycaemia

To assess the association between glycaemia and MMS, linear regression models adjusted for known covariates were employed in total and independent ‘test’ and ‘training’ population (**Tables 6.3-6.6**). The independent ‘testing’ cohort was underpowered (37 individuals self-reported as non-meat consumers of the 2,655 with available FFQ data). To increase sample size, analysis in the total population (n = 7,004) was employed. No significant associations were found between MMS and fasting plasma glucose, even after removal of non-significant covariates (MMS: $p = 0.86$ and $p = 0.37$, respectively; **Table 6.3**) For postprandial glucose, MMS was found to be significantly associated in the unadjusted model (model 1; $p = 0.02$; **Table 6.4**). In a sensitivity analysis, associations between MMS and postprandial glucose persist when gestational age, parity and ethnicity were removed from the model – albeit from significant to marginally significant

(model 2; MMS: $p = 0.067$; **Table 6.4**). Before removal of non-significant covariates, a significant interaction between MMS and ethnicity was found (model 1: $p = 0.041$; **Table 6.4**). Furthermore, age and BMI were shown to be correlated with MMS and postprandial glucose (model 1: $p < 0.0001$ and $p < 0.0001$, **Table 6.4**). These results suggest that MMS could lower postprandial glucose by 0.003 mmol/L (CI: -0.006, -0.005, and $p = 0.02$) and that this score is associated with ethnicity (**Table 6.4**). However, these results should be interpreted with caution, as the effect size is clinically not strong and may be driven by the 'training' population. Therefore, a sensitivity analysis of the independent 'training' and 'test' population was performed.

Table 6.3: Linear regression of the association of the metabolic meat intake score (MMS) with fasting glucose in total population (n=7,004).

Variables	Model 1		Model 2	
	β in mmol/L (95% CI)	p-value	β in mmol/L (95% CI)	p-value
MMS	-0.008 (-0.001, 0.009)	0.86	0.003 (-0.003, 0.009)	0.37
Age	0.013 (0.010, 0.015)	<0.0001	0.014 (0.012, 0.017)	<0.0001
Gestational age	-0.0005 (-0.007, 0.006)	0.90	ns	ns
IMD20	0.003 (-0.015, 0.009)	0.61	ns	ns
Maternal BMI	0.025 (0.023, 0.028)	<0.0001	0.023 (0.021, 0.026)	<0.0001
Parity	-0.004 (-0.016, 0.008)	0.54	ns	ns
Ethnicity	0.364 (-0.076, 0.804)	0.11	ns	ns
MMS * Ethnicity	-0.0004 (-0.002, 0.009)	0.50	ns	ns

Model 1 included all covariates and fasting glucose as outcome. Model 2 only included significant covariates and also reported fasting glucose as outcome. Non-significant variables removed from the model are denoted as 'ns'. Parity was reported as having 0,1,2,3,4,5, or 6 children. IMD was reported in quintiles as 1,2,3,4,5. Ethnicity was self-reported and only included White Europeans (1) and Pakistani (2). CI, confidence interval; meat Y/N, meat – yes '1' or no '2'; MMS, metabolic meat intake score; ns, non-significant; IMD20, index of multiple deprivation in quintiles; BMI, body mass index.

Table 6.4: Linear regression of the association of the metabolic meat intake score (MMS) with postprandial glucose in total population (n=7,004).

Variables	Model 1		Model 2	
	β in mmol/L (95% CI)	p-value	β in mmol/L (95% CI)	p-value
MMS	-0.003 (-0.006, -0.005)	0.02	-0.002 (-0.005, 0.0002)	0.607
Age	0.05 (0.041, 0.057)	<0.0001	0.046 (0.04, 0.053)	<0.0001
Gestational age	0.002 (-0.017, 0.022)	0.835	ns	ns
IMD20	0.032 (-0.002, 0.006)	0.065	0.029 (-0.003, 0.062)	0.077
Maternal BMI	0.047 (0.041, 0.054)	<0.0001	0.046 (0.04, 0.053)	<0.0001
Parity	-0.025 (-0.058, 0.009)	0.151	ns	ns
Ethnicity	-0.837 (-2.08, 0.406)	0.187	ns	ns
MMS * Ethnicity	0.004 (0.002, 0.008)	0.041	0.003 (-0.005, 0.006)	0.098

Model 1 included all covariates and postprandial glucose as outcome. Model 2 only included significant covariates and also reported postprandial glucose as outcome. Non-significant variables removed from the model are denoted as 'ns'. Parity was reported as having 0,1,2,3,4,5, or 6 children. IMD was reported in quintiles as 1,2,3,4,5. Ethnicity was self-reported and only included White Europeans (1) and Pakistani (2). CI, confidence interval; meat Y/N, meat – yes '1' or no '2'; MMS, metabolic meat intake score; ns, non-significant; IMD20, index of multiple deprivation in quintiles; BMI, body mass index.

The sensitivity analysis of the independent 'training' and 'test' population showed that the effect size of MMS on PPG did not change and was comparable (i.e., -0.003 mmol/L; **Tables 6.4, 6.6 and 6.8**), indicating no bias was introduced by incorporating the 'training' set in the total cohort (**Tables 6.4 and 6.6**). Again, no significant associations were found between MMS and fasting plasma glucose, even after removal of non-significant covariates (MMS: $p = 0.62$ and $p = 0.64$, respectively; **Table 6.5 and 6.7**).

Table 6.5: Linear regression of the association of the metabolic meat intake score (MMS) with fasting glucose in ‘training’ population (n=2,655).

Variables	Model 1		Model 2	
	B in mmol/L (95% CI)	p-value	B in mmol/L (95% CI)	p-value
MMS	-0.003 (-0.002 , 0.001)	0.62	0.0002 (-0.001 , 0.001)	0.67
Age	0.011 (0.007 , 0.015)	<.0001	0.012 (0.009 , 0.016)	<.0001
Gestational age	0.008 (-0.003 , 0.019)	0.16	ns	ns
IMD20	-0.013 (-0.031 , 0.005)	0.15	ns	ns
Maternal BMI	0.021 (0.017 , 0.024)	<.0001	0.019 (0.016 , 0.02)	<.0001
Parity	0.002 (-0.017 , 0.020)	0.87	ns	ns
Ethnicity	0.047 (-0.655 , 0.749)	0.90	ns	ns
MMS * Ethnicity	0.0004 (-0.002 0.002)	0.71	ns	ns

Model 1 included all covariates and fasting glucose as outcome. Model 2 only included significant covariates and also reported fasting glucose as outcome. Non-significant variables removed from the model are denoted as ‘ns’. Parity was reported as having 0,1,2,3,4,5, or 6 children. IMD was reported in quintiles as 1,2,3,4,5. Ethnicity was self-reported and only included white Europeans (1) and Pakistani (2). CI, confidence interval; meat Y/N, meat – yes ‘1’ or no ‘2’; MMS, metabolic meat intake score; ns, non-significant; IMD20, index of multiple deprivation in quintiles; BMI, body mass index.

Table 6.6: Linear regression of the association of the metabolic meat intake score (MMS) with postprandial glucose in 'training' population (n=2,655).

Variables	Model 1		Model 2	
	β in mmol/L (95% CI)	p-value	β in mmol/L (95% CI)	p-value
MMS	-0.003 (-0.007 , 0.0001)	0.062	-0.003 (-0.006, 0.0008)	0.056
Age	0.047 (0.036 , 0.058)	<.0001	0.048 (0.037 , 0.058)	<.0001
Gestational age	0.0067 (-0.025 , 0.038)	0.68	ns	ns
IMD20	0.018 (-0.003 , 0.067)	0.49	ns	ns
Maternal BMI	0.040 (0.030 , 0.050)	<.0001	0.040 (0.030 , 0.050)	<.0001
Parity	-0.064 (-0.117 , -0.107)	0.02	-0.067 (-0.12 , -0.14)	0.01
Ethnicity	-2.35 (-4.34 , -0.348)	0.02	-2.38 (-4.37 , -3.81)	0.02
MMS * Ethnicity	-0.007 (0.002 , 0.014)	0.009	0.008 (0.002 , 0.001)	0.009

Model 1 included all covariates and postprandial glucose as outcome. Model 2 only included significant covariates and also reported postprandial glucose as outcome. Non-significant variables removed from the model are denoted as 'ns'. Parity was reported as having 0,1,2,3,4,5, or 6 children. IMD was reported in quintiles as 1,2,3,4,5. Ethnicity was self-reported and only included White Europeans (1) and Pakistani (2). CI, confidence interval; meat Y/N, meat – yes '1' or no '2'; MMS, metabolic meat intake score; ns, non-significant; IMD20, index of multiple deprivation in quintiles; BMI, body mass index.

Table 6.7: Linear regression of the association of the metabolic meat intake score (MMS) with fasting glucose in ‘test’ population (n=4,349).

Variables	Model 1		Model 2	
	B in mmol/L (95% CI)	p-value	B in mmol/L (95% CI)	p-value
MMS	-0.003 (-0.002 , 0.001)	0.62	0.0002 (-0.0007 0.001)	0.64
Age	0.011 (0.007 , 0.015)	<.0001	0.012 (0.009 , 0.016)	<.0001
Gestational age	0.008 (-0.003 , 0.019)	0.16	ns	ns
IMD20	-0.013 (-0.031 , 0.005)	0.15	ns	ns
Maternal BMI	0.021 (0.017 , 0.024)	<.0001	0.019 (0.016 , 0.02)	<.0001
Parity	0.002 (-0.017 , 0.020)	0.87	ns	ns
Ethnicity	0.047 (-0.655 , 0.749)	0.90	ns	ns
MMS * Ethnicity	0.0004 (-0.002 0.002)	0.71	ns	ns

Model 1 included all covariates and fasting glucose as outcome. Model 2 only included significant covariates and also reported fasting glucose as outcome. Non-significant variables removed from the model are denoted as ‘ns’. Parity was reported as having 0,1,2,3,4,5, or 6 children. IMD was reported in quintiles as 1,2,3,4,5. Ethnicity was self-reported and only included white Europeans (1) and Pakistani (2). CI, confidence interval; meat Y/N, meat – yes ‘1’ or no ‘2’; MMS, metabolic meat intake score; ns, non-significant; IMD20, index of multiple deprivation in quintiles; BMI, body mass index.

Table 6.8: Linear regression of the association of the metabolic meat intake score (MMS) with postprandial glucose in 'test' population (n=4,349).

Variables	Model 1		Model 2	
	β in mmol/L (95% CI)	p-value	β in mmol/L (95% CI)	p-value
MMS	-0.003 (-0.007 , 0.001)	0.064	-0.002 (-0.0002, 0.002)	0.88
Age	0.049 (0.039 , 0.059)	<.0001	0.056 (0.048 , 0.064)	<.0001
Gestational age	-0.0005 (-0.025 , 0.024)	0.96	ns	ns
IMD20	0.051 (0.004 , 0.097)	<.0001	-0.047(-0.087 , -0.007)	0.021
Maternal BMI	0.053 (0.044 , 0.062)	<.0001	0.048 (0.039 , 0.057)	<.0001
Parity	-0.006 (-0.050 , 0.037)	0.78	ns	ns
Ethnicity	-0.30 (-1.99 , 1.40)	0.73	ns	ns
MMS * Ethnicity	0.002 (-0.003 , 0.008)	0.34	ns	ns

Model 1 included all covariates and postprandial glucose as outcome. Model 2 only included significant covariates and also reported postprandial glucose as outcome. Non-significant variables removed from the model are denoted as 'ns'. Parity was reported as having 0,1,2,3,4,5, or 6 children. IMD was reported in quintiles as 1,2,3,4,5. Ethnicity was self-reported and only included White Europeans (1) and Pakistani (2). CI, confidence interval; meat Y/N, meat – yes '1' or no '2'; MMS, metabolic meat intake score; ns, non-significant; IMD20, index of multiple deprivation in quintiles; BMI, body mass index.

6.5 Discussion

This study aimed to develop a metabolic meat-intake score based on self-reported FFQ and NMR data and assess associations between this MMS and glycaemia in pregnancy – a period known of disrupted glucose control. Using data from the prospective BiB birth-cohort, the research objectives were tested. A total of 7,004 women at ~26.3 weeks' gestation were retained for final analysis, with an average age of 27.2 years and BMI of 28.4. Overall, this study demonstrated that the developed metabolic 'meat-intake' score is associated with postprandial glucose levels; albeit, not clinically strong.

6.5.1 Metabolite Meat Intake Scores

Thirty-eight metabolites associated with self-reported meat consumption ('yes' or 'no') were identified, including very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and ratios of omega-3, omega-6, poly-unsaturated, mono-unsaturated and saturated fatty acids (PUFA, MUFA, and SFA, respectively)(**Appendix Table D.2**). These 38 metabolites were subsequently used to calculate the MMS. The validity of the MMS was evaluated internally in the 'train' population (n = 2,655) and was confirmed to be significantly associated with self-reported meat consumption. The internal validation assessing the relationship between MMS and self-reported meat-intake reported that with a unit increase of MMS there 12% less likelihood of the individual being a meat consumer. The internal validation revealed that the panel of metabolites that comprised the MMS was able to distinguish between meat consumers and non-meat consumers. Furthermore, other methods of categorising self-reported meat consumption (e.g., red-meat vs no red-meat intake) should be explored to determine the best method of developing a metabolic meat-intake score.

Recently, metabolites haven been shown to be associated with certain dietary patterns, including meat intake. Previous studies reported amino acid metabolites associated with a dietary patterns, including lower isoleucine, leucine, valine and creatine, and higher glutamine, glycine and trimethylamine, in vegans and vegetarians compared to omnivores (Schmidt et al., 2021; Wang et al., 2019). Furthermore, others studies found that creatinine, total cholesterol, LDL, and HDL was higher in meat-eaters compared to non-meat-eaters (Lindqvist et al., 2019). Although our study did not confirm the predictive role of

amino acids, our work is in agreement regarding LDL and HDL metabolites. Moreover, other work shows that diets containing red-meat is correlated with LDL and its subfractions, including total cholesterol, free cholesterol and phospholipids, and that plant-based meat consumers had lower levels of TC and LDL-C (Lindqvist et al., 2021; Schmidt et al., 2021; Yu et al., 2019a; Rosell et al., 2005). Likewise, the present found associations between dietary protein and LDL subfractions, identified via PLS analysis (**Appendix Table D.2**). Reasons for discrepancies with this current studies' results is that most of the diet / metabolite association studies were conducted in men and studies in pregnant women are lacking. Therefore, to our knowledge, this is the first study examining these diet patterns associations in an ethnically diverse population with pregnant women. Furthermore, the Nightingale platform Nightingale platform measures a large number of metabolites which have not yet been studied in relation to dietary protein intake in this cohort of women, including lipoprotein profiling with 14 subfractions (Schmidt et al., 2021).

6.5.2 Metabolite Meat Intake Scores and Glycaemia

Following internal validation, the MMS was regressed against fasting and postprandial glucose to evaluate its role as a moderator dysglycaemia in pregnancy. To minimise confounding, linear regression models included known GDM covariates. This study reports that MMS was not associated with fasting glucose; however, an association with postprandial glucose was observed in the total population ($n = 7,004$). Furthermore, evaluating the role of MMS as a moderator of dysglycaemia in the 'test' population ($n = 4,349$), revealed no change in effect sizes – indicating that no bias was introduced by utilising the total population (including the 'training' and independent 'test' cohort). A likely explanation of the clinically small effect size; utilizing meat consumption as a whole may not be the most effective method of distinguishing groups in this cohort. The BiB cohort utilises FFQs which were not designed to stratify in meat- vs plant-based diet and the number of meat consumers is substantially larger compared to the non-meat consumers ($n = 2,618$ vs 37 , respectively). These factors made it challenging to decide on the categorisation of the self-reported meat intake and perhaps other methods of categorising meat consumption will be of interest.

This study aimed to explore the additional value a metabolic profile score instead of using FFQs. Although FFQs offer a relatively easy and cost-effective way to collect an accurate record of habitual food and nutrient intake for a large group of individuals, these methods take time and certain level of understanding and physical ability to complete, increasing burden on participants and investigators. Additionally, FFQs are known to have several limitations including various types of bias (e.g., recall and social desirability bias), not all foods may be presented on the list (e.g., pre-prepared meals and take aways), difficulty determining portion size in some questionnaires, and little detail on the characteristics of foods (e.g., cooking method or combinations of food in meals) (Cade et al., 2004). Therefore, new methods of capturing dietary intake may be of interest. This study demonstrated an association between MMS and PPG (PPG ~ MMS + covariates; β : -0.003 mmol/L, and $p = 0.02$), but no association between FFQ (i.e., meat consumer 'yes' or 'no') and PPG (PPG ~ FFQ + covariates; β : -0.29 mmol/L, and $p = 0.237$) was demonstrated. The lack of association between FFQ and measures of glycaemia may indicate the beneficial use of metabolite profiles to assess diet-disease relationships. The costs of NMR metabolite analysis is approximately £20 per sample, which is not expensive but could be a limitation for large scale studies and LMICs (Taylor et al., 2021). Using metabolite profiles as a new method of capturing dietary intake may be beneficial for improving misreporting and examining diet-disease relationships. However, future studies should focus on finding more distinctive metabolite profiles, and explore the feasibility and cost-effectiveness of using diet-metabolite profiles.

