Project Title: Determining the Effect of Food ordering on blood glucose In Gestational Diabetes Mellitus (DEFI-GDM) - a randomised crossover study

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Introduction

Gestational diabetes mellitus (GDM) is glucose intolerance with onset or first diagnosis during pregnancy, affecting around 1 in 10 pregnancies in the UK. A major risk factor for GDM is obesity and, with ever increasing rates of obesity, GDM prevalence is also rising. Gestational diabetes increases the risk of adverse perinatal outcomes including macrosomia, preterm delivery, need for caesarean section and even stillbirth. Women with GDM are also 10 times more likely to develop T2DM and twice as likely to develop cardiovascular disease in later life than women without GDM (1,2). Dietary modification, involving incorporating complex carbohydrates into the diet at the expense of refined carbohydrates, is recommended as first line management to optimise blood glucose control and reduce the risk of adverse pregnancy outcomes including neonatal hypoglycaemia and caesarean section (3). However, this often fails, necessitating the use of adjunctive pharmacological agents such as metformin and insulin. There are significant disadvantages to pharmacological management, including hypoglycaemia, weight gain, and raised BMI in offspring (4,5). Low/no carbohydrate diets are gaining popularity among women aiming to improve glucose control by dietary means. However, the safety of these diets is uncertain (6). Furthermore, evidence suggests that post-natal adherence to such diets may actually increase risk of T2DM (7). There is a pressing need to find safe and effective dietary approaches to optimise glycaemic control in GDM which can be easily adhered to in order to improve perinatal and longer-term outcomes.

The impact of the sequence of ingestion of macronutrients on glycaemic control is an emerging area of interest. Compared to carbohydrate, protein and fat induce greater satiety and reduce postprandial glycaemia. Several mechanisms likely mediate this, including influencing gastric emptying and secretion of insulin and incretin hormones such as glucagon like peptide-1 (GLP-1) (8). Recent evidence suggests that the effect magnitude is greater when non-carbohydrate macronutrients are consumed first; in patients with diabetes, consumption of the fat/protein components of a meal prior to the carbohydrate components reduces the post-prandial peak by around 40% (9). Furthermore, overall post-prandial glucose excursion is reduced by around 50-70% (9,10). It has also been demonstrated that sustained non-carbohydrate first meal consumption is beneficial for longer term glycaemic control in patients with T2DM: patients encouraged to eat vegetables before carbohydrate achieved better glycaemic control than patients offered traditional dietary advice over a 2-year period (11).

In women with GDM, sustained consumption of a protein/fat preload before meals improves glucose tolerance compared to an isocaloric carbohydrate preload, with glucose levels approximately 15% lower following a glucose load (12). However, preloads may increase overall energy intake, and acceptability is uncertain. Altering the sequence of macronutrient ingestion within a normal diet, as demonstrated to be effective in T2DM and non-diabetic individuals (13,14), could offer an alternative approach to optimising glucose control in women

with GDM. This could improve acceptability and adherence, whilst ensuring a balanced diet without energy or macronutrient restriction. This study will evaluate the effect of consumption of non-carbohydrate components of a breakfast meal before carbohydrate components on post-prandial glycaemia compared to carbohydrate-first consumption in women with GDM. It will also explore potential mechanisms mediating any observed effect, examining subjective satiety and food intake over the following 24 hours in women with GDM as well as changes in insulin, C-peptide, glucagon, acylated-ghrelin, peptide tyrosine (PYY) and GLP-1, hormones which are involved in glucose homeostasis and appetite regulation. The test meal represents a commonly eaten breakfast in the real world, and results are likely to be repeatable with other meal types.