The importance of mealtime glycaemia and dietary protein have been stipulated in *Chapters 1, 4 and 5*. Mealtime glucose represents a key target for improving glycaemic control in pregnancy, as previous studies have linked uncontrolled postprandial glucose to adverse pregnancy outcomes, for instance LGA is known to be one of the associated adverse pregnancy outcomes (Law et al., 2019; Feig et al., 2017; Zheng et al., 2019). Furthermore, evidence has shown that postprandial glucose levels appear the most effective for the determination of the likelihood of adverse pregnancy outcomes – e.g. fasting glucose levels only explain 12% of the variation of birth weight, and postprandial glucose approximately 40% (Zheng et al., 2019). This might suggest the lack in found associations between MMS and fasting glucose in the present study. Exploratory

analysis in *Chapter 4* demonstrated a favourable association between higher protein intake (+1SD or +92 kcal/day) and lower 24-hr mean glucose (-0.91 mmol/L, $p=0.020$) and total AUC_{glucose} (1209.6 mmol/L.min⁻¹, $p=0.021$) (Dingena et al., 2023b).

Modest associations between metabolite profiles of meat consumption and measures of glycaemic control (i.e., PPG) were found, this panel of 38 metabolites included extremely and very large VLDL triglycerides and ratio of saturated fatty acids/total fatty acids) that could be of interest. The use of metabolites and development of metabolite dietary quality scores may aid in the interpretation of future studies on the associations of diet patterns (e.g., meat consumption) with disease risk (e.g., diabetes or CVD). For instance, prospective studies noted lower risk of T2DM in individuals with higher circulating n-6 linoleic acid (Wu et al., 2017; Imamura et al., 2017) and in vegans compared to other diet groups (Tonstad et al., 2009; Papier et al., 2019). Other studies report similar results, and thus circulating n-6 linoleic acid may play a role in the association between diet group and risk of T2DM, albeit with BMI as a major contributing factor (Schmidt et al., 2021). Unfortunately, linoleic acid was not identified in the associated metabolic profile we found; however, ratio n-6 and PUFA to fatty acids were identified. For fatty acids and cardiovascular disease risk, Pan et al. (2022) (Pan et al., 2022) found no matter total, saturated, or unsaturated fatty acids, each was positively correlated with red meat consumption, and associated with incident cardiovascular disease risk in the same direction. This correlation might be because red meat, while rich in SFAs, also contains unsaturated fatty acids (UFAs), and the proportion SFAs/UFAs is highly dependent on the type of red meat (Pan et al., 2022).

Previous studies in the BiB cohort found evidence that maternal pregnancy NMR metabolites improves prediction of pregnancy-related disorders (McBride et al., 2020; Taylor et al., 2021; Fuller et al., 2022a), highlighting the significance of this line of research. Prediction models for pregnancy disorders (e.g., hypertension and SGA/LGA) consisting of NMR metabolites and known risk factors for GDM (e.g., maternal age, BMI, and ethnicity) performed better than these known risk factors alone (Taylor et al., 2021; McBride et al., 2020). Other work has shown that the distributions of the NMR metabolites differed by ethnicity (Fuller et al., 2022a; Taylor et al., 2019). White European women had higher

levels of most lipoprotein subfractions (i.e., cholesterol, glycerides and phospholipids), MUFA, and creatinine but lower levels of most amino acids, glucose, linoleic acid, n-6 and PUFAs, compared to Southeast Asian women (Taylor et al., 2019; McBride et al., 2020). This present study also found that ethnicity is a confounding factor for a metabolic profile consisting of most lipoproteins (i.e., VLDL, LDL and HDL) and their subfractions (i.e., cholesterol, glycerides and phospholipids), omega-6 and PUFA ratios. Higher BMI and having GDM were associated with higher levels of several lipoprotein subfractions (i.e., triglycerides) (Taylor et al., 2021), this is in agreement with our study as BMI was a significant confounder. Fuller et al. (2022) (Fuller et al., 2022a) showed that the metabolic risk profile for GDM differs between White European and Southeast Asian in the BiB cohort. These studies confirm that additionally assessing metabolic meat-intake profiles stratified per ethnicity may aid in informing targeted strategies.

Focussing more closely on diet, we should note the presence of differences in cultural diet patterns in Southeast Asians and White British women. The Southeast Asian diet is predominantly cereal based, with typical meals consisting of rice, flat breads (e.g., roti and naan), potatoes, vegetables and small amounts of meat (Lee et al., 2013; Jenum et al., 2019). A “traditional British diet” – seen in White British women – is a diet pattern consisting of i) high intake in white bread/refined cereals, butter and high fat dairy products; ii) moderately high intake in tea, prepared meat products, chips/roast potatoes, cakes/pastries and preserves/confectionery, and iii) low intake in vegetables, fruit and wholegrain bread/cereals (Pryer et al., 2001). Therefore, based on these cultural dietary patterns, grouping of meat consumers vs non-meat consumers may be a limitation. However, in the analysis of MMS and glycaemic measures, the model was adjusted for ethnicity. Nonetheless, there is need for future research exploring other grouping categories and differences between Pakistani vs White British women. The Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort (n=11833) found that socioeconomic status (SES) was associated with food choices, whereby women of high SES more likely consumed healthy foods and less processed foods than women of a lower SES (Rogers et al., 2017). Zulyniak et al. (2017) (Zulyniak et al., 2017) found that White European women who adhered most strongly to a plant-based diet represented the highest

socioeconomic group and Southeast Asian women who adhered most strongly to the plant-based diet were more recent immigrants and had a lower SES. As the majority of the women in the BIB cohort have a low SES (IMD in the lowest quintile), future work should take SES in account when developing new dietary strategies. All in all, a better understanding of dietary metabolites related to protein intake and their association to these health risks (e.g., LGA, T2DM and CVD) could inform dietary management and prevention strategies.

6.5.3 Strengths and Limitations

This study has offered insights into development of a novel metabolic meat-intake score and its association with glycaemic control in pregnancy within a diverse cohort; however, the results may not be generalizable across other ethnic groups or geographic regions. Furthermore, metabolomics platforms differ in coverage of the metabolome and dietary behaviour within study populations are likely to differ, making comparisons of results across studies complex. Regarding sample analysis, samples were taken at a single time point at 26-28 week's gestation; therefore, this study was unable to account for differences in fasting duration and day-to-day variation. Also, the results are not generalizable throughout the full course of pregnancy. As with all observational studies, the effect of confounding needs to be taken into account – all analyses were adjusted for known confounders – and causality cannot be inferred. Conducting a longitudinal study could resolve some issues, in *Chapter 5*, a protocol for a longitudinal study including metabolic assessment is described. Similar to other nutritional epidemiological studies, there was an unavoidable recall bias and social desirability bias when estimating meat consumption by FFQ. In addition, comprehensive dietary data on dietary protein intake were not available and non-meat consumers were disproportionately underrepresented, which can influence the quality and efficacy of the MMS.

Even though, this study has several limitations; to our knowledge, this is the first study to use metabolic meat-intake score to characterize the relationship between dietary protein intake and glycaemic control in pregnancy. Although this analysis was underpowered, this is the largest dataset in a diverse population consisting of a comprehensive collection of covariates. Moreover, for metabolite analysis, a wide range of metabolic markers based an NMR platform were

detected, including but not limited to lipoprotein and their constituents, including different particle size, density and chemical structure.

6.5.4 Conclusion

To conclude, this study has identified potential metabolic profiles to characterise meat consumption – using these profiles a metabolic meat-intake score was developed – but no clinically strong associations between this MMS and measures of glycaemia in pregnancy were found. Future analyses of this cohort should consider other identifiers of meat consumption (e.g., red-meat vs no red-meat intake), to determine the most effective method of developing the MMS. Exploring these links between diet, metabolites and glycaemia across gestation will provide a better understanding of the metabolome during pregnancy and how it relates to maternal and offspring health and shed a light on how to improve prevention strategies. However, future work focussing on attaining more comprehensive dietary records and metabolites data at different stages of the pregnancy and in larger cohorts is needed to evaluate the moderating effect of diet on metabolism, maternal glucose control and pregnancy risks.

6.6 Summary

- The association between dietary protein and gestational dysglycaemia is well demonstrated. However, the relationship between protein source, namely meat intake, and glycemic control in pregnancy remains unclear. This study aimed to (i) develop a metabolic meat-intake score (MMS) by identifying metabolites associated with meat intake, and (ii) examine the association of this MMS with measures of glycaemic control in pregnancy.
- 38 metabolites were found to be associated with dietary patterns of meat consumption. The internal validation demonstrated a relationship between MMS and self-reported meat-intake – suggesting that the panel of metabolites was able to distinguish meat consumers from non-meat consumers.
- Associations between MMS and pregnancy dysglycaemia (i.e., OGTT – FPG and PPG) were tested via linear regression adjusted for known confounders in the total (n = 7,004) and independent ‘test’ population (n = 4,349) and found no clinically strong associations – indicating that this MMS may be ineffective in this cohort.

- This study demonstrates that meat consumers can be characterised by a distinct metabolite profile and may be associated with glycaemia in pregnancy. Future analysis exploring other indicators of meat consumption in this cohort and future studies with a larger sample size are warranted. Exploring these links between diet, metabolites and glycaemia across gestation will provide a better understanding of the metabolome during pregnancy and how it relates to maternal and offspring health. Ultimately, improving prevention strategies.

Chapter 7

General Discussion, Future Implications, and Conclusions

The aim of this thesis was to contribute to the overall understanding of mediators of (postprandial) glucose control – including dietary, personal, physiological and environmental parameters – and which management strategies could influence these mediators and improve glycaemic control in diabetes in pregnancy. Four studies were conducted to answer the research objectives by:

- (i) evaluating the current evidence regarding the role of lifestyle interventions in improving dysglycaemia in DIP (*Chapter 3*);
- (ii) analysis of CGM data to identify the times during the day with observed higher levels of dysglycaemia in GDM and identify which dietary mediators are associated (*Chapter 4*);
- (iii) feasibility of a longitudinal study with an embedded RCT on CGM glucose profiles throughout the course of the pregnancy and their association with personal and physiological parameters in DIP (*Chapter 5*), and
- (iv) assessing associations of self-reported meat-intake with plasma metabolic markers and measures of glycaemic control in pregnancy (*Chapter 6*).

The literature review illustrated that DIP places a great risk on public health due to its prevalence and association with adverse short- and long-term pregnancy outcomes for both mother and offspring. Globally, healthcare bodies have developed dietary and lifestyle guidelines to achieve optimal (mealtime) glycaemic control. Unfortunately, women with DIP continue to struggle with achieving optimal (postprandial) glycaemic control, resulting in continued risk of adverse pregnancy outcomes. The body of literature has shown that glycaemia in DIP is influenced by numerous factors (e.g., personal and physiological parameters). Mealtime glucose has been identified as a key target for improving long-term glucose control, as this is the main period of time that blood glucose levels go out of target range, and the more time spent above target range is associated with a higher prevalence of adverse pregnancy outcomes. Current dietary guidelines are suboptimal in assessing glucose responses to meals consumed in real-life, as personal and

physiological factors beyond the characteristics of food play an important role. Identification of these factors has provided insights into lifestyle determinants involved in PPGR and glycaemic control in DIP, namely:

- There is a large discrepancy between lifestyle interventions studies on glycaemia in maternal diabetes, with a significant gap in research involving pre-gestational T1DM and T2DM (*Chapter 3*).
- Nutritional supplements, diet, and exercise show advantageous effects on measures of glycaemia, with maternal age and gestational age as possible modifiers of effect of lifestyle-interventions on maternal glycaemia (*Chapter 3*).
- Glycaemic control significantly differed throughout a 24-hr period in GDM pregnancies, with morning glucose levels demonstrating greatest level of variability (*Chapter 4*).
- Individuals with GDM assigned to diet+metformin have greater difficulty managing their glucose control – mean glucose and AUC_{glucose} were significantly higher in diet+metformin compared to diet alone (*Chapter 4*).
- Higher protein intake was associated with a more favourable (mealtime) glucose control (*Chapter 4*).
- A metabolic meat intake score (incorporating 38 metabolites) was developed to characterise meat consumption by a distinct metabolite profile (i.e., VLDL triglycerides and saturated fatty acids metabolites). However, this MMS distinguished non-meat consumers rather than meat consumers and was associated with postprandial glucose levels, albeit not clinically strong (*Chapter 6*).

The importance and implications of these findings for future research and ultimately contribution in development of new nutritional strategies for optimal glycaemic control in DIP is described below and in **Figure 7.1**.

7.1 Interventions for Improving (Postprandial) Glycaemia

7.1.1 Diet

This thesis has demonstrated that certain dietary options encourage improvements in dysglycaemia in DIP. In *Chapter 3*, a pooled meta-analysis showed that diet interventions (including low GI, restricted energy intake, and

DASH) improve IR and potentially improve FPG in women with GDM, compared to a standard diet. A low/reduced carbohydrate diet for pregnant women with GDM (first-line treatment in GDM pregnancies) has been linked with reduced FPG, decreased risk of postprandial hyperglycaemia, and reduced risk of requiring insulin to manage dysglycaemia (Webber et al., 2015; Major et al., 1998; American Diabetes Association, 2017). Both Yamamoto et al., (2018) and the meta-analysis conducted in *Chapter 3* demonstrated a high heterogeneity, which could have been influenced by differences in baseline FPG, or PPG levels and their relation to the reported outcomes. For example, if FPG and/or PPG levels were within target at recruitment/baseline diet intervention may not have significant effect. To overcome this, individuals with glucose levels in target range would have to be excluded. Furthermore, adherence to the interventions is under reported in the studies of the SRMA in *Chapter 3*. This meta-analysis supports current recommendations that prescribe dietary interventions to manage dysglycaemia during pregnancy. Future work that accounts for personal characteristics and adherence to the diet may allow for better clarity of the effectiveness and feasibility of distinct diets.

In *Chapter 4*, the exploratory nutritional analysis demonstrated a favourable association when protein intake increased by 1 standard deviation (+92 kcal/day) – while holding dietary carbohydrates and fats quantities constant – is associated with lower mean glucose and total AUC_{glucose} (-0.91 mmol/L, $p=0.020$ and 1209.6 mmol/L.h, $p=0.021$, respectively). While current positions and recommendations of major health bodies (National Health Services [UK], Canadian Diabetes Association, the American Diabetes Association, and the European Association for the Study of Diabetes) focus on replacing low-quality processed (i.e., high glycaemic-index) carbohydrates with high-quality (i.e., low glycaemic-index) carbohydrates for patients with diabetes, this thesis positions protein as an additional dietary mediator of gestational dysglycaemia. The influence of protein blunting glycaemic response is likely explained by its stimulating effect on gut hormones (i.e., cholecystokinin, gastric inhibitory polypeptides, and GLP-1) which mediate the slowing of gastric-emptying rates (Meng et al., 2017a; Hutchison et al., 2015). Also, protein may blunt rise in blood glucose levels by ameliorating the insulin response – some amino acids have been shown to stimulate insulin secretion from pancreatic β -cells (van Loon et al., 2000; Gunnerud et al., 2013;

Gannon et al., 1988; Hutchison et al., 2015). Although the study in *Chapter 4* was not designed to investigate interactions between macronutrients consumed and time of day, it shows that future studies need to be appropriately designed to investigate such an interaction, and report on the importance of timing nutritional-quality meals to manage dysglycaemia.

Building on from *Chapter 4* and to address aforementioned issues, the INFORMED study was designed – rationale and protocol are illustrated in *Chapter 5*. Briefly, current evidence suggests that control of (postprandial) glycaemia is key to manage maternal and offspring health in DIP but evidence is limited regarding (i) the role of diet and lifestyle as mediators of glucose variability and control throughout the course of pregnancy, and (ii) what aspects of maternal and offspring health are associated. The overall aim of the INFORMED study was to assess postprandial glucose profile over the full duration course of pregnancy, so that trends and early markers/mediators of dysglycaemia (i.e., personal characteristics and physiological parameters) could be identified. The trial was embedded within standard clinical care and utilised routine data and biological samples collected by health services to minimise participant burden and non-essential participant contact, reducing the risk of bias and will permit the study to continue even with the re-introduction of public restrictions. CGM was integrated to measure glucose levels and volatility over a prolonged periods alongside and experimental breakfast meals to provide a standard food for all participants by which their response could be compared in early- and mid-pregnancy. Recruitment has started but the start was delayed due to (i) the COVID-19 pandemic (i.e., non-essential human studies were suspended and protocol had to be re-designed according to COVID guidelines), (ii) delays in ethics approval and other sign-offs within the NHS research site, and (iii) post-pandemic recruitment struggles for research studies. Unfortunately, no participants had been recruited before and during the thesis write-up, thus no results been obtained.

The results of *Chapter 4* are supported by existing evidence regarding dietary protein as a mediator of glucose control in pregnancy (Dingena et al., 2023b; Gannon et al., 1988; Maslova et al., 2017; Meng et al., 2017a; Allman et al., 2019). To date, however, the relationship between major dietary protein sources, particularly meat-intake, and glycaemic control in pregnancy remains

unclear. Metabolites offer a novel quantitative approach to untangle this association and better understand the role of animal protein metabolism on glucose metabolism in pregnancy. In an attempt to establish whether meat-intake by distinct metabolic profiles is associated with dysglycaemia in pregnancy, *Chapter 6* aimed to i) develop a metabolic meat-intake score (MMS) by identifying metabolites associated with dietary patterns of meat intake using FFQ data, and ii) whether this MMS associated with measures of glycaemic control in pregnancy. This was first study examining the utility of a metabolic meat-intake score to characterize the relationship between dietary protein intake and glycaemic control in an ethnically diverse population of pregnant women. Additionally, the utilised Nightingale platform is able to measure a large number of metabolites (including lipoprotein profiling with 14 subfractions) which have not yet been studied in relation to animal protein intake in this cohort. By combining the NMR metabolite data with the PLS VIP outcomes, an MMS was developed in *Chapter 6*.

Thirty-eight metabolites were found to be associated (VIP [Variable Importance in Projection] score ≥ 1) with meat consumption; extremely and very large VLDL triglycerides and ratio of saturated fatty acids/total fatty acids were most important metabolites (i.e., top 3 VIP scores). This relationship between MMS and self-reported meat-intake was inversed – as non-meat consumers had a higher average MMS score compared to meat-consumers (mean MMS = 340.4 vs 337.3) and the internal validation reported that with a unit increase of MMS there 12% less likelihood of the individual being a meat consumer. This indicating that the MMS distinguished between meat consumers and non-meat consumers. Subsequently, adjusted regression models in the total population found that MMS was associated with a reduction in postprandial glucose, this confirming an advantageous relationship between dietary protein and glycaemia in pregnancy – albeit clinically not strong due to small effect size. The independent cohort reported a similar effect size (PPG: MD -0.003 mmol/L), albeit not significant. This chapter has identified potential metabolic profiles associated with glycaemia in pregnancy; nevertheless, the associations are weak and future analysis would benefit from exploring other methods of grouping self-reported meat consumption.

The significant effect of ethnicity on the association between metabolic profile and glycaemia in pregnancy seen in *Chapter 6*, may be the result of personal differences between individuals of Pakistani and European origin. For instance, differences in dietary patterns and food preparation methods suggest that Southeast Asian cooking methods may alter the macronutrient composition of food by increasing the proportion of total fat relative to other nutrients, this will have an effect on glycaemia (Raynor, 2008; Zulyniak et al., 2017). The majority of previous work has reported that the co-ingestion of carbohydrate with fat from varied sources resulted in a reduction in $iAUC_{\text{glucose}}$ (Sun et al., 2014; Collier et al., 1984; Meng et al., 2017a). In regards to dietary patterns, Southeast Asian women are more likely to consume fruits and vegetables and less likely to consume meat compared to White British women (Jenum et al., 2019; Pryer et al., 2001). Furthermore, a previous study of the BiB cohort reported that Pakistani origin mothers were on average older, shorter, lighter, and less likely to smoke or consume alcohol during pregnancy than White British origin mothers, which are all known confounders of glycaemia (Wright et al., 2013). Therefore, based on these cultural differences, grouping of meat consumers vs non-meat consumers may be a limitation. However, there is need for future research exploring other grouping categories and differences between Pakistani vs White British women.

Metabolomics may aid in providing quantitative evidence of the associations and metabolic mechanisms underlying diet-disease relationships and identifying novel risk factors, as single-nutrient studies may be misleading due to their failure to capture the complex interplay between foods and nutrients consumed as meals and over prolonged periods of time. Exploring these links between diet, metabolites and glycaemia across gestation will provide a better understanding of the metabolome during pregnancy and how it relates to maternal and offspring health. Ultimately, improving management strategies.

7.1.2 Nutritional Supplements

Based on the meta-analyses in *Chapter 3*, nutritional supplements are associated with a reduction in FPG and IR, though the difficulty of generalizability due to heterogeneity and variety of supplements (e.g., alpha-lipoic acid, probiotic, ginger, fish oil, or zinc and vitamins) must be underlined. Nutritional supplements are able to improve maternal dysglycaemia – maternal age, gestational age, body weight and ethnicity – were shown to be confounders of nutritional supplement

interventions. For instance, nutritional supplement interventions were less effective on FPG in women with older maternal age and recommended body weight. Additionally, nutritional supplements were more effective at earlier in the pregnancy; thus, management strategies should aim for early intervention. These results confirm that nutritional supplements can reduce fasting glucose and insulin resistance, though underlines the importance of considering personal characteristics (and their influence of the effectiveness of interventions) and the limited evidence regarding their effect on postprandial and long-term estimates of dysglycaemia (i.e., PPG and HbA_{1c}). Regarding clinical significance (when target levels of ≤ 5.6 mmol/L for FPG and <2.89 for HOMA-IR are reached [Webber et al., 2015; Sokup et al., 2013]), this can be reached in women with mild dysglycaemia, given the magnitude of effect reported in this SRMA (MD - 0.40). Based on the findings, future studies with a more uniform nutritional supplementation approach are warranted to make informed recommendation to care guidelines for management regarding type of supplements and duration.

Overall, larger effect sizes, higher graded evidence and less heterogeneity was reported in the nutritional supplement-based interventions compared to diet- and exercise interventions. This is likely due to ease of adherence and standardisation of supplements compared to diet and exercise, which are likely more susceptible to changes in routine and circumstance (e.g., extended work hours, family commitments, sickness, etc.). As such, diet- and exercise-based interventions may require greater personalisation and flexibility to accommodate patient needs.

7.1.3 Exercise

In addition to dietary modifications, exercise is a vital component in management of dysglycaemia in DIP (Webber et al., 2015; Davenport et al., 2018). The meta-analysis in *Chapter 3* found that exercise was advantageous for modifying fasting glucose levels in women with GDM. These exercise interventions focused on brisk walks, resistance exercise, home-based exercises, and moderate intensity aerobics versus standard antenatal care. Subgroup analysis for this type of intervention was limited due to fewer included studies – maternal age, gestational age, and body weight – were shown to be confounders of exercise interventions. The observed advantageous effect of exercise on FPG is in agreement with previous studies (Allehdan et al., 2019; Cremona et al., 2018), but no significant

effects were reported on PPG or HbA_{1c}. Additionally, the magnitude of effect on FPG was clinically not strong (MD -0.10 mmol/L). As such, future studies are needed to determine the clinical effect of exercise interventions on other measures of glycaemia (including PPG, HbA_{1c}, and HOMA-IR).

7.1.4 Pharmacological

GDM and T2DM management typically consists of dietary and lifestyle education, with pharmacological therapy (initially metformin) incorporated if glycaemic control remains unimproved (Webber et al., 2015; American Diabetes Association, 2020b). However, the relationship between lifestyle treatment with or without metformin to glucose spikes and variability over a 24-hour period in women with GDM remains unclear. Therefore, an exploratory analysis of observational data in women with GDM in *Chapter 4* was conducted – examining associations of treatment with diet alone vs diet+metformin and maternal glycaemia. When comparing treatment methods, individuals assigned to diet with metformin appeared to have higher levels of dysglycaemia, as reflected by elevated mean glucose and total AUC_{glucose}. Confirming that individuals assigned to diet+metformin have greater difficulty managing their glucose control. Possible explanation of not achieving glycaemic control may be the results of certain barriers, such as i) lack of health insurance, a regular primary care or obstetric provider; ii) women and health care providers may be unaware of the existence, the importance of preconception care or it is not seen as a high priority; iv) social and economic challenges, including lack of child care, transportation, geographic isolation, and distrust of health care providers (Kendrick, 2004; Khan et al., 2019; Korenbrot et al., 2002; Owens et al., 2006). Noteworthy, metformin is only prescribed when women are failing to achieve glucose targets with lifestyle modifications; thus, glucose levels in this group are initially higher. The exploratory post-hoc analysis on treatment (diet alone vs diet+metformin) and measures of glucose control adjusted for known confounders (i.e., maternal age, BMI, gestational week, parity and ethnicity) indicated BMI and gestational age as significant confounders – higher BMI and later pregnancy have been previously associated with decreased glucose control (Bashir et al., 2020; Salzer et al., 2015a; Martínez et al., 2017).

Metformin is the most commonly prescribed anti-hyperglycaemic medication for Maternal T2DM and GDM in the UK; however, its effectiveness on glycaemic

control is only recently being documented. Recently, a clinical trial suggested that higher doses of metformin can reduce HbA_{1c} by 1–2% (11– 22 mmol/mol) (Hirst et al., 2012). A promising effect as previous research reported that a 1 % reduction in HbA_{1c} is associated with improved maternal and offspring outcomes in women with GDM (Kiefer et al., 2022). Furthermore, early treatment intensification with diet and metformin is associated with reduced foetal glucose levels, foetal hyperinsulinemia and macrosomia (Bashir et al., 2020). Taken together, this reveals the importance of treatment intensification when dysglycaemia is observed.

7.2 Monitoring and Timing of (Postprandial) Glycaemia

Maternal glucose is dynamic – glucose tolerance and insulin sensitivity vary over a 24-hour period, and evidence suggests that glycaemic peaks, troughs and patterns may be more predictive of disrupted glucose control (Scott et al., 2020a; Law et al., 2015; Tan et al., 2014). Suggesting that CGM can (i) offer new information regarding associations between disrupted glycaemic control and maternal and offspring health, and (ii) be used to inform and direct care more accurately at an earlier point of pregnancy. However, it remains unclear which periods of the day are prone to dysglycaemia. Therefore, *Chapter 4* ventured to demonstrate that (i) CGM offers different methods of assessing glycaemic health; (ii) measures of dysglycaemia vary considerably over a 24-hour period; and (iii) distinct periods of day are prone to lower or higher absolute glucose levels as well as glucose variability.

The secondary analysis conducted in *Chapter 4* found that glycaemic control significantly differed throughout a 24-hr period, with morning glucose levels demonstrating greatest level of variability (CV; 8.4% vs 6.5%, $p < 0.001$). Furthermore, the exploratory analysis did not find any significant associations between the birthweight groups (i.e., SGA, NGA and LGA) and time-of-day (i.e., morning, afternoon, evening and overnight) on maternal glucose control. However, Therefore, this study could not formulate conclusions regarding adverse pregnancy outcomes – expressed as birthweight – and maternal glucose control. Although, other studies have linked temporal dysglycaemia and adverse pregnancy outcomes. Namely, mean glucose and nocturnal glucose (i.e., 00:00–06:00h) were significantly higher in women who delivered an LGA infant compared to women who did not deliver LGA infants (6.2 ± 0.6 mmol/L vs. $5.8 \pm$

0.6 mmol/L, $p = 0.025$ and 6.0 ± 1.0 mmol/L vs. 5.5 ± 0.8 mmol/L, $p = 0.005$, respectively) (Law et al., 2019).

Moreover, *Chapter 4* highlighted that mean glucose levels during the day (i.e., morning, afternoon, and evening) are significantly higher compared to mean glucose levels overnight. This is in agreement with existing understanding of overnight glycaemic control, which postulates that mean glucose levels typically drop overnight (Zaharieva et al., 2020). However, recent work have stipulated that glucose excursions expose a health risk that is independent of mean glucose levels (Zaccardi et al., 2018; Monnier et al., 2008). Therefore, other measures of glucose control should be considered. For instance, glycaemic variability expressed as CV of glucose (Monnier et al., 2008; Danne et al., 2017), which quantifies the magnitude of glycaemic variability standardised to mean glucose levels. Despite seeing no difference in mean glucose levels between afternoon and evening, the secondary analysis shows that CV steadily declines during the day reaching lowest values 'overnight'. Additionally, morning CV was significantly higher compared to other times-of-day. Importantly, previous studies suggest that CV is related to offspring growth in the 2nd trimester in women with T1DM (Kristensen et al., 2019), and may be an indicator of risk of future health complications associated with T2DM – including cardiovascular disease, coronary events, non-cardiovascular mortality, and total mortality (Scott et al., 2020b; Kampmann et al., 2019b).

Glycaemic control and variability depend on a variety of different exogenous and endogenous determinants, such as (i) elevated insulin resistance, hepatic glucose production and insulin antagonistic hormones production, and (ii) increased sedentary lifestyle, unhealthy dietary behaviour and age related metabolic deterioration (Martínez et al., 2017). This thesis stipulates that morning-time control of glucose levels may be a key point of interest for managing maternal and offspring health and that future studies could benefit from incorporating multiple measures of glucose control (including glycaemic variability). Collectively, these insights demonstrate the significance of glycaemic variability and timing of dysglycaemia in respect to improving maternal and offspring outcomes in DIP.

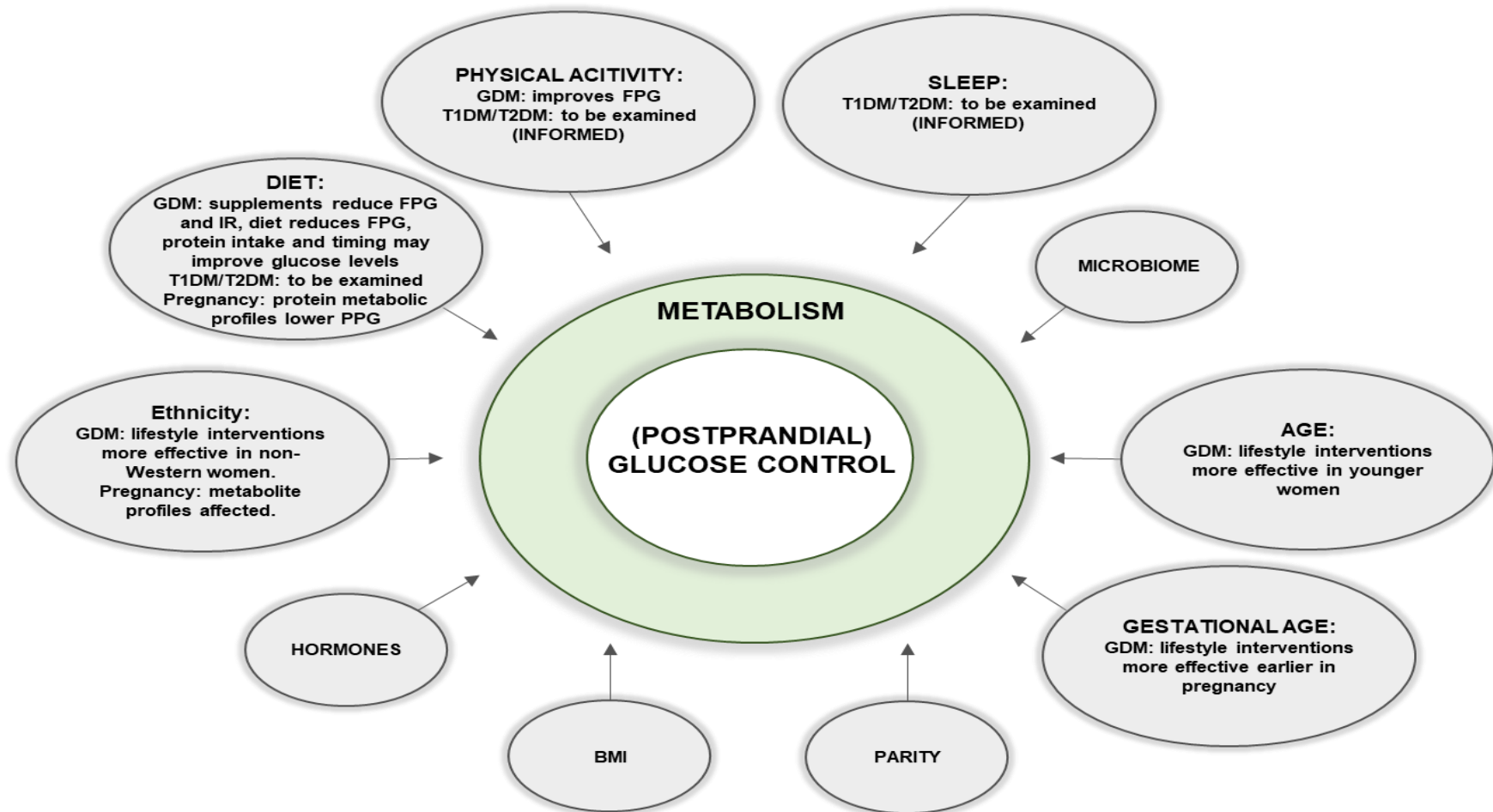


Figure 7.1: Schematic overview of drivers of (postprandial) glucose control in DIP. *This scheme provides an overview of previously postulated and by this thesis identified mediators of glycaemic control in maternal diabetes.*

7.3 Limitations and Methodological Considerations

7.3.1 Chapter 3: Nutritional and Exercise-Focused Lifestyle

Interventions on Glycaemic Control in Maternal Diabetes – an SRMA

Chapter 3 did not find evidence for lifestyle (i.e., nutritional supplement-, diet-, exercise-based) interventions that associate with glycaemia in pre-gestational T1DM and T2DM, highlighting the need for intervention studies in this group of women. Furthermore, *Chapter 3* was unable to find associations between lifestyle interventions and postprandial and long-term estimates of glycaemia (i.e., PPG and HbA_{1c}) in GDM. Noteworthy, caution is warranted when interpreting the findings and exploring their wider application, given the magnitude of effect and due to the individual nature of the studies (i.e., different intervention strategies) within each of the lifestyle categories. Differences in intervention strategies could influence the physiological mechanisms underlying glycaemic control. Yet, based on the findings, the meta-analysis supports current recommendations that prescribe lifestyle interventions to manage dysglycaemia during pregnancy; however, additional research accounting for more uniform intervention approaches and adherence to the interventions may allow for better clarity of the effectiveness and feasibility of distinct lifestyle interventions. Eventually, aiding in making informed recommendation to guidelines for management regarding type and duration of lifestyle intervention.