Aims and Objectives

Aims

Using a randomized crossover study of a breakfast meal in women with GDM:

1. To determine whether the sequence of macronutrient consumption affects post-prandial glycaemia
2. To determine whether the sequence of macronutrient consumption affects post-prandial gut hormone release
3. To determine whether the sequence of macronutrient consumption affects subsequent satiety and food intake

Objectives

Using a randomized crossover study of a breakfast meal in women with GDM:

1. To determine whether the sequence of macronutrient consumption affects the magnitude of the post-prandial rise in plasma glucose as measured by Flash Glucose Monitoring (FGM)
2. To determine whether the sequence of macronutrient consumption affects the pattern of post-prandial glucose excursion as measured by FGM
3. To determine whether the sequence of macronutrient consumption affects changes in pre- to post-prandial serum insulin, C-peptide and glucagon levels
4. To determine whether the sequence of macronutrient consumption affects changes in pre- to post-prandial ghrelin, GLP-1 and PYY release
5. To determine whether the sequence of macronutrient consumption affects satiety scores as measured by Visual Analogue Scales (VAS)
6. To determine whether the sequence of macronutrient consumption affects subsequent calorie and macronutrient intake as detailed in self-recorded 24-hour food diaries

Project design

The proposed study is a randomized crossover study involving women with current GDM.

Subjects

Thirty-five pregnant women with GDM, aged 18-50 years old, will be eligible to be recruited for the study.

Exclusion criteria include:

- History of type 1 or type 2 diabetes mellitus
- Dietary restrictions or clinically confirmed food allergies that may affect study requirements
- Pharmacologically managed GDM at the point of study entry
- Hyperemesis gravidarum at the point of study entry (i.e. prolonged/severe nausea and vomiting)
- Using antiemetic medication (e.g. dimenhydrinate, prochlorperazine, promethazine)
- Any other problems or medical conditions that would substantially limit their ability to complete the study requirements

Participants can be recruited onto the study if they have previously had GDM in another pregnancy. Participants can also be recruited if they are involved in other research studies, but this will depend on the study type and the decision will be made by the CI.

Research methods

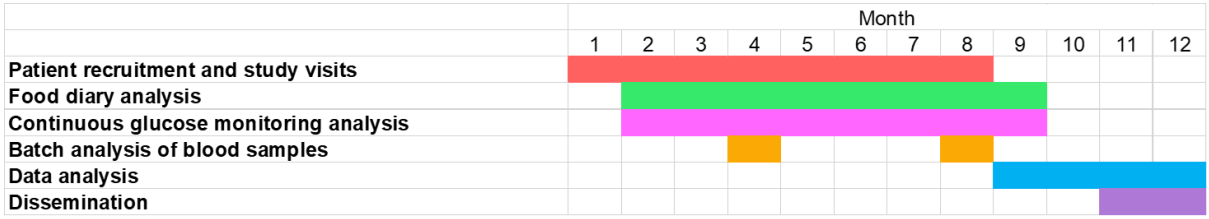
Recruitment

Participants will be recruited from endocrine antenatal clinics within the South-Eastern Health and Social Care Trust (SEHSCT) via different methods. Following a positive diagnosis from the oral glucose tolerance test (OGTT), patients are routinely referred to the Ulster Hospital for further care. A member of the direct care team in the SEHSCT antenatal clinic will be required to screen their patient database (i.e. age, history of type 1 or type 2 diabetes mellitus, and pharmacologically managed GDM) to determine whether patients meet the main inclusion/exclusion criteria for the study. Other criteria will be checked during a screening call with the researcher as this information may not be within medical records (i.e. Dietary restrictions or clinically confirmed food allergy that may affect study requirements, prolonged/severe nausea and vomiting at point of study entry and use of antiemetic medication). At the participants first appointment, a member of the direct care team will provide study details and contact details for the research team to those patients who may be eligible for the study. This will either be given verbally (if it is a remote consultation) or in person by the clinical care team, with the additional provision of the participant information sheet being emailed or given in person if the patient is happy to receive this.

Study posters will also be on display in the waiting rooms at locations where OGTTs are being carried out across the SEHSCT (i.e. Ulster Hospital, Newtownards Hospital, Lagan Valley Hospital and Downe Hospital). Any interested patients will be asked to speak to their healthcare professionals in the antenatal clinic, who will be able to provide information on the study and contact details for the research team if they are potentially eligible.

The SEHSCT locations are only involved in identifying potentially eligible participants and in advertising the study via displaying posters in areas where OGTTs are being conducted. It will only be the direct care team at the Ulster Hospital approaching patients about the study. Interested participants will contact the research team to discuss the study and determine their eligibility. They will be emailed a copy of the participant information sheet if they haven't already received this from the antenatal clinic and given at least 48 hours to decide whether they wish to take part. The researcher will follow up after 48 hours to answer any questions. If they are still interested in being involved in the study, the researcher will complete screening to confirm their eligibility which should last no longer than 30 minutes. If they are eligible and provide their consent, a letter of participation will be sent to their antenatal team in the SEHSCT as their healthcare provider to inform them of participation in the intervention.

Within the study population, 60-70% of women commence pharmacological management, usually within 4 weeks of diagnosis. The study is designed to allow visits in close succession, allowing sufficient time for the visits to occur before medication is likely to be started. With approximately 400 women diagnosed with GDM in SEHSCT each year, 35 participants represents a recruitment rate of less than 9%. The timeline for recruitment, and other study activities is depicted in the below Gantt chart.



Data collection

Eligible participants will be invited to attend the research facility in the Centre for Public Health at Queen's University Belfast on two mornings, following an overnight fast, with an **interval of at least two days between the visits** to ensure the participant has a break before the next fasting period. The maximum number of days between the visits should ideally be no more than 2 weeks. At each visit, participants will be asked to consume a 440Kcal breakfast meal approximately 1.5 hours after arrival on their first study visit and approximately 30 minutes after arrival on their second study visit. This time may increase to 1.5 hours after arrival if the flash glucose monitor needs to re-inserted on the participants arm. The breakfast meal should be consumed within 10 minutes, under different conditions at each visit. On one visit they will be asked to eat the protein/fat-based component of the meal (scrambled egg) before the carbohydrate-based component (wholemeal toast) and on the other visit they will be asked to eat the meal in the reverse order.

Scrambled egg was chosen as the protein and fat will be homogenously distributed (compared to boiled egg, for example). Wholemeal toast was chosen as, although lower glycaemic index and therefore less likely to expeditiously raise blood glucose than toasted white bread, it will be more consistent with the participants' existing dietary needs. Meals will be prepared by members of the research team who have completed Food Safety training. The scrambled egg will be prepared in the microwave without additional cooking fat and the toast will be prepared with a single serving of low-fat vegetable oil-based spread (see Table 1 for nutritional information). The order of the meals will be randomly allocated using block randomization.

Table 1: Nutritional information for breakfast components

	Scrambled egg	Wholemeal toast (Hovis wholemeal thick sliced)	Vegetable oil-based spread (I can't believe it's not butter 'light')	Total
Quantity	140 g (2 large eggs)	2 slices (100g)	14 g	254 g
Energy (kcal)	183	222	35	440
Fat (g)	12.6	1.8	4	18.4
Protein (g)	17.6	10	Trace	27.6
Carbohydrate (g)	Trace	37.8	Trace	37.8

Study Design

The following activities will occur at the time points indicated in Figure 1 (following calibration of the device at the first study visit and second study visit if required):

- **Anthropometry:**
  - Height and weight will only be measured at the **start of visit 1** using calibrated scales and stadiometer respectively.

- **Self-administered questionnaires:**
  - ***Screening/background information*** – socio-demographics, lifestyle and medical history
  - ***Self-reported appetite*** – VAS, 100mm in length with words anchored at each end, expressing the most positive and the most negative rating, will be used to assess hunger, satiety, fullness, prospective food consumption and desire to eat both pre- and post-consumption and palatability (at 10 and 40 minutes).