A high percentage of studies included were conducted in LMICs, this could be indicative of barriers to achieve or improve glycaemic control. Because lack of resources, trained personnel, and other priorities related to reducing maternal, foetal, and neonatal mortality, providing care to women with GDM is not high on the priority lists in many LMICs (Nielsen et al., 2012; Goldenberg et al., 2016). In some cultures the woman herself does not make the decisions concerning her own health - those decisions are generally made by her husband and/or in-laws (Nielsen et al., 2012). All these factors contribute to managing glycaemic control in GDM in LMICs.

Other limitations of *Chapter 3* were that six of the included studies were pilot studies or underpowered, thus future studies should increase the number of participants. Additionally, subgroup analysis based on common moderators of

GDM and glycaemic control could not be performed for some of the outcomes. More studies including multiple outcome measures of glycaemic control, especially PPG and HbA_{1c}, are required. Moreover, the short duration of some of the interventions and late gestational age at which the interventions were started may have limited their impact on glycaemic outcomes, this could be areas of improvement. Finally, most of the outcomes had a very- or low-GRADE quality score due to limitations in the design of included studies (e.g., lack of allocation concealment, blinding of either outcome assessors or participants, reporting of adherence to the intervention) which are all of importance to consider for designing future interventions. In case of the INFORMED study (*Chapter 5*), allocation to intervention was concealed, intervention was double-blinded, and adherence to the intervention will be recorded during the phone call with the participant.

7.3.2 Chapter 4: Relationship of Dietary and Pharmacological Treatment on 24-hr Continuous Measures of Glycaemia in Gestational Diabetes Mellitus – an Observational Study

Chapter 4 observed that (i) glycaemic control differed throughout a 24-hr period, (ii) individuals assigned to diet+metformin have greater difficulty managing their glucose control, and (iii) higher protein intake was associated with a more favourable (mealtime) glucose control in GDM. Although the study population was ethnically diverse ($\approx 40\%$ non-European ancestry), there was inadequate power to test for ethnic-specific associations. Thus, designing ethnically diverse studies is of interest. In this thesis, the INFORMED study (*Chapter 5*) planned to recruit participants from the Leeds area and *Chapter 6* covers the BiB cohort (i.e., 50.2% White European and 49.5% Pakistani), both accounting for diversity in ethnicity. Furthermore, the study was conducted within the NHS; therefore, the findings may less be generalizable to other nations or government health services.

Also, CGM data and dietary intake (food dairies via myfood24) were obtained at one time-period during gestation – glycaemia and dietary patterns are likely to fluctuate over time which would not be captured by measuring at one specific time-point in pregnancy. Therefore, the results may not be representative of other times during the pregnancy and generalisability of the findings may be

limited. In addition, the dietary logs were only available in a small group of participants and their mealtimes were not recorded. Future studies should aim to conduct multiple measurements of glycaemia and dietary intake throughout pregnancy to better understand how glucose control and dietary patterns change throughout pregnancy, specifically as it is known that these change during pregnancy. The INFORMED study in Chapter 5 accounts for these factors, by administering dietary logs at 2nd and 3rd trimester in all if not most participants and recording habitual meal times. Further, treatment duration did not vary greatly, as participants were diagnosed and recruited at the similar times; however, duration of treatment may modify dysglycaemia, this may be evident in a larger sample size recruited at a wider range of gestation. Due to unequal number of total measurements between days and participants, more advanced analysis of CGM data (e.g., time-warping or functional data analysis) could not be conducted. While this prevented from assessing glucose shifts over multiple days or comparing weekdays and weekends, it did allow for identification of time-points in a 24-hour period where glucose excursions were common. Furthermore, no physical activity data were available, thus this modifier could not be evaluated. The INFORMED study (*Chapter 5*) planned on examining these glucose shifts, comparing weekdays and weekends and incorporating data on physical activity. Nonetheless, the results of *Chapter 4* suggest a role of dietary protein, carbohydrate quality and timing on dysglycaemia.

7.3.3 Chapter 5: Individualised Patient Care and Treatment for Maternal Diabetes (INFORMED) – Evaluation of an observational and Randomised Crossover Trial Embedded within Routine Care

To address shortcomings of the other studies, the INFORMED study in *Chapter 5* was designed. The most prominent limitation of this study is the absence of recruited participants. Several reasons for the lack of participants can be provided. First and foremost due to the COVID-19 pandemic; (i) the protocol had to be significantly changed to be COVID-safe, (ii) the HRA/NHS ethics application for all non-essential studies was suspended for several months, (iii) once it all started again there were considerable backlogs, and (iv) a new process for acquiring the Research Passport and Capacity & Capability was being piloted. This eventually resulted extensive delays of commencing the INFORMED study.

Mitigating through the pandemic important lessons were learnt – i) how to adapt the design and protocol of a study; ii) how the process of ethic approval works and changes during a pandemic; and iii) how to work with and communicate with third party institutions.

Furthermore, to ensure patient and staff safety, face-to-face contact for external researchers was restricted. Therefore, a research nurse was assigned for initial recruitment of the participants at the Diabetes in Pregnancy Clinic. A while into the recruitment phase, this indirect recruitment process was proven to make recruitment difficult with loss-to-follow-up of potential participants. To simplify the recruitment process, amendments to the protocol were made – the research nurse instead of the external researcher could take consent. Despite these amendments, the recruitment process remained slow and no participants have been recruited. If there was more time, amendments would be made for the external researcher to come to the clinic and directly recruit the participants. Also, some form of incentive for the participants should be considered. During this study it became evident that recruitment is one of the most challenging, if not most challenging aspects of conducting a study. This requires a lot of flexibility and creative thinking, an essential skills for a researcher to have.

Other potential limitation should be noted, the participant recall data regarding sleep, physical activity and diet would be subject to social desirability bias. To account for this social desirability bias, repeated and complementary measures (i.e., metabolites) are included. Lastly, INFORMED is being conducted within the NHS (UK); therefore, generalisability of the findings to other nations or government health services may be limited. Unfortunately, no further conclusions or recommendations can be drawn. Regardless of the limitations of this chapter, this chapter has provided a novel study design and describes a ready-to-go study that can be implemented in future studies.

7.3.4 Chapter 6: Association of Dietary Protein Intake and Glucose Control in Pregnancy – an Observational Analysis of the Born in Bradford cohort Study

Chapter 6 provided evidence of distinct metabolic meat intake profiles present in pregnancy. The metabolite samples were taken at single time-point and the same time as OGTT (i.e., 26-28 week's gestation); therefore, it is possible

that the results cannot represent changes in the metabolome that have occurred together with metabolic changes throughout gestation and with longitudinal GDM development. Also, this study was unable to account for differences in fasting duration and day-to-day variation. A prospective cohort (*Chapter 5*) where serum samples are repeatedly collected in early and late pregnancy would offer a better understanding of the role of metabolites in pregnancy. Further, improve understanding of the temporal relationship between metabolites, diet and glycaemic control in pregnancy, and whether this relationship changes throughout the course of gestation. Confirmation of associations between metabolite values / profiles and measures of glycaemia (e.g., FPG and PPG) may aid in the development of dietary interventions designed to be implemented at different stages of the pregnancy.

In addition, examining different metabolite panels to evaluate the metabolite profiles may be beneficial. The Nightingale © panel employed by BiB is affordable and inclusive, covering a large range of metabolite classes, ideal for exploratory analyses. However, it comprises different classes of fatty acids (e.g., total n-3 fatty acids, total SFAs and ratios of these) rather than individual fatty acids. Thus, this study was unable to determine which individual fatty acids of each class are responsible for the identified associations. More inclusive panel of fatty acids may aid in a better understanding of the biological pathways and identify targets in dietary interventions in management of dysglycaemia in DIP. For instance, mass-spectrometry data covers a larger panel of the metabolome, including energy metabolism; however, it is more expensive ~£80-£200 (depending on how many samples are assayed at a time) compared to ~£20 per sample for NMR data (Taylor et al., 2021). By having access to both datasets here, we can have broader coverage of the metabolome.

A key strength of BiB cohort dataset is that it detailed socioeconomic, education, and mental and physical wellbeing data (Taylor et al., 2021). Moreover, the large number of Southeast Asian and White European families in this cohort, allows for future exploration of the ethnic diversity and better understanding ethnic differences in the developmental origins of disease risk (i.e., T2DM, CVD, and GDM). However, generalisability of the findings may also be limited due to the fact that only two ethnic groups were included, thus the results

may not be applicable to other ethnic groups or geographic regions. As with all observational studies causality cannot be inferred and the effect of confounding needs to be taken into account – all analyses were adjusted for known confounders. Conducting a longitudinal study could resolve some of these issues. Similar to other nutritional epidemiological studies, there was an unavoidable recall bias and social desirability bias when estimating meat consumption by FFQ. To minimise these types of bias questionnaires could self-reported without the presence of an investigator. In addition, comprehensive dietary data on protein intake were not available and the distribution of meat-intake vs no meat-intake was disproportionate resulting in underpowered results. Future analyses of this cohort should also consider utilising different methods for categorising different sources of dietary protein intake (e.g., red-meat, dairy, eggs). To this date, this is the largest available diverse cohort. Hence, future work should include comprehensive dietary data and larger sample sizes to be in a position to make more conclusive dietary recommendations.

7.3.5 Overall

This thesis provides a consistent notion that factors beyond the characteristics of food (e.g., carbohydrate intake) play an important role in dysglycaemia in DIP. In *Chapter 3*, lifestyle interventions were more effective in younger, non-Western women, and earlier in pregnancy. In *Chapter 4*, glycaemic variability and timing of dysglycaemia was identified. Additionally, higher protein intake was associated with a more favourable (mealtime) glucose control. The INFORMED study, in *Chapter 5*, was designed to identify novel personal, lifestyle, and physiological parameters associated with (postprandial) glycaemia in pre-gestational diabetes. Finally, in *Chapter 6* distinct metabolic meat intake profiles associated with modestly improved postprandial glucose levels were identified.

When considering all results, an RCT aiming to increase dietary protein intake in women with DIP may be effective in reducing dysglycaemia – the INFORMED study touches upon incorporating meal replacement (added protein vs no added protein to a shake) intervention. However, wide application of metabolic profiling might be difficult due to the costs of analysis for large scale studies and LMICs. Although, longitudinal study or more elaborate interventions may be methodologically challenging to implement due to difficulties that arise with participation (e.g., recruitment and adherence) and when implementing an RCT

for an extended time period. Moreover, when designing a dietary intervention personal characteristics (e.g., ethnicity and maternal / gestational age) and physiological parameters (e.g., timing of dysglycaemia and metabolite profiles) should be considered – more personalised interventions are of note for future studies.

7.4 Future Implications

Although several important findings have emerged from this thesis, questions remain which should be addressed in future studies. *Chapter 3*, concluded that the magnitude of effect was clinically not strong for some lifestyle interventions categories and that forthcoming investigations should focus on larger well-designed RCTs in order to clarify the most effective lifestyle intervention or combination across a range of outcomes (e.g., multiple measures of glucose control and pregnancy outcomes) in women with all diabetes types during pregnancy. Ideally, incorporating longer term outcomes in mothers and offspring. With these means, more suitable lifestyle recommendations for diabetes in pregnancy can be developed.

In *Chapter 4*, the findings highlight that prospective work investigating the benefit of increased intake and timing of dietary protein on management of dysglycaemia in DIP is required. To assess the importance of diet and factors beyond the intrinsic properties of food in during pregnancy on (postprandial) dysglycaemia in DIP: a prospective cohort and RCT evaluating dietary intake, other parameters (regarding lifestyle, personal characteristics and physiology) and at multiple time points during gestation could be utilised to assess how these factors relate to dysglycaemia. This could aid the formation of recommendations that would need to be adopted for clinical trials and management guidelines to achieve optimal glycaemic control in DIP. Noteworthy, the INFORMED study (*Chapter 5*) aimed to answer these research questions and to the address these gaps in knowledge; although, no results were obtained. Thus, no propositions regarding advancement of glycaemic management in DIP could be formed. However, future studies could implement parts of the design of the INFORMED study.

For this reason, a final study (*Chapter 6*) exploring the associations between source of protein intake (i.e., meat-intake), metabolite profiles, and glycaemic

control in pregnancy was conceived. Exploring these links between diet, metabolites, and glycaemia will provide a better understanding of the metabolome during pregnancy and how it relates to maternal and offspring health and shed a light on how to improve prevention strategies. However, future work focussing on attaining more comprehensive dietary records and metabolites data at different stages of the pregnancy in a large sample is needed to evaluate the moderating effect of diet on metabolism, maternal glucose control and pregnancy risks – this was one of the secondary objectives of *Chapter 5*.

Another avenue is the exploration of relationships between maternal pregnancy and their offspring cord blood metabolites. To date, there is no published work using the offspring metabolomics data. Future studies should also focus on exploring the different sources of dietary protein as dietary patterns differ between ethnicities amongst others. This thesis did not further examine the effect of nutritional supplements, which limits to inform future research recommendations, but does highlight the importance of future investigations. The aforementioned future explorations would be critical to improve management of glycaemia in DIP (research and clinical practice), since knowing the mechanisms linked to (postprandial) glucose dysregulation would allow for the conception of better suited management strategies to attenuate the consequences arising from dysglycaemia in DIP.

7.5 Conclusions

The findings from this thesis provide novel insight into the factors driving (postprandial) glycaemia in DIP. First, this thesis found that nutritional supplements, diet, and exercise can be used to support the management of dysglycaemia in women with DIP, but additional evidence is required before this association can be recognised in women with pre-existing T1DM and T2DM. Second, the findings confirm that CGM provides a rich source of information that can detect and quantify periods of dysglycaemia (i.e., morning time and glycaemic variability). Furthermore, individuals assigned to diet with metformin appear to have the greatest difficulty managing glycaemia (increased 24-hr mean glucose and total AUC), suggesting the need for more directed care. Also, increased dietary protein intake may assist with dysglycaemia management. Lastly, this thesis has demonstrated that meat consumption can be characterised by a distinct metabolite profiles (i.e., VLDL triglycerides and saturated fatty acids)

and that this profile is associated with lower postprandial glucose response in pregnancy – albeit modestly. Furthermore, the INFORMED study protocol is ready-to-go and can be used for future studies, to gain novel insights. Clinically, the findings of this thesis emphasise personal, lifestyle and clinical parameters are important for the management of (postprandial) glycaemia in DIP – personalised care may be the way forward incorporating these parameters. Also, adopting healthy dietary changes earlier during pregnancy, and ideally pre-conception for women with DIP is of importance as NICE recommendations state. For the women themselves, the findings highlight the importance of awareness of modifiable risk factors (such as protein intake) and adopting lifestyle changes as early as possible to achieve euglycaemia which may contribute to their health risk and that of their offspring. Future work should aim to better understand the relationship of aforementioned factors with glycaemia in DIP. Ultimately, aiding in the creation of more appropriate and effective recommendations for improving DIP management.