- **Flash glucose monitoring:**
  - Serum glucose levels will be obtained using FreeStyle Libre 2 (Abbott), a FGM device allowing for multiple sampling without multiple blood draws. Following participant consent, a researcher will follow manufacturer's instructions and apply the device to the back of the participant's arm. The researcher will then dispose of the needle (that is discharged from the device) into a sharps bin. The participant will then wait for 1 hour to allow the device to calibrate.
  - Glucose levels will be recorded by the researcher 5 minutes prior to consumption of the breakfast meal and every 5 minutes thereafter until the end of the study visit, using a reader connected to the device. Participants will be informed that the use of the device and reader are only for study purposes. They will also be informed that glucose readings from the device cannot be provided by the researcher, and that they should continue with their usual clinical care method for glucose monitoring readings.

- **Intravenous blood sampling:**
  - This will be carried out pre- and post-consumption (at 20 and 50 minutes) to allow measurement of serum and/or plasma glucose, insulin, C-peptide, glucagon, ghrelin, GLP-1 and PYY. At each blood draw, 13ml of blood will be collected (i.e. 26ml blood sample in total at each study visit).
  - Samples will be centrifuged and stored at -80 degrees Celsius until analysis in the CPH laboratory. All samples will be batch analysed using validated assays (16–18). Hormone analysis will take place at Imperial College London, whilst glucose, insulin and C-peptide will be analysed in the CPH, QUB. This will take place once all participants have completed the study.

- **Food diaries:**
  - Participants will be asked to document the remainder of their food and drink consumption for the day of their study visits in food diaries (i.e. two 1-day food diaries) (19). The first diary may be returned at the second study visit and the second food diary may be returned by post in a pre-paid envelope. If the participant prefers to send the completed food diary back via email this will be

facilitated. Energy and nutrient intakes will be estimated using Nutritics dietary assessment software.

Participants will be paid an honorarium of £200 to cover time and travel costs on completion of the visits.

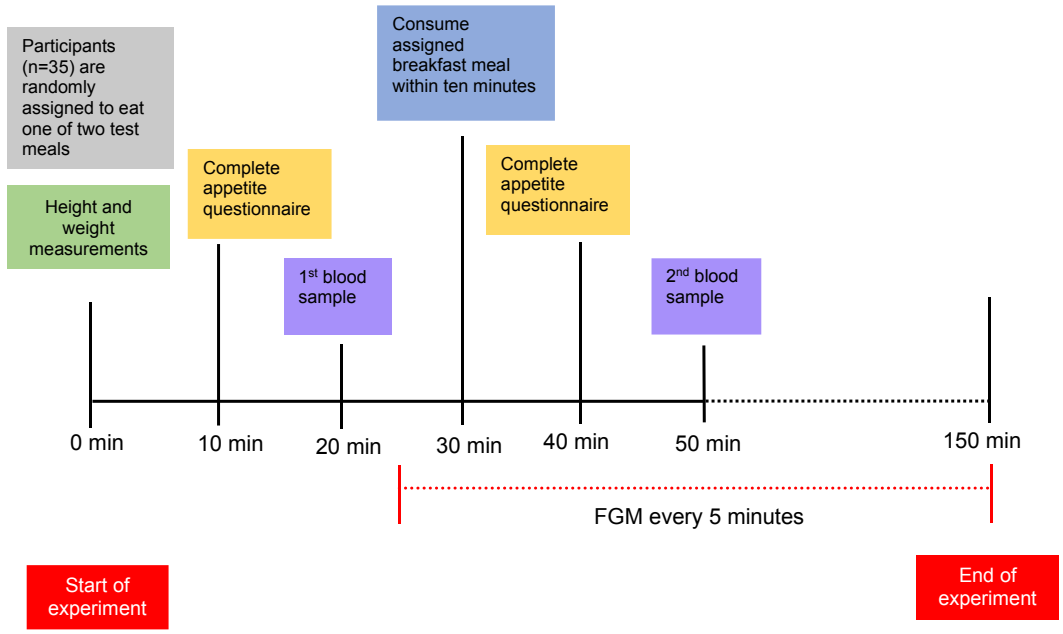


Figure 1: Timeline of study activities

Patient and public involvement

The research question was formulated following discussions with women with a history of GDM, particularly those who had required medication after dietary modifications were ineffective. They described frustration that the recommended dietary advice hadn't helped them to control their blood sugars, despite feeling restricted in what they ate. There was a high level of interest in research that might help women and clinicians better understand how to control blood sugars with diet alone.

The study design was co-constructed with Patient and Public Involvement and Engagement (PPIE) representatives with experience of a pregnancy affected by GDM, with key elements of the study design informed by their involvement. The use of FGM was felt to be an attractive component of taking part in the study. However, it was felt that only having access to FGM devices for the duration of the study was unsatisfactory. After discussion with Research Governance and QUB contracts, it was not considered possible to provide devices to participants after the study ends due to indemnity and insurance issues. This is because using devices provided during a study for standard care in the antenatal clinics would not be covered.

Study Outcomes

Primary outcome:

- The difference in the magnitude of postprandial rise in blood glucose between the two test meals.

Secondary outcomes:

- The difference in the magnitude of postprandial change in serum levels of gut hormones between the two test meals.
- The difference in mean change in pre-post ingestion satiety scores between the two test meals.
- The difference in 24 hour energy and macronutrient intake following the two test meals.

Power Calculation

A simplified sample size calculation was conducted based upon a paired samples t-test using PASS (PASS 2009, NCSS LLC, Kaysville, Utah). In this sample size a standard deviation of 0.9 mmol/L was used, based upon the standard deviation of the plasma glucose peak from a crossover study which evaluated glucose levels following consumption of a rice-first or rice-last meal in non-diabetic individuals (15). This will be an over-estimate of the within-person standard deviation which wasn't available. This study was used as it evaluated the difference in glucose peaks following consumption of a carbohydrate-first or carbohydrate-last meal, as opposed to total post-prandial glucose excursion. This calculation indicated that, with 28 individuals, the study would have over 80% power to detect as statistically significant, a difference in mean of 0.5 mmol/L peak glucose between the two groups (carbohydrate-last versus carbohydrate-first). This is smaller than seen in a previous study for the carbohydrate-last group compared to the carbohydrate-first group (15). As this is based on a study of a non-diabetic cohort, it is anticipated that there will likely be a higher divergence in tolerance to carbohydrate in the cohort in the current study. Recruitment of 35 women will allow for a 20% drop-out rate.

Statistical Analysis

The primary objective is to determine whether the sequence of macronutrient consumption affects the magnitude of the post-prandial rise in plasma glucose. We do not anticipate any carry over effect. The height and time after consumption of the glucose peak will be compared between groups using a paired t-test to calculate a difference in mean and 95% Confidence Interval (CI) (20). The total AUC will also be compared between the two groups using a paired t-test to calculate a difference in mean and 95% Confidence Interval (CI).

To explore potential mechanisms by which any observed effects occur, the following exploratory analyses will be carried out using paired t-tests: differences in the mean pre- to post-prandial change in insulin, C-peptide, glucacon, PYY, GLP-1 and ghrelin compared between groups, the mean change in hunger, satiety, fullness, prospective food consumption and desire to eat ratings between pre- and post-consumption as recorded by VAS compared between groups, and subsequent caloric and macronutrient intake compared between groups. Probability values <0.05 will be considered statistically significant.

Confidentiality and Data Storage

The data collected will be treated securely and with the strictest confidence as necessary under the General Data Protection Regulations and stored as required by QUB. All data collected will be kept in a pseudonymised format so that participant results can be linked to the study ID. Participant ID numbers will be used to keep the identity of the participants

anonymised.