Chapter 8

Scientific Contributions from this PhD

8.1 Published Peer Review Articles

1. **Dingena C.F.**, Mahendra A., Holmes M.J., Clements N., Scott E.M., and Zulyniak M.A. (2023). Protocol for the INFORMED (Individualised Patient Care and Treatment for Maternal Diabetes): A randomised controlled trial embedded within routine care. *BMJ Open*. 13(2): e065388. <https://doi.org/10.1136/bmjopen-2022-065388>
2. **Dingena C.F.**, Holmes M.J., Campbell M.D., Cade E., Scott E.M., Zulyniak M.A. (2023). Observational assessments of the relationship of dietary and pharmacological treatment on continuous measures of dysglycemia over 24 hours in women with gestational diabetes. *Frontiers in Endocrinology*. 14:e1065985. <https://doi.org/10.3389/fendo.2023.1065985>
3. **Dingena C.F.**, Arofikina D., Campbell M.D., Holmes M.J., Scott E.M., Zulyniak M.A. (2023). Nutritional and Exercise-Focused Lifestyle Interventions and Glycemic Control in Women with Diabetes in Pregnancy: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. *Nutrients*. 15(2): 323. <https://doi.org/10.3390/nu15020323>

8.2 Peer Review Articles in Progress

1. **Dingena C.F.**, Mahendra A., Holmes M.J., Scott E.M., and Zulyniak M.A. Development and Association of Metabolite Meat-Intake Scores with Glycemia in Pregnancy: An Observational Study of the Born in Bradford Cohort.

8.3 Conference Oral Presentations and Invited Talks

1. **American Society for Nutrition (NUTRITION), 2023:** Development and Association of Metabolite Meat-Intake Scores with Glycemia in Pregnancy: An Observational Study of the Born in Bradford Cohort. **Dingena, C.F.**, Mahendra, A, Holmes, MJ, Scott, EM, & Zulyniak, MA.

2. **The 9th PhD Conference in Food Science and Nutrition, 2022:** Dietary Mediators and New Nutritional Strategies for the Management of Diabetes in Pregnancy. Dingena C.F., Holmes M.J., Scott E.M., and Zulyniak M.A.
3. **UK Congress on Obesity, 2022:** Unravelling (postprandial) dysglycaemia in diabetes during pregnancy. Dingena C.F., Mahendra A., Holmes M.J., Clements N., Scott E.M., and Zulyniak M.A.
4. **Diabetes in Pregnancy Clinic (LTHT), May 2022:** Protocol for the INFORMED (Individualised Patient Care and Treatment for Maternal Diabetes) study.
5. **The 8th PhD Conference in Food Science and Nutrition, 2021:** New Nutritional Strategies for the Management and Treatment of Diabetes during Pregnancy. Dingena C.F., Mahendra A., Holmes M.J., Scott E.M., and Zulyniak M.A.
6. **Nutrition Society Nutrition Futures, 2021:** Temporal fluctuations and dietary moderation of dysglycaemia in women with gestational diabetes. Dingena C.F., Holmes M.J., Campbell M.D., Scott E.M., and Zulyniak M.A.
7. **Nutritional Epidemiology Group (NEG) Seminar, University of Leeds, May 2021:** Continuous Glucose Monitoring in Gestational Diabetes.
8. **The 7th PhD Conference in Food Science and Nutrition, 2020:** Demystifying (postprandial) glycaemia in diabetes during pregnancy. Dingena C.F., Campbell M.D., Scott E.M., Zulyniak M.A.

8.4 Conference poster presentations

1. **American Society for Nutrition (NUTRITION), 2023:** Development and Association of Metabolite Meat-Intake Scores with Glycemia in Pregnancy: An Observational Study of the Born in Bradford Cohort. Dingena, C.F., Mahendra, A, Holmes, MJ, Scott, EM, & Zulyniak, MA.

2. **IDF World Diabetes Congress, 2022:** Assessment of dietary and pharmacological treatment on continuous measures of glycemia over 24-hr in gestational diabetes. **Dingena C.F.**, Holmes M.J., Scott E.M., and Zulyniak M.A.

3. **UK Congress on Obesity, 2022:** Protocol for the INFORMED (Individualised Patient Care and Treatment for Maternal Diabetes) study: A randomised controlled trial embedded within routine care. **Dingena C.F.**, Mahendra A., Holmes M.J., Clements N., Scott E.M., and Zulyniak M.A.

4. **American Society for Nutrition (NUTRITION), 2022:** Individualised Patient Care and Treatment FOR Maternal Diabetes (INFORMED); Intensive Profiling of Dysglycemia in Maternal Diabetes—A Longitudinal Study Protocol. **Dingena C.F.**, Holmes M.J., Scott E.M., & Zulyniak M.A. Current Developments in Nutrition, 6, 635-635.

5. **American Society for Nutrition (NUTRITION), 2022:** The Effect of Lifestyle Interventions on Glycemia in Diabetes Mellitus During Pregnancy: A Systematic Review. **Dingena C.F.**, Arofikina D., & Zulyniak M. Current Developments in Nutrition, 6, 636-636.

6. **American Diabetes Association, 2020:** Postprandial Glucose Variability in People with Type 1 Diabetes Is Individual and Impacted by Physiological and Clinical Parameters. **Dingena C.F.**, Marsh A., Ajjan R.A., & Campbell M.D. Diabetes, 69.

7. **The 6th PhD Conference in Food Science and Nutrition, 2019:** Preliminary Data Analysis shows reduced Morning-Time Glycaemic Control in Women with Gestational Diabetes Mellitus. **Dingena C.F.**, Scott E.M., Campbell M.D.

8.5 Scientific collaborations

8.5.1 Conference abstracts

1. **European Association for the Study of Obesity, 2020:** Interrupted Sitting Improves 24-hour Glucose Control in People with Type 1 Diabetes. Alobaid, AM, Dingena C.F., Marsh A., Coales E.M., Ajjan R.A., Campbell M.D.
2. **European Association for the Study of Obesity, 2020:** Interrupted Sitting Improves Acute Postprandial Glucose Control without Increasing Risk of Hypoglycaemia in People with Type 1 Diabetes. Alobaid A.M., Dingena C.F., Marsh A., Coales E.M., Ajjan R.A., Campbell M.D.
3. **European Association for the Study of Obesity, 2020:** Personal characteristics influence rapid-acting insulin pharmacokinetics in individuals with type 1 diabetes treated with multiple daily injections. Coales E.M., Alobaid A.M., Dingena C.F., Marsh A., O'Mahoney L.L., Dempsey P., Francois M., Ajjan R.A., Campbell M.D.

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Appendix A

Supplementary materials for Chapter 3

Table A.1: Questions per Domain for Risk of Bias Assessment.

	<i>Sub-questions</i>
<u><i>Domain 1</i></u>	
Randomisation process	<p>1.1 Was the allocation sequence random?</p> <p>1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?</p> <p>1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?</p>
<u><i>Domain 2</i></u>	
Deviations from intended intervention	<p>2.1 Were participants aware of their assigned intervention during the trial?</p> <p>2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?</p> <p>2.3 If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?</p> <p>2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?</p> <p>2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?</p> <p>2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?</p> <p>2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?</p>
<u><i>Domain 3</i></u>	
Missing outcome data	<p>3.1 Were data for this outcome available for all, or nearly all, participants randomized?</p> <p>3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?</p> <p>3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?</p> <p>3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?</p>

<p><u>Domain 4</u></p> <p>Measurement of outcome</p>	<p>4.1 Was the method of measuring the outcome inappropriate?</p> <p>4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?</p> <p>4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?</p> <p>4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?</p> <p>4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?</p>
<p><u>Domain 5</u></p> <p>Selection of the reported result</p>	<p>5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?</p> <p>Is the numerical result being assessed likely to have been selected, on the basis of the results, from...</p> <p>5.2 ... multiple eligible outcome measurements (e.g., scales, definitions, time points) within the outcome domain?</p> <p>5.3 ... multiple eligible analyses of the data?</p>

The questions for RoB were derived from the Cochrane RoB2 tool (Sterne et al., 2019).

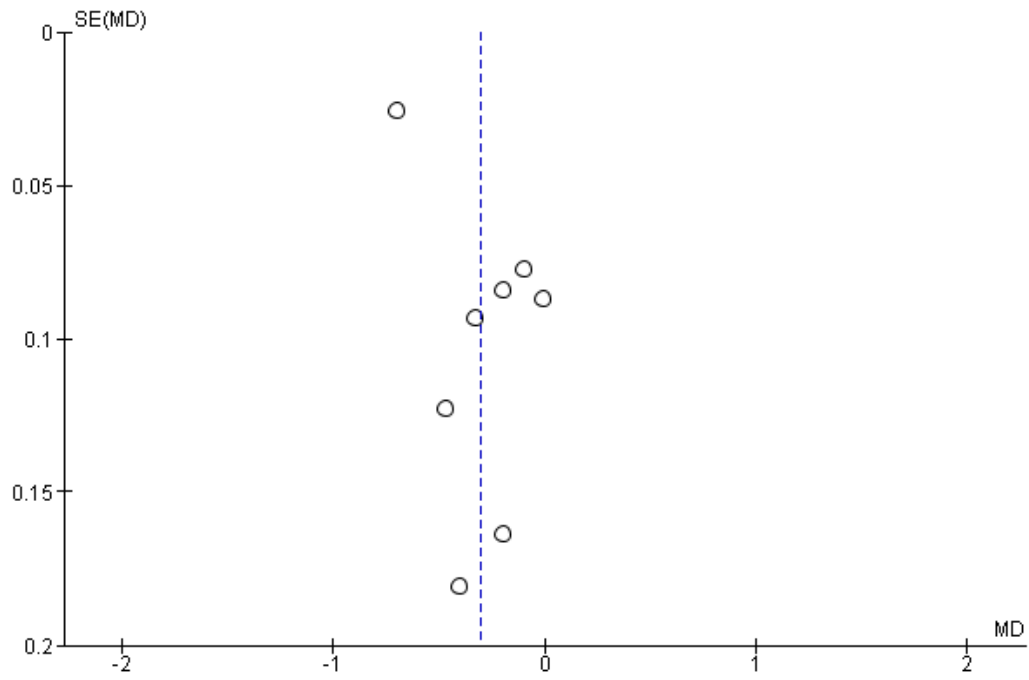


Figure A.1: Funnel plot of fasting plasma glucose (mmol/L) in nutritional supplement interventions.

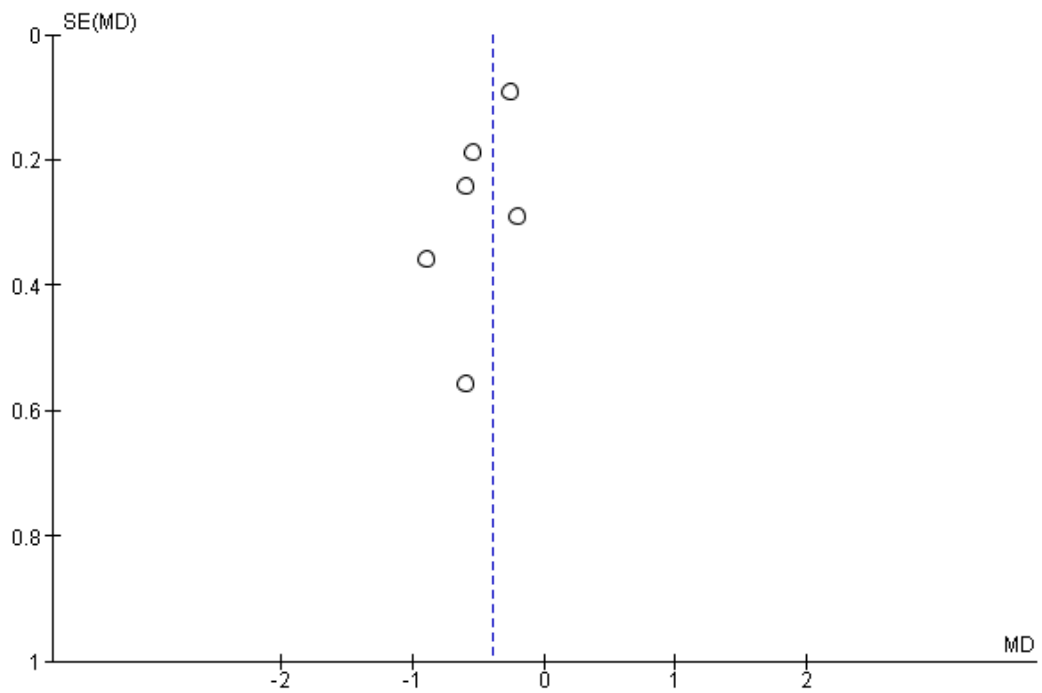


Figure A.2: Funnel plot of HOMA-IR in nutritional supplement interventions.

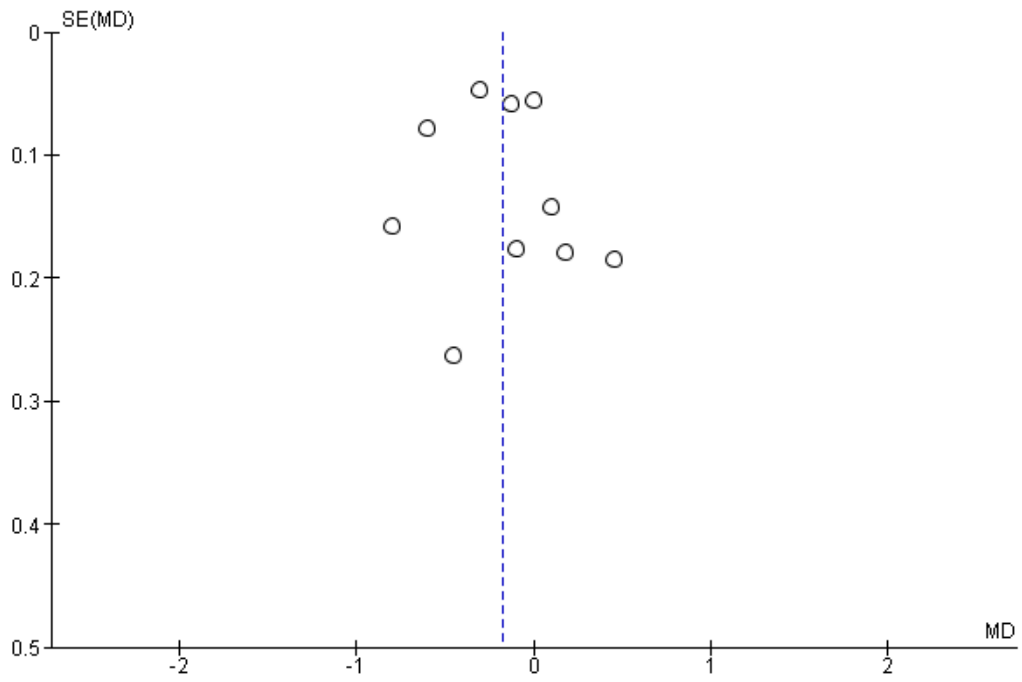


Figure A.3: Funnel plot of fasting plasma glucose (mmol/L) in dietary interventions.

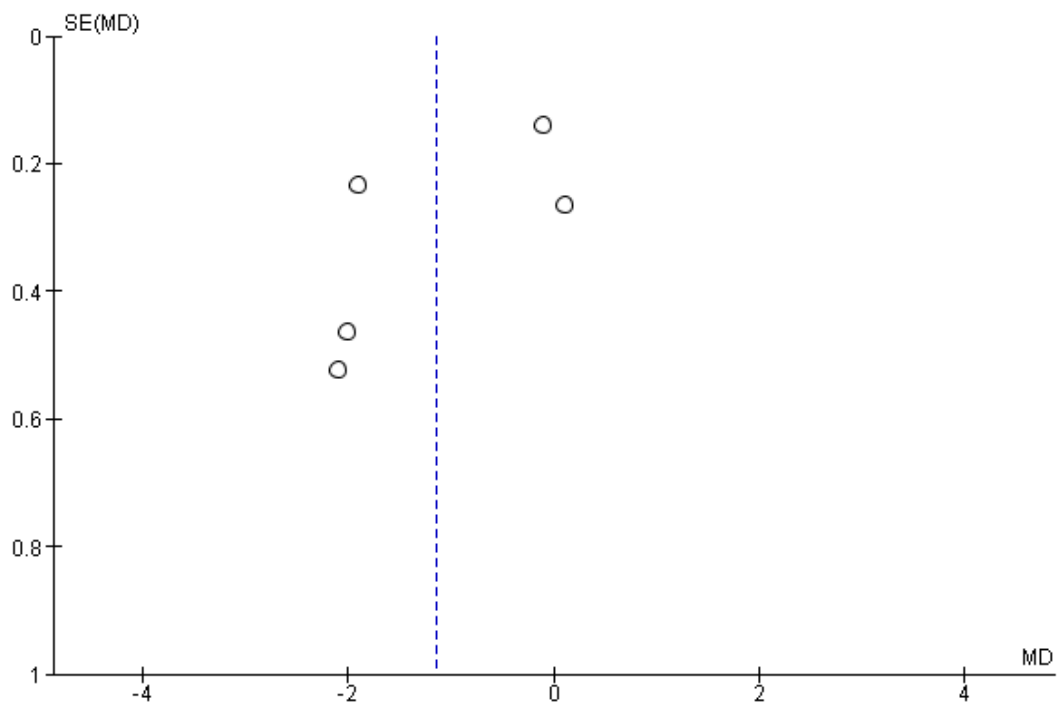


Figure A.4: Funnel plot of HOMA-IR in dietary interventions.

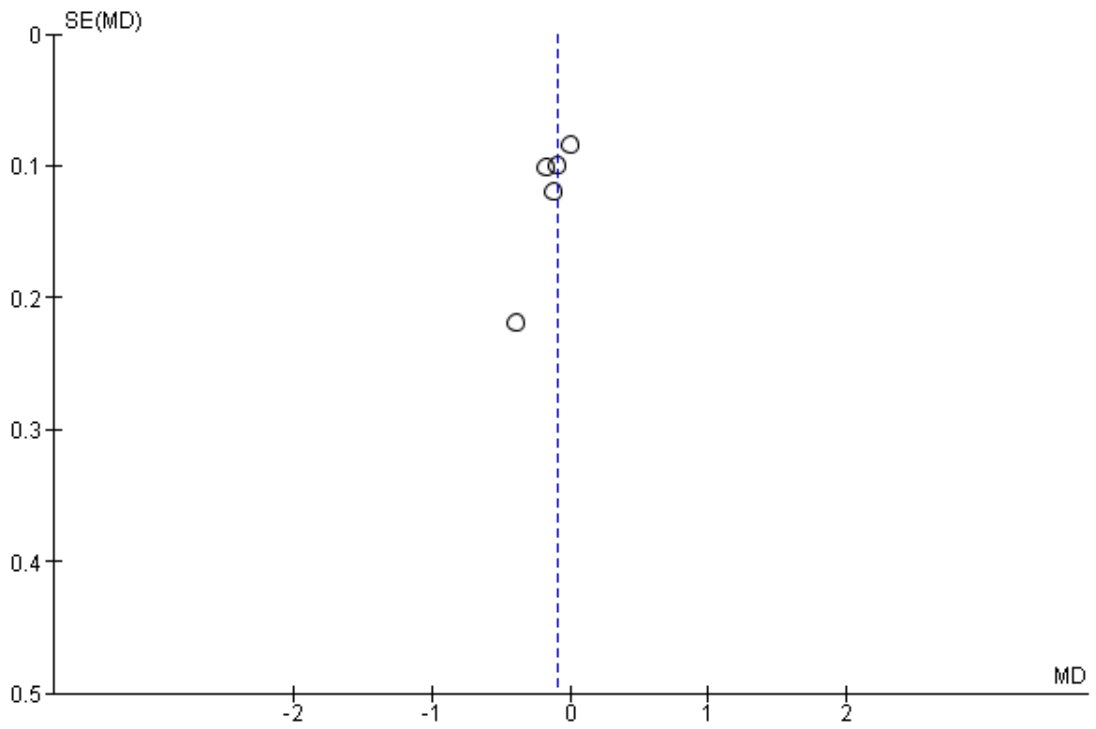
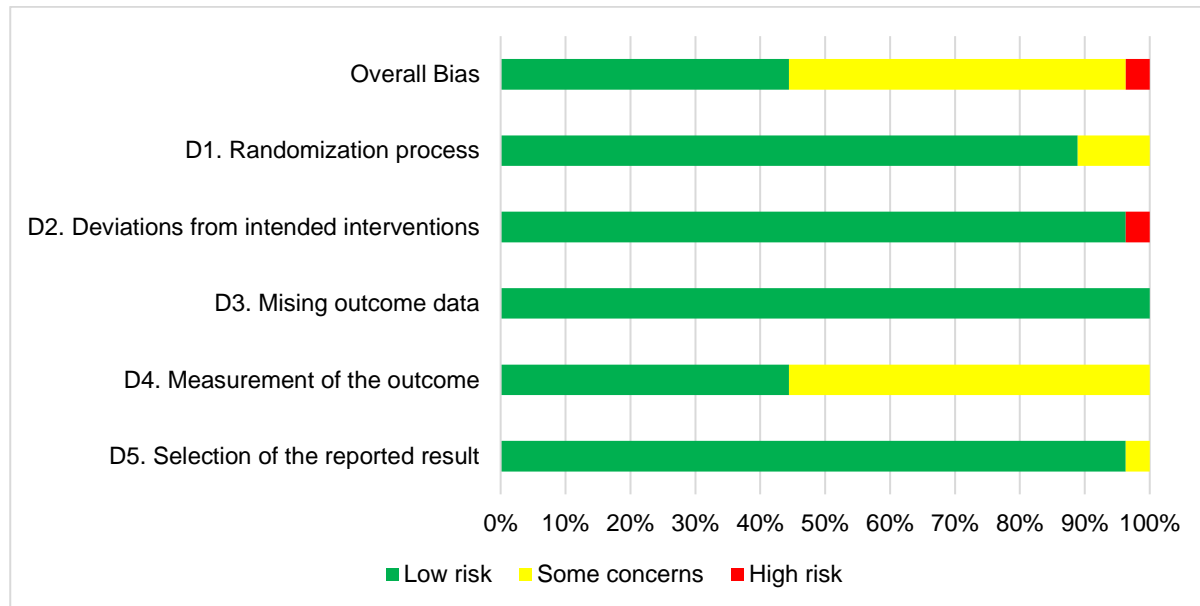


Figure A.5: Funnel plot of fasting plasma glucose (mmol/L) in exercise interventions.

Table A.2: Risk of Bias Assessment

<u>Study ID</u>	<u>Experimental</u>	<u>Comparator</u>	<u>Outcome</u>	<u>Weight</u>	<u>D1</u>	<u>D2</u>	<u>D3</u>	<u>D4</u>	<u>D5</u>	<u>Overall</u>	
Asemi (2013)	DASH diet	Standard care diet	FG, PPG, HbA1c	NA	+	+	+	+	+	+	+
Asfalalah (2020)	ALA supplement	Cellulose acetate	FG, HbA1c	NA	+	+	+	+	+	+	+
Bo (2014)	Brisk walks 20 min/day	Standard care diet	FG, PPG, HbA1c	NA	+	+	+	+	+	+	+
Brankston (2004)	Resistance exercise	Standard care diet	FG, PPG	NA	+	+	+	!	+	!	
de Barros (2010)	Resistance exercise	Standard antenatal care	FG	NA	+	+	+	!	+	!	D1 Randomisation process
Fei (2014)	SBOS supplement	Standard antenatal care	FG, HOMA-IR	NA	+	!	+	!	+	!	D2 Deviations from interventions
Grant (2011)	Low GI diet	Standard care diet	FG, PPG	NA	+	+	+	!	+	!	D3 Missing outcome data
Hajimoosayi (2020)	Ginger supplement	Placebo supplement	FG, PPG, HOMA	NA	+	+	+	!	+	!	D4 Measurement of the outcome
Halse (2014)	Home-based exercises	Standard antenatal care	FG, PPG, HbA1c	NA	+	+	+	!	+	!	D5 Selection of the reported result
Hernandez (2014)	Higher complex CHO	Standard care diet	FG, PPG	NA	+	+	+	!	+	!	
Hernandez (2016)	Higher complex CHO	Standard care diet	HOMA-IR	NA	+	+	+	!	+	!	
Jamilian (2015)	Soy diet	Control diet	FG, HOMA-IR	NA	+	+	+	+	+	+	
Jamilian (2018)	Fish oil supplement	Placebo supplement	FG, HOMA-IR	NA	+	+	+	+	+	+	
Jamilian (2019)	Mg-zinc-calcium-Vit D	Placebo supplement	FG	NA	+	+	+	+	+	+	
Jamilian (2020)	Flaxseed oil/ ALA supplement	Placebo supplement	FG, HOMA-IR	NA	+	+	+	+	+	+	
Kokic (2018)	Exercise programme / walks	Standard antenatal care	FG, PPG	NA	+	+	+	+	+	+	
Lindsay (2015)	Probiotic (Lactobacillus salivarius)	Placebo supplement	FG, HOMA	NA	+	+	+	+	+	+	
Louie (2011)	Low glycemic index (target GI⁵⁰)	Standard care diet	HOMA, HbA1c	NA	+	+	+	+	+	+	
Ma (2015)	Intensive low glycemic load	Standard care diet	FG, PPG, HbA1c	NA	+	+	+	!	+	!	
Ostadmohammadi (2019)	Zinc Gluconate / Vit E supplement	Placebo supplement	FG, HOMA	NA	+	+	+	+	+	+	
Perichart-Perera (2012)	Low glycemic index	Standard care diet	FG	NA	+	+	+	!	+	!	
Qazi (2020)	Moderate intensity aerobics	Standard antenatal care	HbA1c	NA	+	+	+	!	+	!	
Rae (2000)	Moderately energy restricted diet	Standard care diet (not restricted)	FG, HbA1c	NA	+	+	+	+	+	+	
Rasmussen (2020)	Low CHO morning intake	High CHO morning intake	FG, HOMA	NA	+	+	+	!	+	!	
Valentini (2012)	"Ethnic meal plan"	Standard care diet	FG, PPG, HbA1c	NA	!	+	+	!	-	-	
Wang (2015)	Oil-rich diet	Standard care diet	FG, PPG	NA	!	+	+	!	+	!	
Yao (2015)	DASH diet	Standard care diet	FG, HOMA	NA	!	+	+	!	+	!	

Table A.3: Scores of Risk of Bias per domain.

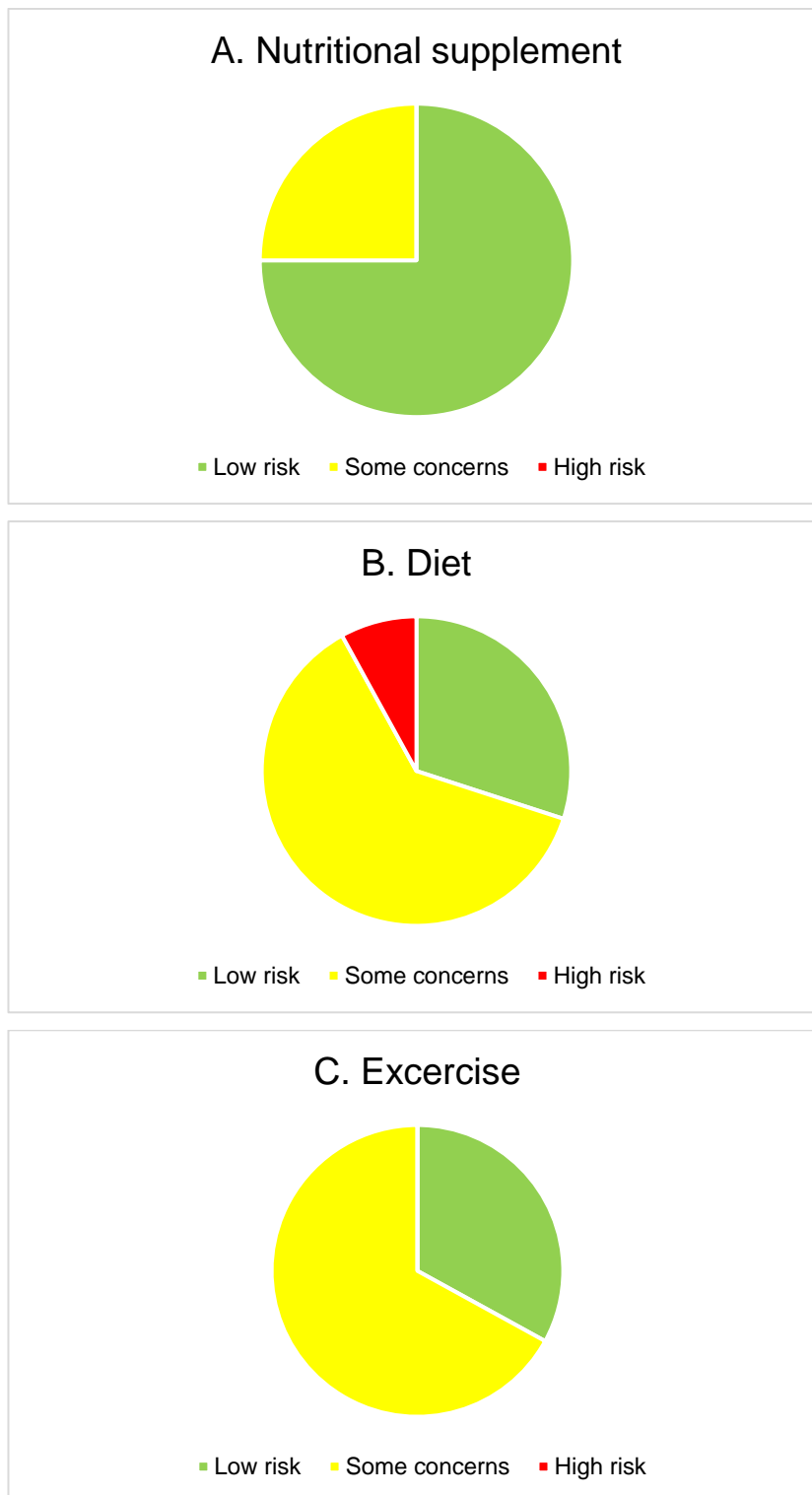


Figure A.6: Breakdown of Risk of Bias scores per intervention type.

Table A.4: GRADE Assessment Table for Nutritional Supplement-based Interventions.

OUTCOME	No OF STUDIES	No OF PARTICIPANTS		CERTAINTY ASSESSMENT					EFFECT ESTIMATE	GRADE
		Diet-based interventions	Placebo or standard care	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations		
FASTING GLUCOSE (MMOL/L)	8	264	268	Not serious	Serious ^a	Serious ^b	Not serious	None	-0.3 [-0.55 , -0.06]	⊕⊕○○ Low
POSTPRANDIAL GLUCOSE (MMOL/L)	1	37	33	Not serious	Not serious	Not serious	Very serious ^c	Publication bias strongly suspected ^d	-0.1 [-0.66 , 0.46]	⊕○○○ Very low
HBA1C (%)	1	30	30	Not serious	Not serious	Not serious	Not serious	Publication bias strongly suspected ^d	-0.15 [-0.22 , 0.08]	⊕⊕⊕○ Moderate
HOMA-IR	6	204	208	Not serious	Not serious	Serious ^b	Not serious	None	-0.04 [-0.58 , -0.22]	⊕⊕⊕○ Moderate

a. Due to high unexplained heterogeneity.

b. Due to substantial differences in interventions and comparisons.

c. The 95% CI included benefits and harms.

d. Only reported by 1 study.

Table A.5: GRADE Assessment Table for Diet-based Interventions.

OUTCOME	No OF STUDIES	No OF PARTICIPANTS		CERTAINTY ASSESSMENT					EFFECT ESTIMATE	GRADE
		Diet-based interventions	Placebo or standard care	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations		
FASTING GLUCOSE (MMOL/L)	10	309	296	Serious ^a	Serious ^b	Not serious	Not serious	None	-0.17 [-0.35 , 0.01]	⊕⊕○○ Low
POSTPRANDIAL GLUCOSE (MMOL/L)	5	125	133	Not serious	Serious ^b	Not serious	Very serious ^c	None	-0.23 [-0.69 , 0.24]	⊕○○○ Very low
HBA1C (%)	4	167	158	Not serious	Serious ^b	Serious ^d	Serious ^c	None	-0.08 [-0.23 , 0.08]	⊕○○○ Very low
HOMA-IR	5	107	107	Not serious	Serious ^b	Not serious	Not serious	None	-1.15 [-2.12 , 0.17]	⊕⊕⊕○ Moderate

a. Problems with allocation concealment and blinding of participants/researchers/outcome assessors.

b. Due to high unexplained heterogeneity.

c. The 95% CI included benefits and harms.

d. Due to substantial differences in interventions.