Documents including questionnaires and blood results will be stored securely in a locked filing cabinet in a locked office within CPH at QUB. The document containing individual names and contact telephone numbers that will be needed throughout the study will be stored on a QUB encrypted password protected computer held at QUB and will be destroyed on completion of the study. Consent forms will be stored securely in a locked filing cabinet in a locked office, separate to where the research data is stored. When outcome data has been collected and entered in Nutritics, the research team will export it and this will also be stored on a QUB encrypted password protected computer held at QUB. The reader is only used alongside the FGM device to obtain glucose results during the study visits. These results are recorded by the researcher for each participant. Personal information will not be shared with Abbott (device and reader supplier).

All such files will be password protected and stored on devices only with sign in password protection and fully updated security software. Only encrypted password protected external storage devices will be used if needed and all data will be backed-up on the QUB server. Personal data will be stored over 3 years is in line with QUB policy. For example, consent forms containing personal data will be retained. Research data generated by the study will be stored for 5 years based on University Policy. Long term arrangements for the storage of research data after the study has ended will involve the data being stored by the data custodian, Professor Jayne Woodside, on QUB password protected computers and within QUB locked offices in keypad access buildings that are not accessible to the public. Samples will also be stored in the Laboratory in CPH, QUB.

Ethical and Regulatory Considerations

Queen's University Belfast (QUB) will act as Sponsor for the study and the CI will take overall responsibility for the conduct of the study.

The study will comply with the requirements and standards set out in the UK Policy Framework for Health and Social Care Research. Prior to initiating the study, a favourable opinion will be sought from and NHS/HSC Research Ethics Committee for the study protocol, informed consent forms and other relevant documents. Approval will also be sought from the South Eastern HSC Trust to act as a Participant Identification Centre in the study.

Substantial amendments that require review by NHS REC and approval from the sponsor will not be implemented until that review is in place and other mechanisms are in place to implement at site. The CI will notify the REC of the end of the study, which is defined as completion of data analysis. If the study is ended prematurely, the Chief Investigator will notify the REC and sponsor, including the reasons for the premature termination. Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC and Sponsor.

Indemnity

QUB as Sponsor will provide indemnity for the management, design and conduct of the study.

Dissemination

Dissemination of the overall study results will occur via multiple avenues with target audiences including the women who took part in the study, women with current, or a history of, GDM throughout the UK, health care professionals, academics and policy makers. Dissemination

will occur via publication in relevant peer-reviewed publications, presentation at regional scientific meetings and relevant national / international scientific conferences (e.g. Diabetes in Pregnancy conference). The research team also regularly promote their research activities via social media, media interviews and newspaper articles etc. It is anticipated that similar approaches will be utilised in dissemination of this work via press releases, relevant social media and additional streams as identified by PPIE contributors. No one will be identifiable in any data produced from this study.

Potential of research for patient care

The primary objective of this study is to determine whether the order of consumption of macronutrients within a meal influences blood glucose levels post-prandially in women with GDM. If consumption of non-carbohydrate components of the meal first was shown to reduce post-prandial glucose excursion in women with GDM, it would have significant implications for women and their babies. Well controlled blood sugars in GDM are associated with a reduction in perinatal complications, including pre-eclampsia, macrosomia, shoulder dystocia and neonatal hypoglycaemia (3). Data from this study could inform investigation of the effects of sustained non-carbohydrate first eating patterns on glucose control throughout pregnancy, the requirement for pharmacological treatment and the risk of adverse perinatal outcomes. If demonstrated to be beneficial, it would empower women with GDM to eat a balanced diet, without restricting individual food groups or macronutrients. It would represent an opportunity to offer simple advice, applicable to a large cohort of women at little cost to beneficial effect, and could inform future health policy.

The reduced glucose excursion associated with non-carbohydrate first meal plans has also been demonstrated in normoglycaemic and pre-diabetic individuals. It is possible that this dietary approach could have a subsequent effect on the risk of future T2DM in this at-risk population. During pregnancy and the post-partum period, women are particularly receptive to dietary and lifestyle changes and so this would also be an opportunity to instil dietary habits that could have a prolonged beneficial effect on health.

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