Table A.6: GRADE Assessment for Exercise-based Intervention

OUTCOME	No OF STUDIES	No OF PARTICIPANTS		CERTAINTY ASSESSMENT					EFFECT ESTIMATE	GRADE
		Diet-based interventions	Placebo or standard care	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations		
FASTING GLUCOSE (MMOL/L)	5	181	185	Not serious	Not serious	Serious ^a	Not serious	None	-0.1 [-0.20 , -0.01]	⊕⊕⊕○ Moderate
POSTPRANDIAL GLUCOSE (MMOL/L)	4	149	153	Not serious	Serious ^b	Serious ^a	Serious ^c	None	-0.24 [-0.59 , 0.11]	⊕○○○ Very low
HBA1C (%)	3	144	146	Not serious	Serious ^b	Serious ^a	Serious ^c	None	0.04 [-0.19 , 0.27]	⊕○○○ Very low
HOMA-IR	1	99	101	Not serious	Not serious	Not serious	Serious ^c	Publication bias strongly suspected ^d	0.00 [-0.88 , 0.88]	⊕⊕○○ Low

a. Due to substantial differences in interventions and comparisons.

b. Due to high unexplained heterogeneity.

c. The 95% CI included benefits and harms.

d. Only reported by 1 study.

Appendix B
Supplementary Materials for Chapter 4

No supplementary materials for *Chapter 4*, all relevant tables and figures are presented in the body of text.

Appendix C

Supplementary Materials for Chapter 5

Appendix C.1: Infographic



INFORMED
INDIVIDUALISED PATIENT CARE AND TREATMENT FOR MATERNAL DIABETES



UNIVERSITY OF LEEDS



The Leeds Teaching Hospitals NHS Trust

THE MAIN ISSUE?

A mother's blood glucose changes after mealtimes and throughout the day, affected by her personal characteristics, daily lifestyle and the pregnancy itself. Too much or uncontrolled glucose in your blood during pregnancy can lead to a large baby and can cause problems during pregnancy and labour. Also, babies exposed to higher glucose levels are more likely to become obese and get type 2 diabetes when they grow up.

WHAT WE WILL INVESTIGATE?

Recent studies have shown that other factors beyond the characteristics of food play an important role in how glucose is absorbed after a meal during pregnancy. These factors include your personal characteristics such as age, ethnicity and BMI and genetics.

Using continuous glucose monitoring (CGM), which measures glucose levels every few minutes, we will investigate:

- 1) How diet quality affects glucose control in type 1 and 2 diabetes pregnancy?
- 2) Which personal characteristics are most strongly related?
- 3) How does glucose control evolve during pregnancy?

INTERESTED?

If you have type 1 or type 2 diabetes and are within the first 12 weeks of pregnancy and interested in taking part, please contact us for more information.

 +31627072821
 fscd@leeds.ac.uk

WHAT WE WILL ASK FROM YOU?

During your routine care, medical details are recorded and you will wear a CGM device. We ask for your permission to safely access and assess this data.

Furthermore, we ask you to complete short questionnaires on diet, physical activity and sleep at three occasions during your pregnancy (after each routine care visit). These questionnaires will be partly online and via phone calls.

To gain more insight in mealtime glucose responses, we ask you to consume standardised breakfast meals on two separate occasions (optional), no additional clinical visits needed. These meals will be delivered at your home.

HOW LONG DOES THE STUDY LAST?

We will collect data throughout the pregnancy, including birth outcomes.

WHAT WILL THIS TELL US?

We think that all the information we gather will help to develop new ways in which women can reduce their risk of having uncontrolled glucose, reduce still births, pregnancy complications and improve the long term health of their children.

INFORMED Infographic (version 1.0) IRAS No: 297276 Date: 09-08-2021

Appendix C.2: Participant Information Sheet



Individualised patient care and treatment **FOR Maternal Diabetes**

Understanding the glycaemic profile of maternal diabetes using continuous glucose monitoring: intensive glucose profiling to inform patient care and treatment

Participant Information Sheet

Thank you for considering your participation in our study called INFORMED, which is part of a PhD research project at the School of Food Science and Nutrition. We - as research team - would like to provide you with details about the study, what your role will involve, and other key information before you decide.

Please ask us (*contact details at the end of the handout*) if there is anything that is not clear or you would like more information. Take time to decide whether or not you wish to take part.

Study information

What is the purpose of the study?

During pregnancy, a mother's blood glucose level changes constantly across 24-hour period, and is affected by her physical characteristics, lifestyle, and the pregnancy itself. While many factors affect the way babies grow in the womb, one of the easiest to measure and modify, is the amount of glucose that they get from their mother. Uncontrolled or too much glucose in their mother's blood during pregnancy, usually leads to a large baby and can increase the chance of problems during pregnancy, labour, and immediately after birth for both mother and child. Being born too small can also be problematic and has been linked to increases the chances of obesity and type 2 diabetes.

Glucose levels of the mother rise after meal consumption and, if uncontrolled, can contribute to some of these health concerns. While the type of food being eaten is vital, recent studies have shown that other factors (such as age, ethnicity, activity levels, and sleep duration) also play a part. However, despite knowing these factors, we currently do not know how to modify a meal to match a mother's characteristics and how a mother's diet effects glucose levels throughout pregnancy. As part of your routine care you are wearing a continuous glucose monitor. With this study we are investigating the impact of diet and lifestyle effects on glucose control throughout pregnancy as there is currently very little information on how diet and lifestyle affects glucose levels measures during pregnancy.

Therefore, as a first step, we want to monitor and study how 24-hour and mealtime glucose levels change in response to diet and across pregnancy in

women with pre-existing type 1 or type 2 diabetes. Most information on glucose control and medical data will be requested via your medical records, if you give us permission for us to access your data. To decrease the burden of participation, we will use existing data as much as possible. However, to be able assess your diet and lifestyle during pregnancy, we will ask you to complete questionnaires via phone calls. These questionnaires are detailed below. None of the information obtained via the questionnaires will be shared with your clinical care team. This data on lifestyle will be anonymised and is solely for the purpose of this study.

Why have I been invited?

We are approaching women with type 1 and type 2 diabetes, who are early in their pregnancy, to help us with this study. You do not have to take part – it is completely up to you — and does not affect the quality of care you receive from the NHS. Also, if you choose to take part and later decide to withdraw (for any or no reason), it will not affect your quality of care from the NHS.

What could my participation do?

By taking part in this study, you will help us to better understand how glucose levels change during pregnancy in women with type-1 and type-2 diabetes and the role of diet. By knowing this, we can design special diets and nutritional strategies to minimise the chances of babies being exposed to abnormal glucose levels and their risk of future health problems.

What would taking part involve?

Recruitment. As part of the recruitment process, we ask you to complete a screening questionnaire and medical history questionnaire. The screening questionnaire will consist of questions about education and employment level, health status, current diabetes treatment, previous smoking status and alcohol intake, use of supplements, access to internet and your participation in other studies. The medical history questionnaire will enquire about diagnosis of other diseases such as hypertension, asthma etc. This information will be used to check your eligibility and to set up a database of participant characteristics.

Medical information. We ask you to give us consent to access selected parts of your medical record that reflect your general health and the health of your pregnancy (e.g., blood pressure, blood/urine test results, current medication, diabetes related pregnancy outcomes) and your diabetes health risks (e.g., age, body weight, ethnicity). Additionally, once you have given birth, your baby's birthweight, and any pregnancy complications will be copied from your medical records.

Urine samples. You will be providing urine samples regularly during pregnancy to your clinical team. Once they have been tested, rather than throwing them away we would like your consent to keep the remaining sample for future metabolic analysis. These samples will be processed and anonymised with your unique study number by a member of the clinical staff and research team and subsequently transported to the University of Leeds in designated Human Tissue Act approved and compliant facilities for storage and further analysis. Only researchers directly involved in the study will have access to the samples. Results of samples analyses will only be used for the purpose of the research study. Excess of the samples not used in the analysis for this study will be stored long-term at the University of Leeds for future research.

Blood Samples. You will be having blood taken regularly during this pregnancy for your clinical care. On three of your routine visits to the Diabetes in Pregnancy Clinic we would like your consent to take an additional 10ml of blood (the equivalent to approximately two teaspoons) for the study. We will store this to look at molecular and genetic markers that may be involved in metabolism and diabetes later. No infant blood samples are requested. These samples will be collected, processed and anonymised with your unique study number by a member of the clinical staff and research team and subsequently transported to the University of Leeds in designated Human Tissue Act approved and compliant facilities for storage and further analysis. Only researchers directly involved in the study will have access to the samples. Results of samples analyses will only be used for the purpose of the research study. Excess of the samples not used in the analysis for this study will be stored long-term at the University of Leeds for future research.

Glucose Data. We ask for your consent for us to access your clinical glucose data throughout pregnancy. This will require no additional work on your part.

Lifestyle Questionnaires. On three occasions during your pregnancy, at weeks ~10-12, ~18-20, and ~28-34, we will contact you at your convenience by phone or video call to complete some short questionnaires about your habitual physical activity, sleep quality / patterns and mealtimes. Also, following your three clinical visits at week ~10-12, ~18-20, and ~28-34, we will ask you to keep track of your diet for 3 days (2 weekdays and 1 weekend day) using an online dietary tracker called MyFood24. During the phone call, we will explain you how to use this dietary tracker. The phone calls will last not more than 30 minutes. The dietary tracker will take approximately 10 minutes per day to complete. This data will be anonymised and none of this data will be shared with your clinical care team.

Breakfast replacements. To gain more insight into mealtime glucose responses, we would like you to consider taking part in additional part of the study, where we will provide you with two different breakfast shakes to drink instead of your usual breakfast for 4 days on two separate occasions (one during your 2nd and one during your 3rd trimester). The two different breakfast shakes (e.g. Shake 1 and Shake 2) have the same amount of carbohydrate as that recommended during pregnancy, but one is designed to be absorbed slower, and the other faster so we can see how this affects your glucose measures on the continuous glucose monitor. Dependent on your randomization you will consume Shake 1 for two days followed by Shake 2 or vice versa. They are vegan friendly and adhere to religious requirements. The shakes will be delivered to your home with instructions for you to prepare. You can still participate in the main study without having to take the breakfast shakes.

This study will tell us, in detail previously unseen, how (mealtime) glucose changes across pregnancy and how diet can best be used to manage glucose levels and minimise maternal and infant health risks.

What are the possible risks of taking part?

Although we have designed the meals to not contain allergens and to release the same amount of glucose as you would usually eat for breakfast there is a possibility that you may experience an allergic reaction or higher glucose levels than normal after the standardised meal consumption. We will check that you have no allergies before taking part and ask you to contact the research team if

you have these reactions to the meal. You will be advised to monitor and manage your blood glucose levels like you normally would and feel most comfortable with. However, if blood glucose levels surpass $>18\text{mmol/L}$ for more than 90-minutes you are advised to administer a corrective dose of insulin or contact your GP/clinical care team and inform a member of the research team via email. The meals are designed to minimize risk of hyperglycaemia. Blood samples are part of your routine clinical care and will be performed by qualified clinical staff, so any discomfort should be minimal.

What are the possible benefits of taking part?

There are no specific benefits to you of taking part, but participating in this study will give us important information about how to assess glucose in relation to personal characteristics, pregnancy outcomes, and newborn health. We anticipate that this will then help us to identify and develop new diet strategies to help women reduce their risk of small or large babies, stillbirths, pregnancy complications, and improve the long-term health of their children.

Further Information

What will happen if I don't want to carry on with the study?

You are free to withdraw at any time without explanation. If you decide not to carry on, it will not affect your care in anyway.

What if something goes wrong?

During the study, you will be covered by the Sponsor's Insurance, the University of Leeds is acting as Sponsor for this study. The University of Leeds has insurance cover in force, which meets claims against it and where those claims arise from the Universities own negligence in its role and activities relating to the study (and which is subject to the terms, conditions and exceptions of the relevant policy). Clinical negligence indemnification will rest with the participating NHS Trust under standard NHS arrangements.

If you are unhappy about any part of the study, you are encouraged to discuss this with the research team or with the Patient Assistance and Liaison Services (PALS) at your hospital. Normal legal processes are also open to you. We foresee minimal risks as most data will be collected from your routine clinical records and specific study risks are limited to questionnaires and meal replacements.

What will happen to my additional blood samples and urine sample?

Your blood and urine samples will be labelled with your unique study number and stored in freezers at the University of Leeds for storage. Analysis of the samples will be undertaken for molecular and genetic factors that may contribute to a mother's metabolism, glucose control and babies growth. Only researchers directly involved in the study will have access to the samples. Results of samples analyses will only be used for the purpose of the research study. Excess of the samples not used in the analysis for molecular and genetic factors will be stored at the University of Leeds in designated Human Tissue Act approved and compliant facilities for long-term storage and future research.

How will we use information about you?

We will need to use information from you and from your medical records (including the continuous glucose monitoring data) for this research project. This

information will include your initials/ NHS number/ date of birth/ name/ contact details. This data will be destroyed after the end of the study. Unless, you consented us to keep your contact details to contact you about future studies. This data will be stored securely on University of Leeds password encrypted computers and be destroyed after 5 years. Your study information will be given an unique anonymised study number, so that the study information cannot be linked to your personal information. One member of the research team, who is authorised by the NHS, will be able to access the medical data that you consent to. Only this NHS authorised member of the research team will manage your data on secure university computers. Other members of the research team will not be able to link your contact details and health records. This anonymised research data for analysis, writing up of the results, and study validation will be stored on password encrypted computers and be destroyed after 15 years. We will keep all information about you safe and secure. All analyses and reports will be written in a way that no-one can work out that you took part in the study.

How will my information be kept confidential?

All information which is collected about you will be held securely and treated in accordance with the Regulation (EU) 2016/679 (General Data Protection Regulation) and the Data Protection Act 2018.

We will be using information collected by your local hospital from you and your medical records in order to undertake this study. No personal identifiable data will leave the NHS hospital without your consent; Data leaving the hospital will be labelled with your unique study number and will not have your name or any other identifying details on it. We refer to this as linked anonymised data as it is linked to you by a code. The code will only be known by key research team members, which will be kept securely.

Data which leaves the NHS Trust where you are being treated will be held securely in a database, operated by the data analysis team at the University of Leeds. This includes only linked anonymised study data and will not have your name or any other identifying details on it.

If you join the study, the data collected for the study, together with any relevant medical records, may be looked at by authorised persons from University of Leeds, the Research and Development Department of your local hospital and the Regulatory authorities to check the study is being carried out correctly. They all have a duty of confidentiality to you as a research participant.

Other third party researchers (e.g. universities, NHS organisations or companies involved in health and care research) may wish to access anonymised data (including samples) from this study in the future (anonymised data do not include names, addresses, or dates of birth, and it is not possible to identify individual participants from anonymised data). If this is the case, the Chief Investigator will ensure that the other researchers comply with legal, data protection and ethical guidelines. This may include research outside of the UK and EU and/or research that is commercial in nature. Your data will be stored securely for a period of 15 years after the end of the trial before being destroyed.

What are your choices about how your information is used?

If you withdraw consent during the study, no further data will be collected on you. However, any data (including samples) already collected by the research team may be retained and subsequently analysed for the purposes of the study. Your right to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. To safeguard your rights we will use the minimum personally-identifiable information possible.

The University of Leeds as the Sponsor, is the data controller for this study. This means that we are responsible for looking after your information and using it properly.

The University of Leeds is the data processors for this study. The lawful basis for processing personal data collected in this study is that it is a task in the public interest. You can find out more about how we use your information at <https://dataprotection.leeds.ac.uk/wp-content/uploads/sites/48/2019/02/Research-Privacy-Notice.pdf>; and <https://dataprotection.leeds.ac.uk/wp-content/uploads/sites/48/2019/09/HRA-transparency-wording.pdf>. or by contacting University of Leeds Data Protection Officer's (e-mail: dpo@leeds.ac.uk).

What will happen to the study results?

The study is part of a PhD project and the result will be used for writing the doctorate thesis. The study results may be presented at meetings or published in scientific journals but individuals will not be identifiable. After the study has ended we will send a newsletter with the study results to your research team, which they will be able to share with you.

Who is organising and funding the research?

The research study has been primarily funded and sponsored by the University of Leeds with additional support from the Wellcome Trust. The Chief Investigators (Dr Michael Zulyniak and Professor Eleanor Scott) are University of Leeds researchers. Professor Scott is also one of the senior NHS consultants providing clinical care in the Diabetes Pregnancy Clinic.

Who has reviewed the study?

Before any research goes ahead it has to be checked by an Ethics Committee. This study has been reviewed by the Leeds East Research Ethics Committee.

What happens now if I agree to do the study?

The study procedures will be explained to you in more detail by the research team. You will be able to ask questions and voice any queries. If you agree to take part we will ask you to sign a consent form and complete screening questionnaires online to confirm eligibility, this will take approximately 10 minutes. The research team will then co-ordinate with you the dates for completing the lifestyle questionnaires, food diary, and consuming the breakfast replacements. If you are not eligible to participate, information provided prior to participation will be destroyed. Unless, you opted 'Yes – My email can be kept on file and I am willing to be contacted about future studies', this information will be destroyed after 5 years.

Please, contact the research team for more information:

Co-Investigator

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Name: Professor Eleanor Scott

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Leeds, LS9 7TF

Email: eleanor.scott9@nhs.net

Telephone 0113 2065014

Appendix C.3: Consent Form



Individualised patient care and treatment **FOR Maternal Diabetes**

Understanding the glycaemic profile of maternal diabetes using continuous glucose monitoring: intensive glucose profiling to inform patient care and treatment

IRAS Project ID: 297276

Participant ID for this study:

Name of Researcher:

CONSENT FORM

Please **initial** all boxes that apply:

1. I confirm that I have read and understand the Participant Information Sheet dated (Version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected in any way.

3. I understand that relevant sections of my medical records of me and my baby, after their birth until either of us is discharged from hospital and data collected during this study may be looked at by members of the research team, from regulatory authorities or from the NHS Trust / sponsor, in this case the University of Leeds, where it is relevant to taking part in this research. I give permission for these individuals to have access to my records.

4. I understand that my name will not be linked with the research materials, and I will not be identified or identifiable in the report or reports that result from the research.

5. I agree for any unused urine (up to 2.5 mL) collected as routine care during this study to be stored securely long-term at University of Leeds for later analysis for research purposes only. These are considered a 'gift' from me, and may be used in relevant future research (in an anonymised form). I understand that this may involve co-operation with researchers outside of the UK) (*optional*).

6. I agree for any additional blood samples collected during this study to be stored securely long-term at University of Leeds for later analysis for research purposes only. These are considered a 'gift' from me, and may be used in relevant future research (in an anonymised form). I understand that this may involve co-operation with researchers outside of the UK) (*optional*).
- a. I give my consent for the taking of an additional blood sample for molecular analysis (*optional*).
- b. I give my consent for the taking of an additional blood sample for genetic/DNA analysis (*optional*).
7. I understand that the information collected about me and my baby may be used to support other ethically approved research in the future, and may be shared anonymously with other researchers. This may include research outside of the UK and EU and/or research that is commercial in nature.
8. I agree for my GP to be informed of my participation in this study.
9. I give consent to the research team to keep my contact details for them to contact me during and after the study (*optional*).
10. I am happy to be contacted about longer term follow up after ending of the study of myself or my baby (*optional*).
11. I agree to take part in the standardised meals study (*optional*).
12. I agree to take part in the study to the sections I have consented to.

Name of participant Date E-Signature

Name of person taking consent Date* E-Signature*

*To be signed and dated after the participant has signed and dated. Signed copies of this form will be stored in a secure online database (MS OneDrive) and one copy will be for participant to keep.

Appendix C.4: Screening Questionnaire



Individualised patient care and treatment **FOR MatE**rnal **D**iabetes

Understanding the glycaemic profile of maternal diabetes using continuous glucose monitoring: intensive glucose profiling to inform patient care and treatment

Screening questionnaire

Thank you for considering your participation in our study named INFORMED. We would like to fill out this questionnaire, so we can assess your eligibility.

First name/s:.....

Last name:.....

Address:.....

Postcode:.....

Phone number:.....

E-mail address:.....

Date of birth:.....

General Practitioner (GP)/Family doctor

Name:.....

Address:.....

Postcode:.....

Phone number:.....

Medical specialist (if applicable)

Name:.....

Address:.....

Postcode:.....

Phone number:.....

1. What is your ethnic origin?
 - White (Caucasian)
 - Black (African-American)
 - Asian
 - Mixed

2. What is the highest educational qualification that you have?
 - No qualifications
 - Achieved GCSE grades D-G, NVQ Level 1, Skills For Life level 1, BTEC-award Certificate or diploma level 1, OCR National
 - GCSE grades A*-C, NVQ Level 2, BTEC Award Certificate OR diploma level 2
 - AS & A level, NVQ Level 3, Advanced Extension award, International Baccalaureate, OCR National
 - NVQ Level 4, BTEC Professional award, Certificate of Higher Education
 - BTEC Award Advanced professional / Bachelors Degree / Graduate Diploma
 - University Masters Degree / Postgraduate diploma / NVQ Level 5 / BTEC Advanced Professional Award Certificate and Diploma level 7
 - Doctorate (e.g. PhD, DClin.)

3. What do you do?
 - I am a student
 - I am employed
 - I am self employed
 - I am a housewife, househusband
 - I am unemployed
 - I am unable to work (e.g. due to a disability)
 - I am retired
 - I do something else (e.g. volunteering), namely.....

4. When is your expected due date?
If you do not know exactly, try to estimate it as well as possible.

Day	Month		Year				

5. How long have you been pregnant? (weeks)
If you do not know exactly, try to estimate it as well as possible.

6. Do you have children?
 - Yes, I have child(ren).
 - No

7. Do you have a singleton pregnancy?
 Yes, I am expecting an single child
 No, I am expecting twins, triplets etc.

8. Would you say your general health is.....?
 Excellent
 Very Good
 Good
 Fair
 Poor
 Don't know/Not sure
 I rather not say

9. For how long have you been diagnosed with diabetes?
If you do not know exactly, try to estimate it as well as possible.

Please, specify in years

10. Did you take any medication (including diabetes medication) in the last month?
 No
 Yes

If yes, please specify which medication, the dose and how many times a day.
For example: Routine: Omeprazole 40mg once a day for 7 days or one-time
treatment: amoxicillin 500mg once a for 7 days.

.....
.....
.....
.....
.....

11. How many units of alcohol did you normally (before pregnancy) consume during the week? (A

Guide to the number of units of alcohol in some typical alcoholic drinks is provided).

<https://www.nhs.uk/Livewell/alcohol/Pages/alcohol-units.aspx>

- I do not use any alcohol
- Less than 1 unit a week
- 1 - 5 units a week
- 6 - 7 units a week
- 8 - 15 units a week
- 16 - 30 units a week
- More than 30 units a week



12. Do you currently smoke or use e-cigarettes?

- Yes - smoke cigarettes
- Yes - smoke cigars
- Yes - use e-cigarettes
- No, I quit smoking
- No, I never smoked

→ go on to question 15

→ go on to question 16

13. How many cigarettes/cigars do you normally smoke?

- 1-5 each day
- 6-10 each day
- More than 10 each day
- None, I smoke pipe or vape

14. What year did you start and quit smoking?

Started: (yyyy) |_|_|_|_|
 Quit: (yyyy) |_|_|_|_|

15. Do you have any food allergies?

- No
- Yes

If yes, please tick any allergies that apply below:

- Tree Nuts (e.g. walnuts, almonds, pine nuts, brazil nuts, and pecans)
- Peanuts
- Cow's milk
- Other milk
- Eggs
- Wheat
- Barley
- Oats
- Molluscs
- Lupin
- Sesame
- Sulphites
- Soy
- Mustard
- Celery
- Fish, shellfish and crustaceans
- Other, namely.....

16. Do you use any dietary supplements? (i.e. vitamin supplements, minerals, fibres or probiotics). Examples of commonly used probiotics include:

- Actimel drink
- Activia yogurt
- Benecol yogurt drink
- Yakult drink
- Arla Skyr yogurt drink
- Creamier bio-live Irish yogurts
- Probiotics from a local chemist or herbalist (e.g. Acidophilus Capsules)

- No
- Yes

If yes, what products have you used: How often do you use them (e.g. once per day):

.....

.....

17. Do you have internet access on a computer, tablet or smartphone at home?

- Yes
- No

18. Are you currently participating in any other research studies?

Yes

No

If yes, please specify which study and give brief explanation.

.....

.....

.....

.....

.....

Can we keep your email on file and contact you about future studies? Yes / No

Appendix C.5: Medical History Questionnaire



Individualised patient care and treatment **FOR Maternal Diabetes**

Understanding the glycaemic profile of maternal diabetes using continuous glucose monitoring: intensive glucose profiling to inform patient care and treatment

Medical History Questionnaire

1. Do you currently have or have you had history of any of the following diseases? If you have selected yes for any of the following conditions, please indicate which (if any) you are currently receiving treatment for? Please also state whether the condition limits you in your daily activities.

Diseases	Have you/ are you currently suffering from any of the following?		Are you receiving treatment for this disease?		Does this disease limit you in your daily activities?	
	No	Yes	No	Yes	No	Yes
Disease of heart/coronary arteries (angina pectoris, heart attack)						
Hypertension						
Lung disease						
Asthma						
Diabetes						
Stomach ulcers or other stomach disorders						
Kidney disease						
Liver disease						
Anaemia or any other blood disease						
Cancer						
Depression						
Eating disorder (e.g. anorexia/bulimia)						
Chronic fatigue syndrome (CFS)						

Stroke				
Gastrointestinal disease				
Oesophageal disease				
Thyroid disease				
High cholesterol				

<p>If you are suffering from any other medical problems, please specify below:</p> <p>.....</p> <p>.....</p> <p>.....</p> <p>.....</p> <p>.....</p>

2. In the past, have you had any major abdominal surgery (please provide dates)?

Not had any major abdominal surgery in the past

Yes, had major abdominal surgery in the past, namely:

Laparoscopic (or key-hole): appendectomy Cholecystectomy

Open surgery: appendectomy Cholecystectomy

Other abdominal surgery,
namely:.....

Date/s of any surgery:.....

Appendectomy: removal of the appendix

Cholecystectomy: removal of the gall bladder

Appendix C.6: Physical Activity in Pregnancy Questionnaire (PPAQ)

Individualised patient care and treatment **FOR MatE**rnal **D**iabetes

Understanding the glycaemic profile of maternal diabetes using continuous glucose monitoring: intensive glucose profiling to inform patient care and treatment

Physical Activity Questionnaire

During this trimester, how much time do you usually spend on:

1. Preparing meals (cook, set table, wash dishes)

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

2. Taking care of an older adult

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

3. Sitting and using a computer or writing, not for work

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

4. Sitting at work or in class → If the participant does not work or study, skip to question 7.

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

5. Standing or walking at work while carrying things (heavier than a 1 gallon milk jug)

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

6. Standing or walking at work while not carrying anything

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

7. Sitting and reading, talking or on the phone, not for work

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

8. Watching TV or video

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

9. Light cleaning (make beds, laundry, ironing, putting things away)

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

10. Heavier cleaning (vacuum, mop, sweep, wash windows)

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

11. Shopping (for food, clothes, or other)

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

12. Gardening

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

Please, fill out the next section if you have children. If you do not take care of children, you do not need to complete the this section.

During this trimester, taking care of children, how much time do you usually spend on:

13. Dressing, bathing, feeding children while you are sitting

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

14. Dressing, bathing, feeding children while you are standing

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

15. Playing with children while you are standing or sitting

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

16. Playing with children while you are walking or running

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

17. Carrying children

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

During this trimester, how much time do you usually spend on exercising:18. Walking to go places (such as to the bus, work, visiting). Not for fun or exercise

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

19. Walking for fun or exercise

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

20. Jogging

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

21. Prenatal exercise class

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

22. Doing other things for fun or exercise (such as swimming or dancing)?

None

Less than ½ hour per day

½ to almost 1 hour per day

1 to almost 2 hour per day

2 to almost 3 hour per day

3 or more hours per day

Appendix C.7: Leeds Sleep Evaluation Questionnaire (LSEQ)



Individualised patient care and treatment **FOR Maternal Diabetes**

Understanding the glycaemic profile of maternal diabetes using continuous glucose monitoring: intensive glucose profiling to inform patient care and treatment

Sleep Questionnaire

Modified Leeds Sleep Evaluation Questionnaire

How would you describe the way you currently fall asleep in comparison to usual?

1. More difficult than usual ----- Easier than usual
2. Slower than usual ----- More quickly than usual
3. I feel less sleepy than usual ----- More sleepy than usual

How would you describe the quality of your sleep compared to normal sleep?

4. More restless than usual ----- Calmer than usual
5. With more wakeful periods than usual ----- With less wakeful periods

How would you describe your awakening in comparison to usual?

6. More difficult than usual ----- Easier than usual
7. Requires a period of time longer than usual ----- Shorter than usual

How do you feel when you wake up?

8. Tired ----- Alert

How do you feel now?

9. Tired ----- Alert

How would you describe your balance and co-ordination upon awakening?

10. More disrupted than usual ----- Less disrupted than usual]

Appendix D

Supplementary Materials for Chapter 6

Table D.1: Self-completed food frequency questionnaire at baseline.

Red meat consumed in last 4wks pregnancy	
Beef	1 : 2-3 times a week 2 : 4-6 times a week 3 : Less than 1 a week 4 : Once a week 5 : Rarely or Never
Pork	***
Lamb/mutton/goat	***
Sausage	***
Cured/dried sausage	***
Burger	***
Hotdog/ hotdog/frankfurters/saveloys	***
Bacon	***
Meat pies/pastries	***
Ham	***
Beef/lamb/mutton/goat with sauce	***
Pork with sauce	***
Gravy made in pan or with meat juices	***
Processed meat in last 4wks pregnancy	
Sausage	1 : 2-3 times a week 2 : 4-6 times a week 3 : Less than 1 a week 4 : Once a week 5 : Rarely or Never
Cured/dried sausage	***
Burger	***
Hotdog/frankfurters/saveloys	***

Bacon	***
Meat pies/pastries	***
Ham	***
Nuggets	***
Kebab	***

Poultry in last 4wks pregnancy

Chicken/turkey	1 : 2-3 times a week 2 : 4-6 times a week 3 : Less than 1 a week 4 : Once a week 5 : Rarely or Never
Chicken/turkey with sauce	***
Nuggets	***

Fish in last 4wks pregnancy

Fresh fish or tinned oily fish like sardines, mackerel	1 : 2-3 times a week 2 : 4-6 times a week 3 : Less than 1 a week 4 : Once a week 5 : Rarely or Never
White fish not in batter or breadcrumbs	***
White fish in batter or breadcrumbs	***
Tinned tuna	***
Smoked fish like smoked salmon, mackerel, kippers	***
Salted or dried fish e.g. 'Bombay duck'	***

Dairy and eggs in last 4wks pregnancy

Cheese	1 : 2-3 times a week 2 : 4-6 times a week 3 : Less than 1 a week 4 : Once a week 5 : Rarely or Never
Yoghurt	***
Egg	***
Quiche	***
Egg curry	***

Table D.2: Metabolites with VIPs ≥ 1 for characterising meat-intake.

Metabolites	VIP	Metabolites	VIP
XXL VLDL PL	1.59	XS VLDL TG	1.65
XXL VLDL C	3.81	L LDL C	1.09
XXL VLDL CE	2.99	L LDL CE	1.22
XXL VLDL FC	1.53	M LDL PL	1.14
<u>XXL VLDL TG</u>	<u>4.35</u>	M LDL C	1.94
XL VLDL PL	1.28	M LDL CE	2.62
XL VLDL C	3.74	S LDL PL	1.53
XL VLDL CE	1.95	S LDL C	2.25
XL VLDL FC	1.98	S LDL CE	2.28
<u>XL VLDL TG</u>	<u>4.55</u>	L HDL PL	1.18
L VLDL CE	1.13	L HDL C	1.25
L VLDL TG	1.35	S HDL PL	2.07
M VLDL C	2.11	S HDL C	2.14
M VLDL CE	2.33	S HDL CE	2.58
M VLDL TG	2.15	Ratio omega-3 FA	1.26
S VLDL PL	1.61	Ratio omega-6 FA	1.31
S VLDL C	1.95	Ratio PUFA/FA	2.40
S VLDL CE	2.04	Ratio MUFA/FA	1.81
S VLDL TG	2.53	<u>Ratio SFA/FA</u>	<u>3.86</u>

Summary table of metabolites with $VIP \geq 1$, three highest scoring VIP are denoted in 'italic' and underlined. XXL, extremely large; XL, very large; L, large; M, medium; S, small; XS, very small; VLDL, very-low density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PL, phospholipids; C, total cholesterol; CE, cholesterol esters; FC, free cholesterol; TG, triglycerides; FA, fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids.