Towards understanding ethnic differences in the progression to type 2 diabetes: a preliminary phenotyping study in people with recent-onset diabetes in SE London

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# Study Summary

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<tr>
<th><strong>Title</strong></th>
<th>Towards understanding ethnic differences in the progression to type 2 diabetes: a preliminary phenotyping study in people with recent-onset diabetes in SE London</th>
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<tbody>
<tr>
<td><strong>Short Title</strong></td>
<td>SouL-DeEP: the South London Diabetes and Ethnicity Phenotyping Study</td>
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</table>
| **Protocol Numbers** | Version #1 31/10/2012  
REC# 12/LO/1859 |
| **Study Sponsor** | Sponsored by Kings College London (Dr Keith Brennan) |
| **Principal Investigator** | Dr Louise Goff |
| **Study Design** | Single time-point observational comparison study of two ethnic groups |
| **Study Duration** | 18 months |
| **Study Center(s)** | King’s College London, Franklin-Wilkins building, Stamford Street, London SE1 9NH |
| **Objectives** | The primary objective is to assess, in people of black West African and white European origin with type 2 diabetes, insulin secretory reserve by graded glucose infusion insulin action. Secondary objectives are to assess liver and peripheral tissue insulin sensitivity for glucose metabolism; insulin sensitivity of lipolysis; visceral, intramyocellular, intrahepatocellular and intrapancreatic fat accumulation; metabolic flexibility during the euglycaemic clamp using indirect calorimetry; and post-prandial insulin and incretin secretion during a meal tolerance test. |
| **Number of Subjects** | 20 BWAO and 20 WEO (recruit n=23 per group, allowing for 10% dropout) |
| **Main Inclusion / Exclusion Criteria** | Participants must be males, 18-65 years of age, have a body mass index of 25-40 kg/m² and be able to provide informed consent. Type 2 diabetes must have been diagnosed (according to WHO criteria) within the previous 5 years, be treated with nothing more than lifestyle +/- metformin and have an HbA1c of 8.0% or less.  
Participants will be excluded if treated with thiazolidinediones, insulin, oral steroids or beta-blockers, or drugs/conditions considered by the investigators to have significant impact on the study protocol or outcomes; with a serum creatinine of >150 mmol/l; with a serum alanine transaminase level >2.5 fold above the upper limit of the reference range; contraindications for magnetic resonance imaging; positive auto-antibodies for anti-insulin, anti-GAD or anti-A2; sickle cell disease (trait permitted); or unwillingness/unable to follow protocol. |
| **Statistical Methods** | Summary statistics will be presented as means, standard deviations and proportions. The t-test will be used to compare the 2 groups for the primary analysis. Further exploratory analyses using multiple regression will look at potential
confounding factors namely anthropometric, biomedical and clinical variables. Skewed data will be transformed and presented as unadjusted and adjusted means, confidence intervals, and regression coefficients.
## List of Abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>BWAO</td>
<td>Black West African Origin</td>
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<tr>
<td>CRF</td>
<td>Clinical Research facility</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
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<td>GGI</td>
<td>Graded glucose infusion</td>
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<td>ISR</td>
<td>Insulin secretory reserve</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>MRU</td>
<td>Metabolic research unit</td>
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<tr>
<td>MTT</td>
<td>Meal tolerance test</td>
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<tr>
<td>PIS</td>
<td>Participant information sheet</td>
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<tr>
<td>REC</td>
<td>Research Ethics Committee</td>
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<td>SAE</td>
<td>Serious Adverse Event</td>
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<td>SouL-D</td>
<td>South London Diabetes study</td>
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<td>T2DM</td>
<td>Type 2 diabetes</td>
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<td>WEO</td>
<td>White European Origin</td>
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</table>
1. **Study Contact Information**

1.1 **Sponsor Contact Information**

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2. Scientific Background

The prevalence of T2DM in UK black communities is 2-3 times higher than in the white European community, and the age of onset is younger (1). There are phenotypic differences in the presentation of T2DM in black patients that have implications for clinical management but these are not fully understood. People of BWAO are characteristically insulin resistant (2-4) and hypertensive (5), but with a cardioprotective lipid profile (low total and LDL-cholesterol, high HDL-cholesterol and low triglycerides in blood) (2, 6) which may relate to the lower rates of myocardial infarction (heart attack) seen in this group. Other key differences in the pathophysiology of T2DM in black people have been recognised including lower visceral fat accumulation or ‘central adiposity’ (6-9). Studies of other ectopic fat depots in BWAO people are limited but suggest intramyocellular and intrahepatocellular lipids are relatively low (10). There has been extensive investigation of pancreatic β-cell function in African-American (AA) populations, suggesting a higher compensatory insulin secretory response to early insulin resistance, predisposing to more rapid β-cell failure in this ethnic group (11-13). We have recently demonstrated this hyperinsulinaemia in UK-dwelling African-Caribbean people, using the intravenous glucose tolerance test (14). Studies of AA children and adolescents have suggested that hyperinsulinemia in this group is a function of reduced hepatic insulin extraction rather than increased pancreatic secretion, which would lead to peripheral hyperinsulinemia; such an hepatic effect would effectively conserve β-cell function (12). These studies present persuasive initial evidence that the pathophysiological development of T2DM in BWAO populations is different from that of other groups but require further investigation in the UK BWAO population. In the UK, the BWAO population has preserved to date the cardioprotective lipid profile and lower cardiovascular disease rates even in T2DM patients described above, which has been lost in African American populations. The UK BWAO population is less genetically mixed and has shorter and less complete acculturation of diet and lifestyle. Furthermore, existing studies in African Americans have been small, used surrogate markers of important metabolic processes and different aspects of metabolism have been studied in different groups. Our hypothesis is that, compared to people of WEO, people of BWAO with early T2DM will have (1) markedly greater insulin deficiency associated with (2) exaggerated peripheral insulin resistance. Additionally (3) low visceral and hepatic fat accumulation will be associated with (4) relatively preserved hepatic insulin sensitivity in BWAO people. It should be noted that this study is a first phase of a wider investigation of metabolic responsiveness in BWAO and WEO people with lesser degrees of glucose intolerance than frank T2DM, which ultimately will allow us to describe the pathogenesis of progression to diabetes in the two populations.

Hypothesis

We hypothesise that, compared to people of WEO, people of BWAO with early T2DM will have (1) markedly greater insulin deficiency associated with (2) exaggerated peripheral insulin resistance. Additionally (3) low visceral and hepatic fat accumulation will be associated with (4) relatively preserved hepatic insulin sensitivity in BWAO people. It should be noted that this study is a first phase of a wider investigation of metabolic responsiveness in BWAO and WEO people with lesser degrees of glucose intolerance than frank T2DM, which ultimately will allow us to describe the pathogenesis of progression to diabetes in the two populations.
accumulation will be associated with (4) relatively preserved hepatic insulin sensitivity in BWAO people.

3. **Study Objectives**

3.1 **Primary Objective**
The primary objective is to assess insulin secretory reserve (ISR) by graded glucose infusion (GGI).

3.2 **Secondary Objectives**
Secondary objectives are to assess hepatic and peripheral insulin sensitivity for glucose metabolism; insulin sensitivity of lipolysis; visceral, intramyocellular, intrahepatocellular and intrapancreatic fat accumulation; metabolic flexibility; and post-prandial insulin and incretin secretion.

4. **Study Design**

4.1 **Overview of Study Design**
This is single time-point observational comparison of β-cell insulin secretory function; whole body insulin sensitivity; hepatic and peripheral insulin sensitivity of glucose metabolism; insulin sensitivity of lipolysis and intrahepatocellular, intrapancreatic and intramyocellular lipid storage in males of black West African (n=20) and white European origin (n=20), aged 18-65 years, with newly-diagnosed T2DM (within 5 years of diagnosis).
Figure 1: a schematic of trial design and subject flow

Identification of newly-diagnosed type 2 diabetes (nT2DM)
Eligibility: diagnosed within previous 5 years, HbA1C ≤ 8.0%, Black West African (BWAO) or White European (WEO) ethnicity

Soul-D participants & completers
Send invitation letter (L1) or telephone invitation

Community recruitment
Posters & leaflets in community practices, advert in community literature

Potential participants contact researchers
Send Participant Information Sheet (PIS) & invite to screening

Informed consent
Screening assessment of eligibility (Visit S)

Eligible
Send L2 to participant and L4 to GP.

Not eligible
No further inclusion. Send L3 to volunteer and L5 to GP.

Participants:
Black West African Origin (BWAO), n=20
White European Origin (WEO), n=20

Food diary (4-day)
Completed and returned prior to assessment visits

VISIT A
Meal Tolerance Test (MTT)
Anthropometry
3-day activity assessment

VISIT B
Magnetic resonance imaging (MRI) and spectroscopy (MRS)

VISIT C
Hyperinsulinaemic-euglycaemic clamp including stable isotope infusions of glucose + glycerol

VISIT D
Graded glucose infusion (GGI)
4.2 Anticipated Duration of the Investigation

The study is anticipated to last 14 months from the enrolment of the first participant to the completion of the final report to Diabetes UK.

Planned recruitment rate

Preliminary data from the Soul-D study indicates that 76% of participants meet our inclusion criteria; 40% of these are of black ethnicity and 50% are of white ethnicity. We expect to screen 4 participants per week and recruit 2 per week for 5-6 months.

Timescale of project

On the basis of being able to have participants undertake Assessments A, B and D in pairs and Assessment C being performed individually we will need 100 assessment days. We intend to perform assessments on 3 days per week, totalling 34 weeks. Therefore the following represents the planned timescale of the project:

July – October 2012
   Preparation of protocol

November 2012
   Ethics & R&D application

December 2012
   Ethics approval

January – February 2013
   Subject identification
   Commence recruitment and screening

March 2013
   Commence outcome assessments

August 2013
   Complete recruitment

October 2013
   Complete outcomes assessment

November – January 2014
   Sample analysis

January – March 2014
   Data analysis

March 2014
   Final report
4.3 Outcome Measures

4.3.1 Primary Outcome
The primary outcome measure upon which the study is powered is insulin secretory reserve (ISR) assessed via the graded glucose infusion (GGI).

4.3.2 Secondary Outcomes
Our secondary outcome measures are:
1. weight, height, BMI, waist circumference;
2. β-cell insulin secretory function, using a meal tolerance test;
3. whole body, hepatic and peripheral insulin sensitivity of glucose metabolism, using the 2-step euglycaemic-hyperinsulinaemic clamp with infusion of labelled di-deuterated glucose;
4. insulin sensitivity of lipolysis, using stable isotope methods;
5. intrahepatocellular, intrapancreatic and intramyocellular lipid accumulation using magnetic resonance imaging and spectroscopy (MRI/MRS);
6. total body, visceral and subcutaneous fat distribution using MRI.

4.4 Study Population
Our participants have T2DM. The South London Diabetes Study (Soul-D) shows that in the SE London region 40% of people with newly diagnosed diabetes are of black African-Caribbean origin and 50% are of white European origin.

4.4.1 Sample Size
The aims of this study are to assess differences, between two ethnic groups, in the following biomarkers: 1.) insulin secretory reserve 2.) whole body insulin sensitivity 3.) hepatic insulin sensitivity 4.) peripheral insulin sensitivity. Our primary outcome, upon which our study is powered, is insulin secretory reserve. As this study is novel there are no directly comparable data to use for sample size calculations however we have used the limited adult data from Zoratti et al. (African-Caribbeans 1.13 (SD 0.6) vs white-Europeans 0.80 (SD 0.4) (2) to calculate our sample size which indicates we will have 90% power to detect a 40% difference (approx. 0.5 SD) with a sample size of 20 in each group. Using the more conservative data from Stefan et al. (15) we will have 80% power. We will recruit ~23 per group, to get 20 to completion, allowing for a 10% drop-out. We have additionally performed power calculations using lipolysis and insulin sensitivity data and are confident that this sample size will detect differences in these also (16-18).

4.4.2 Subject Recruitment
Participants will be identified principally through the South London Diabetes (Soul-D) Study database. Participants of Soul-D who have documented consent to be approached about new research in diabetes will be invited by letter (L1) or telephone to participate in the study. Other people with recent onset diabetes may be recruited
in community (consenting SouL-D practices) and hospital diabetes clinics by poster and leaflet and by open advertisement in relevant community literature; these forms of recruitment will require the potential participant to contact the study team. Interested parties will be sent an participant information sheet (PIS) providing details of the study and those who provide informed consent will be invited to a health screening assessment (visit S) which will assess eligibility.

4.4.3 Subject Screening

Potential participants will be invited to attend a screening assessment (visit S). Participants will be instructed to attend the research facility at either King’s College Hospital or King’s College London Waterloo Campus, depending on participant convenience, in a fasted state (10hr overnight fast). The screening visit will start with taking informed consent from the participants, followed by:

1. Screening questionnaire (S1) including medical and drug history, self-declared ethnicity of self, parents and grandparents, MRI contraindication checklist (e.g. claustrophobia, metal implants or prostheses).

2. Anthropometric measures, including height and weight, waist circumference and seated blood pressure.

3. Fasting blood test including full blood count, renal and liver function, HbA1c, lipid profile, sickle cell trait and auto-antibodies for anti-insulin, anti-GAD and anti-A2.

The screening assessment will take 30 minutes and afterwards the participants will be provided with a light breakfast meal.

Participant eligibility will be assessed against the inclusion/exclusion criteria and ‘fitness’ to participate will be assessed and signed off by a clinician within the research team.

Potential participants will be informed, by telephone or email, of their eligibility to participate within 3 weeks of their screening appointment and appointments for their metabolic assessment days will be made – a letter (L2) will be sent to confirm the appointments and to instruct the participant on pre-study requirements. Non-eligible participants will be sent a copy of their screening results (L3) and their GP will be informed of their results. Following screening, a GP letter L4 (eligible) and L5 (not eligible)) will be written informing the GP of the nature of the study and the participants screening results (anthropometrics, blood pressure and biochemistry), a copy of the PIS will be included in the letter.

4.4.4 Inclusion Criteria

Subjects will be eligible to participate in the study if all of the following conditions exist:

1. 18-65 years of age
2. BMI of 25-40 kg/m²
3. Black West African (BWAO) or white European ethnicity (WEO) (self-declared)
4. T2DM diagnosed (according to WHO criteria) within 5 years
5. T2DM treated with nothing more than lifestyle +/- metformin*
6. HbA1c of ≤8.0%
7. Able to provide informed consent

*Metformin will be stopped 7 days before any procedure.

4.4.5 Exclusion Criteria
Subjects will be excluded from participation in the study if any of the following conditions exist:
1. Thiazolidinedione treatment
2. Insulin treatment
3. Oral steroids treatment
4. Beta-blockers treatment
5. Drugs or conditions thought by the investigators to have significant impact on the study protocol or outcomes.
6. Serum creatinine of >150 mmol/l
7. Serum alanine transaminase level >2.5-fold above the upper limit of the reference range
8. Contraindications for MRI
9. Positive auto-antibodies for anti-insulin, anti-GAD or anti-A2
10. Sickle cell disease (trait permitted)
11. Unwillingness/inability to follow the protocol

4.4.6 Exit / Discontinuation Criteria
Subjects will exit the study if any of the following conditions exist:
1. Subject voluntarily withdraws from the study.
2. Subject death.
3. Subject acquires any of the listed exclusion criteria.
4. Subject completes the protocol.
5. Subject is non-compliant with the protocol (see Section 5.5 for definition of non-compliance)
6. Subject’s well-being, in the opinion of the investigators, would be compromised by study continuation.
7. Subject experiences an adverse event

5. Study Procedures

5.1 Informed Consent
All participants for this study will be provided with a PIS and consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The PIS and consent form will be submitted with the protocol for review and approval by the Research Ethics Committee (REC) for the study. The formal consent of a participant, using the REC-approved consent form, will be obtained before that participant is submitted to any
study procedure. The participant must sign this consent form, and the investigator-designated research professional obtaining the consent. The participant will be required to sign two copies of the consent form, one to be given to the participant and the other copy will be with the investigator for records. A blank copy of the REC-approved form will be kept on-site and by the sponsor-investigator.

Consent will be taken by Dr Cynthia Mohandas, Dr Louise Goff, Mr Andrew Pernet or Professor Stephanie Amiel who have undergone GCP and consent training and are experienced in patient communication and consent taking. All potential participants will have been given a PIS and will have had adequate time to read it, understand the protocol, the risks and burdens and benefits, and will have had time to ask questions. The researchers will reiterate this information in person prior to taking consent. Only participants who have capacity to provide consent for themselves will be recruited.

Potential participants will have at least 24 hours to consider participation. However, if the patient prefers to consent at the time of obtaining the information sheet and consent form, they will be able to provide consent at that time. If this is the case, the researcher will contact the patient 24 hours later to confirm their decision to be involved.

It is essential that participants fully understand the requirements of the study and therefore participants without a good understanding of verbal or written English will not be included in the study as there is not the provision for translation or interpreters.

5.2 Study Procedures
All potential participants will complete a screening assessment (visit S). Consented participants will be allocated an anonymised study ID code which will be used on all study documents. Eligible participants will go on to complete four assessment visits, detailed below.

5.2.1 SCREENING (Visit S)
Prior to the screening visit (S) the potential participant will have been provided with a copy of the PIS. The investigator will begin the screening visit by explaining the requirements of the study and offering to answer any questions. The participant will be asked to give formal written consent in the presence of the researcher. The participant will be given a copy of the PIS and signed consent form for their records.

A screening assessment will be performed to include:

- Age
- Self-declared ethnicity of self, parents and grandparents
- Date of diabetes diagnosis
- Relevant past medical history & comorbidities
- Current medications
- Contraindications for MRI (e.g. metal implants/prostheses, claustrophobia, pace maker)
• Body weight
• Height
• Waist circumference, as the mid-point between the lowest rib and the iliac crest.
• Seated blood pressure assessed with an automated sphygmomanometer, using the average of 3 measurements.
• Blood sample will be taken for assessment of electrolytes and creatinine (renal function), liver function tests, full blood count and sickle cell trait, full lipid profile, fasting glucose, HbA1c and auto-antibodies for anti-insulin, anti-GAD and anti-A2.

The screening assessment is expected to take 30 minutes. The participant will be offered a light meal after the screening appointment to avoid ill effects of prolonged fasting. Following telephone confirmation of eligibility the participants will be sent their screening results in the post (L2), non-eligible participants will be sent a copy of their screening results (L3) and their GP will be informed of their results. Following screening, a GP letter (L4 (eligible) and L5 (not eligible)) will be written informing the GP of the nature of the study and the participants screening results (anthropometrics, blood pressure and biochemistry), a copy of the PIS will be included in the letter. The eligible participants will be instructed on keeping a 4-day food diary prior to their first assessment visit. This diary will include at least 1 weekend day. Participants will be instructed to return the diary by post before their first assessment visit.

**ASSESSMENT VISITS (A-D)**

Assessment visits will be scheduled in random order.

Participants will be required to undertake the following instructions in preparation for ALL assessment visits:

• Refrain from strenuous exercise and physical activity in the 48 hours preceding the visit.
• Refrain from consuming alcohol in the 24 hours preceding the visit.
• Consume a standardised diet the day prior to the assessment visits. The diet will be matched to the participant’s daily energy requirements and provide ~50% of calories from carbohydrates (30-35% fat, 15-20% protein) which will be spread evenly throughout the day, ensuring that no more than 30% of the daily carbohydrate load is consumed in the evening meal.
• Refrain from eating or drinking anything other than water after 10pm on the evening before the visit or on the morning of the visit.

5.2.2 **ASSESSMENT A – Meal Tolerance Test (MTT) (19)**

The meal tolerance test (MTT) will be performed in the Metabolic Research Unit (MRU), Franklin-Wilkins Building, King’s College London. The meal tolerance test consists of the consumption of a specified volume, based on body weight, of Ensure Plus milkshake drink (Abbott Nutrition, UK) in a 5 minute period.

The following protocol will be undertaken:
• Upon arrival at the Metabolic Research Unit participants will have the meal tolerance test procedure explained to them in brief.
• Seated blood pressure will be assessed using an automated sphygmomanometer. A series of THREE measurements, each 2 minutes apart, will be recorded.
• An antecubital fossa cannula will be inserted into the non-dominant arm using intradermal lignocaine (1%) to anaesthetise the skin and standard aseptic techniques. Two fasting samples 10 minutes (-10, 0 minutes) apart will be drawn and cannula flushed with 0.9% NaCl solution. The participant will consume the standardised test drink (Ensure Plus). The volume of drink will be calculated based on 6cals (4mls) per kg body weight. The drink will be consumed within a 5-minute period and no other liquids or foods will be consumed for the duration of the MTT.
• Blood samples will be taken at 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 minutes for assessment of glucose, C-peptide and insulin. GLP-1 and GIP will be assessed at -10, 0, 30, 60, and 120. At time points -10, 0, 30, 60 and 120 the blood sample volume will be 10mls and at all other time points the volume will be 7.5mls. The total volume of blood collected will be 110 ml.
• Between 150 and 180 minutes, participants will be fitted with the Actiheart activity monitor, which will be worn for 3 days, and instructions for removal and return will be explained.
• Two urine samples will be taken at the start (fasting) and end of the study period for urinary C-peptide (UCPCR) evaluation.
• At the end of the meal tolerance test, the intravenous cannula will be removed and the participants will be provided with a light lunch meal.

5.2.3 ASSESSMENT B – MRI scanning (20, 21)
Participants will report to the Clinical Imaging Department at Guy’s Hospital, London Bridge between 7.30 and 8am on the day of their appointment.

The following protocol will be undertaken: upon arrival participants will be asked to complete a standard MRI safety screening form and will be instructed to change in to a hospital gown in order to ensure they are free from metal objects on their clothing.

Following screening, the participant will be positioned lying supine in the scanner with MRI coils positioned around the body. Ear defenders will be worn, through which music can be played during the scan.

The following imaging sequence will be acquired:
- A whole-body imaging scan to provide fat and water images. Some images may be acquired in conjunction with the patient holding their breath hold, as is routinely performed during clinical MRI scans of the abdomen. In these cases the subject will be given clear instructions by the radiographer at the beginning and end of the breath hold period.
- Spectroscopy from the soleus muscle in the lower leg to provide a localised assessment of intracellular and extracellular muscle fat.
If we find unexpected abnormalities we will inform the participant and their GP about it. If anything else needs to be done we will ensure that a referral to the correct clinic for further investigations or treatment is made.

5.2.4 ASSESSMENT C – 2-Step Hyperinsulinaemic-Euglycaemic Clamp With Stable Isotope Infusions (22)

The 2-step hyperinsulinaemic-euglycaemic clamp will involve a 120 minute infusion of insulin at a dose of 10mU/m²/min (LOW dose) followed by a 120 minutes infusion of insulin at a dose of 40mU/m²/min (HIGH dose), with an adjusted infusion of 20% glucose in order to maintain euglycaemia. Each insulin infusion will be primed to produce a “square wave” hyperinsulinaemia, in which the steady state plasma concentration is achieved rapidly. In order to measure endogenous glucose production, a primed-continuous infusion of [6,6 ²H₂] glucose will be started 2 hours before the first insulin infusion and run for 360 minutes. To measure lipolysis, a continuous infusion of [²H₅] glycerol will be started at the same time and run for 240 minutes. Fuel oxidation prior to and during the glucose infusion will be assessed using the Delta Trac ventilated hood indirect calorimeter.

The timeline for these infusions is shown in the schematic diagram below:
**Preparation procedures**

- Procedures will be carried out in the morning at the Clinical Research Facility of either King’s College Hospital, St Thomas’ Hospital or Guy’s Hospital. The participant will be admitted to the CRF at 07.30 am, having fasted from 22:00 hours the previous evening.
- Upon arrival at the CRF the participant will be weighed, without shoes and with light clothing. The body surface area of the participant will be calculated using the Gehan and George formula, and insulin infusion rates determined.
- The participant will have a cannula inserted into an antecubital fossa vein for infusions and a second cannula inserted retrogradely into the dorsum of the hand for blood taking. Intradermal lignocaine (1%) will be used to anaesthetise the skin and strict aseptic technique used. 0.9% NaCl solution will be used for flushing and patency.
- Baseline plasma glucose will be assessed using the YSI glucose analyser. If baseline plasma glucose is above 5mmol/l, the participant will follow the protocol for priming regimen in hyperglycaemic patients. Plasma glucose will be measured before commencing the glucose infusion and the procedure will be initiated only if the plasma glucose is ≤5 mmol/l (± 10%).
- The hand cannula, which is to be used for sampling, will be placed in the heated hand warming unit (~60°C), to achieve arterialized venous blood.
- The insulin infusion will be prepared by drawing up 40 mls 0.9% NaCl into a 50ml syringe, adding 2ml autologous blood, and the insulin dose and made up to 55mls with 0.9% NaCl.

**Clamp procedures**

- The labelled glucose and glycerol infusions will be started at -120 minutes.
- Baseline blood samples will be drawn at time points -30, -20, -10 and 0 minutes.
At 0 minutes a blood glucose reading will be taken using the YSI glucose analyser to confirm the blood glucose is ≤5 mmol/l ± 10%.

Then a primed-continuous infusion of insulin (LOW dose, 10 mU/m2/min) from 0 to 120mins followed by re-primed HIGH dose (40 mU/m2/min) from 120 to 240min.

Plasma glucose will be clamped at 5 mmol/l throughout by adjusting an infusion of 20% glucose, according to 5min readings of plasma glucose concentration using a glucose oxidase method (Yellow Springs Analyser).

Plasma glucose will be assessed at time points -120, 0 and every 5 minutes thereafter until 240 minutes. Blood samples will also be taken at 30 min interval, increased to 10min during steady state periods (-30 –0; 90-120 and 210-240min) for measurement of plasma glucose and glycerol concentrations and enrichments; NEFA, insulin, C-peptide and glucagon.

At 240 minutes, when all the measurements have been taken, the isotope and insulin infusions will be stopped.

A final plasma glucose reading, after the participant has consumed a light lunch, will be performed making a total of 51 blood glucose readings will be taken throughout the procedure. Sampling for isotope enrichments, NEFA, insulin, C-peptide and glucagon will occur at -30, -20, -10, 0, 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230, 240 minutes: a total of 16 samples will be taken. The total volume of 205 ml of blood will be taken during the whole procedure.

During this period, blood pressure, heart rate will be assessed approximately every 30 minutes.

**Post-infusion procedures**

- The patient will be given a carbohydrate containing meal and blood glucose will be checked. All cannulae will be removed when the blood glucose is stable or increasing. Once the patient is feeling well they will be able to leave, but will be advised to avoid heavy exercise for 24 hours.

**Indirect calorimetry**

- At time point -60 - -30 minutes, an assessment of fuel oxidation will be performed. The ventilated hood will be placed over the participant’s head and the participant instructed to remain rested and awake. The duration of this assessment will include a settling period and 15 minutes of steady state recording.
- At time point 60 – 90 minutes a repeat assessment of fuel oxidation will be performed.

**5.2.5 ASSESSMENT D – Graded Glucose Infusion (GGI) (23-25)**

Participants will receive graded intravenous infusions of glucose at progressively increasing rates (1, 2, 3, 4, 6, and 8 mg/kg/min), for 40 minutes per step. Glucose, insulin, and C-peptide concentrations will be measured at fasting and 10, 20, 30, and 40mins into each glucose infusion, additionally insulin will be measured at 2,4, and 8 minutes in the 1,3 and 8 mg/kg/min infusion phases only.
• Procedures will be carried out in the morning at the Clinical Research Facility of either King’s College Hospital, St Thomas’ Hospital or Guy’s Hospital. The participant will be admitted to the CRF at 8 am having fasted from 22:00 hours the previous evening. Upon arrival the participant will be weighed, without shoes and with light clothing, to allow calculation of the glucose infusion volumes.

• The participant will have a cannula inserted into an antecubital fossa vein for the administration of the glucose infusion and a second cannula inserted retrogradely into the dorsum of the hand for blood sampling. Intradermal lignocaine (1%) will be used to anaesthetise the skin and strict aseptic technique used. 0.9% NaCl solution will be used for flushing and patency.

• The sampling hand will be placed in the heated hand warming unit, to achieve arterialized venous blood.

• Plasma glucose will be measured before beginning the infusion, using the YSI analyser. If above 5 mmol/l a bolus of rapid acting insulin (0.007U/kg) will be administered followed by a low-dose continuous infusion for 20 minutes. Plasma glucose will be measured again before commencing the glucose infusion and the procedure will only be initiated if the plasma glucose is ≤5 mmol/l (± 10%).

• A baseline/fasting blood glucose sample will be taken at 0 minutes. The glucose infusions will be in the form of 20% dextrose and started at time 0 minutes.

• The infusion rates are 1, 2, 3, 4, 6, and 8 mg/kg/min and each infusion rate will run for 40 minutes. At the end of each infusion period, the 40 minute sample will be drawn and the glucose infusion rate will be immediately increased to the next rate.

• Blood sampling will occur at 10, 20, 30, and 40 minutes. Additionally, at time points 2, 4 and 8 minutes, only in the 1,3 and 8 mg/kg/min steps, insulin concentrations will also be analysed. At all other time points glucose, insulin and c-peptide concentrations will be analysed. A total of 204.5mls will be taken.

• At the end of the 240 minute infusion period the intravenous cannulae will be removed and the participants should be provided with a light lunch meal.
5.3 Study Timetable

There is no requirement to space the visits over a specified period. The minimum period between assessment visits will be 1 week. A reasonable spacing for the visits is one per month so it is expected that participants will take between 6 weeks and 4 months to complete the study. The maximum period that a participant will be able to take to complete the study is 12 months as beyond this period there is considerable chance of phenotypic changes; participants who have not completed within this period will be withdrawn from the study.

5.3.1 Study flowchart

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<th>Visit C – Euglycaemic Hyperinsulinaemic clamp</th>
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## 5.3.2 Study Gantt Chart

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<tr>
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<tr>
<td>64</td>
<td>23/12/13</td>
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<tr>
<td>65</td>
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<tr>
<td>66</td>
<td>6/1/14</td>
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<tr>
<td>67</td>
<td>13/1/14</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>68</td>
<td>20/1/14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>27/1/14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>3/2/14</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>10/2/14</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>17/2/14</td>
<td></td>
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<td></td>
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<tr>
<td>73</td>
<td>24/2/14</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>3/3/14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>10/3/14</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.4 Laboratory Testing Procedures

The following laboratories will undertake analysis of the samples:

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening assessments</td>
<td>King’s College Hospital&lt;br&gt;Clinical Biochemistry&lt;br&gt;Denmark Hill, London, SE5 9RS</td>
</tr>
<tr>
<td>Insulin</td>
<td>Dr Roy Sherwood (Head of Clinical Biochemistry Department)&lt;br&gt;<a href="mailto:roysherwood@nhs.net">roysherwood@nhs.net</a>&lt;br&gt;Tracy Dew&lt;br&gt;<a href="mailto:tracydew@nhs.net">tracydew@nhs.net</a>&lt;br&gt;0203 299 3726</td>
</tr>
<tr>
<td>c-peptide</td>
<td></td>
</tr>
<tr>
<td>GLP-1</td>
<td></td>
</tr>
<tr>
<td>GIP</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>King’s College London&lt;br&gt;Diabetes &amp; Nutritional Sciences Division&lt;br&gt;Franklin-Wilkins Building, Laboratory 4.</td>
</tr>
<tr>
<td>Lipids</td>
<td>Anne-Catherine Perz (technician)</td>
</tr>
<tr>
<td>NEFA</td>
<td></td>
</tr>
<tr>
<td>Isotope enrichments</td>
<td>University of Surrey&lt;br&gt;Diabetes &amp; Metabolic Medicine&lt;br&gt;Postgraduate Medical School&lt;br&gt;Guildford&lt;br&gt;Tel: 01483 688579&lt;br&gt;Email: <a href="mailto:m.umpleby@surrey.ac.uk">m.umpleby@surrey.ac.uk</a></td>
</tr>
</tbody>
</table>

5.4.1 Specimen Preparation

Labelling

All participant documentation and specimens will be labelled with a unique study ID code which is a pseudo-anonymised code used on all study documents and samples, no identifiable information should be recorded on participant documents or samples. The ID code will take the following format: SDIIXXXS, which refers to:

SD – SouL-DeEP study
II – participant first and last initial
XXX – study number e.g. 001
S – screening visit (S), visit A (A), visit B (B), visit C (C), visit D (D)

The labelling should also record information on the time point of collection and the analyte e.g. GLUC -30, INS 120
**Sample collection and processing**

Samples will be collected at time points shown in the sampling schedules below:

**Meal tolerance test – sampling schedule**

<table>
<thead>
<tr>
<th>Time</th>
<th>-10</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A1, A2</strong></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Glucose (2ml fluoride oxalate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A3, A4</strong></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Insulin &amp; c-peptide (5ml plain clotted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A5</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>GIP &amp; GLP-1 (2.5ml EDTA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A6</strong></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
### Euglycaemic hyperinsulinaemic clamp – sampling schedule

| Time (min) | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 | 105 |
|------------|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| **Glucose YSI analyser (0.5ml)** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B1, B2 Glucose (2ml fluoride oxalate)** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B3, B4 Insulin & c-peptide (5ml plain clotted)** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B5, B6 NEFA** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B7 Glucagon** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B8, B9 Isotope enrichment** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |

### Additional Table

| Time (min) | 110 | 115 | 120 | 125 | 130 | 135 | 140 | 145 | 150 | 155 | 160 | 165 | 170 | 175 | 180 | 185 | 190 | 195 | 200 | 205 | 210 | 215 | 220 | 225 | 230 | 235 | 240 |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| **Glucose YSI analyser (0.5ml)** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B1, B2 Glucose (2ml fluoride oxalate)** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B3, B4 Insulin & c-peptide (5ml plain clotted)** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B5, B6 NEFA** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B7 Glucagon** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **B8, B9 Isotope enrichment** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
Graded glucose infusion – sampling schedule

<table>
<thead>
<tr>
<th></th>
<th>-10</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C1, C2</strong></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Glucose (2ml fluoride oxalate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C3</strong></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Insulin (2.5ml plain clotted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C4, C5</strong></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>c-peptide (2.5ml plain clotted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*only during 1,3 and 8mg/kg/min steps

The samples require the following preparation and processing:

### Processing screening samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analysis</th>
<th>Preparation requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>4ml EDTA (purple top)</td>
<td>FBC, HbA1c, sickle cell trait</td>
<td>Send to laboratory without processing – whole blood analysis</td>
</tr>
<tr>
<td>2ml Fluoride oxalate (grey top)</td>
<td>Glucose</td>
<td>Spin in centrifuge at 3000 rpm for 20 minutes at 4°C where possible. Plasma aliquoted into 2 cryovials and labelled accordingly.</td>
</tr>
<tr>
<td>8.5ml Serum (gold top)</td>
<td>Full lipid profile, U&amp;Es, LFTs, anti-GAD, anti-insulin, anti-A₂</td>
<td>Stand at room temperature for 30 minutes. Spin in centrifuge at 3000 rpm for 20 minutes at 4°C where possible. Serum aliquoted into 2 cryovials and labelled accordingly.</td>
</tr>
</tbody>
</table>

### Processing visit A, B, C, D samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sampling tube</th>
<th>Preparation requirements</th>
<th>Volume requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Fluoride oxalate (grey top)</td>
<td>Spun within 2 hours plasma can be frozen at -20°C</td>
<td>200uL plasma</td>
</tr>
<tr>
<td>Insulin</td>
<td>Plain clotted tube (gold top)</td>
<td>Spun within 2 hours plasma can be frozen at -20°C</td>
<td>200uL plasma</td>
</tr>
<tr>
<td>C peptide</td>
<td>Plain clotted tube (gold top)</td>
<td>Spun within 2 hours</td>
<td>200uL plasma</td>
</tr>
</tbody>
</table>
plasma can be frozen at -20°C

NEFA
EDTA (Lilac top)
Spun within 2 hours
plasma frozen at -80°C
200uL plasma

GIP
EDTA (Lilac top)
Spun within 2 hours
plasma can be frozen at -20°C
200uL plasma

GLP-1
EDTA + DDPIV additive on ICE (Lilac top)
Spun immediately at 4°C
plasma frozen immediately at -40°C
200uL plasma

Glucagon
EDTA PLASMA plus Trasylol
Samples will be taken on ice or into chilled tubes. Spun at 4°C. Stored at -80°C.
200uL plasma

Isotope enrichment
D5 Glycerol
Lithium Heparin(green top)
Mix the tubes gently, keep on ice. Spin at 4°C 3000rpm 10 mins. Stored at -80°C.
2 separate 1 ml plasma

D2 Glucose
Fluoride oxalate(grey top)

5.4.2 Specimen Handling and Storage
The screening samples will be analysed at King’s College Hospital Clinical Biochemistry Department. The whole blood sample (EDTA tube), and one aliquot of plasma and one aliquot of serum will be sent to the labs for analysis. A second aliquot of plasma and of serum will be stored as a spare at -20°C in case of lost samples.

The assessment visit samples will be spun immediately (within 2 hours) at 3000 rpm and at 4°C where possible. Aliquoting will be performed according to the following plan for each visit:

Visit A samples and aliquots

<table>
<thead>
<tr>
<th>Type</th>
<th>Volume (ml)</th>
<th>Measurement</th>
<th>Set #</th>
<th>Aliquot volume (mls)</th>
<th>Aliquot cap colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride oxalate</td>
<td>2.5</td>
<td>Glucose</td>
<td>A1</td>
<td>0.5</td>
<td>Yellow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spare plasma</td>
<td>A2</td>
<td>2 x 0.5</td>
<td>Yellow</td>
</tr>
</tbody>
</table>
Aliquots will be racked in 10x10 cryogenic storage boxes. Each aliquot will be labelled with the participant study ID code, the time point of collection and the aliquot set number. The storage boxes will be labelled with the set number to allow sets to be stored together. The sample storage will be mapped on an excel spreadsheet and a printed copy of this box map should be inserted into completed boxes. The storage boxes will be stored in -80°C freezers at Franklin-Wilkins building. The 'spare' aliquots will be used if there are any losses during analysis or for future studies assessing adiponectin, leptin, resistin, TNF-α, vitamin D, bile acids, proteomics, lipidomics and total fatty acids. Samples of urine will be stored in the HTA licensed -80°C freezer in the Metabolic Research Unit on 4th floor of the Franklin-Wilkins building (HTA license # 12523).
Long-term storage of spare aliquots will be in Franklin-Wilkins building, 4th floor laboratory. The sample custodian is Dr Louise Goff, who will be responsible for the safe keeping, analysis and disposal of the samples.

5.4.3 Specimen Shipment

**Screening Samples**

Blood samples collected from participants attending the screening assessment should be couriered to King’s College Hospital the same day for analysis. All samples will have the appropriate accompanying documents. Each sample will be labelled with the participant study ID code. Tubes will carry the date and the trial code and the participant’s date of birth. For each participant form S2 will be completed and sent with the samples. Samples will be appropriately packaged and sent to Tracy Dew/Dr Roy Sherwood, Dept. Clinical Biochemistry, King’s College Hospital, Denmark Hill, London SE5 9RS. Upon receipt of samples a copy of T1 will be faxed by King’s College Hospital to acknowledge receipt of samples. Spare aliquots will be stored in a -20°C freezer in the Diabetes & Nutritional Sciences Division.

**Assessment visit samples**

The following sets of samples will be analysed at King’s College Hospital, Dept. Clinical Biochemistry: A3, A5, B3, B7, C3, C4. These sets will be couriered to King’s College Hospital upon completion of the study. The samples must remain frozen during transit therefore an igloo box and dry ice will be used for transfer. Upon receipt of samples a copy of T1 will be faxed by the King’s College Hospital Dept. of Clinical Biochemistry to acknowledge receipt of samples.

Blood samples collected during Visit B for analysis of isotope enrichment will be analysed at the University of Surrey. Sets B8 and B9 will be couriered to the University of Surrey upon completion of the study. The samples must remain frozen during transit therefore an igloo box and dry ice will be used for transfer. Upon receipt of samples a copy of T1 will be faxed by the University of Surrey to acknowledge receipt of samples.

5.4.4 Laboratory Testing: Normal Values

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Normal Values / Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (fasting-plasma)</td>
<td>3-7 mmol/L</td>
</tr>
<tr>
<td>Urea</td>
<td>3.3-6.7 mmol/L</td>
</tr>
<tr>
<td>Creatinine</td>
<td>40-130 μmol/L</td>
</tr>
<tr>
<td>Total protein</td>
<td>60-80 g/L</td>
</tr>
<tr>
<td>Albumin</td>
<td>35-50 g/L</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>3-20 μmol/L</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>30-130 IU/L</td>
</tr>
<tr>
<td>AST</td>
<td>10-50 IU/L</td>
</tr>
<tr>
<td>Gamma GT</td>
<td>5-55 IU/L</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>&lt;5.2 mmol/L</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt; 2.0 mmol/L</td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>&gt; 1.0 mmol/L</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>1-3 mmol/L</td>
</tr>
<tr>
<td>Total:HDL ratio</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>WBC</td>
<td>4.0-11.0 x 10^9/l</td>
</tr>
<tr>
<td>RBC</td>
<td>4.50-5.80 x 10^9/l</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>13-16.5 g/dl</td>
</tr>
<tr>
<td>HCT</td>
<td>0.40-0.54 l/l</td>
</tr>
<tr>
<td>MCV</td>
<td>77-95 fl</td>
</tr>
<tr>
<td>MCH</td>
<td>25-34 pg</td>
</tr>
<tr>
<td>MCHC</td>
<td>32-37.0 g/dl</td>
</tr>
<tr>
<td>RDW</td>
<td>11.0-15.0%</td>
</tr>
<tr>
<td>Platelets</td>
<td>150-450 x 10^9/l</td>
</tr>
<tr>
<td>MPV</td>
<td>7.4-10.4 fl</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.2-6.3 x 10^9/l</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.3-4.0 x 10^9/l</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.2-1.0 x 10^9/l</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.0-0.4 x 10^9/l</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.0-0.1 x 10^9/l</td>
</tr>
</tbody>
</table>

### 5.5 Study Protocol Compliance

The study protocol must be completed by each participant within 12 months of screening, if a participant fails to do so they will be withdrawn from the study and a replacement participant recruited and will undertake the entire protocol. Data from withdrawn participants will be included in the analysis.

### 5.6 Deviations from the Clinical Protocol

#### 5.6.1 Protocol Deviations

When a deviation from the protocol is necessary for an individual subject, the investigator must complete a description of the deviation from the protocol and justification on the Protocol Deviation Form (PDF).

If a participant finds one procedure within the protocol unacceptable they will be able to continue in the study and withdrawn only from that procedure, a PDF form will be completed.

#### 5.6.2 Protocol Amendments

Should changes in the study plan or protocol become necessary in the course of the study, those specific changes will be discussed and agreed upon by the Sponsor, its acting representative if appropriate, Investigator, and appropriate ethical approval obtained before the changes are implemented. All changes will be documented as protocol amendments.
5.7 Subject Withdrawal

5.7.1 How to Withdraw Subjects
Criteria for withdrawal of subjects from the study
This study is completely voluntary at all times. Participants are free to withdraw from the study at any time without giving a reason. Withdrawal from the study will not affect future care at King’s College Hospital in any way.

Subjects will be withdrawn from the study if they:
- develop any of the exclusion criteria listed above
- fail to complete the protocol within 12 months of screening
- adverse event associated with the study protocol
- subject consent withdrawal

The reason for subject withdrawal will be documented (W1 form).

Replacement of withdrawn subjects
We will aim to complete the study with 20 participants in each group. In order to achieve this, we may need to recruit up to a maximum of 25 per group. Once 23 subjects have been recruited in a group we will recruit further subjects to that group only in event of subject withdrawal or incomplete data collection.

5.7.2 Data Collection and Follow-up for Withdrawn Subjects
Data collected up to the time of participant withdrawal will be analysed where possible (unless the participants withdraw their consent).

5.8 Participant honorarium
Subjects will receive a payment for participation in this study as follows:
People who attend the screening appointment, regardless of eligibility, will be able to claim travel expenses up to £5. There will be no other payment for the screening appointments.

Participants who attend the assessment visits will be able to claim travel expenses, up to £5, for each visit (4 visits) and this will be reimbursed to all participants who do and do not complete the protocol. An honorarium of £40 for visits A and B and £80 for visits C and D, totalling £240, will be paid to participants completing the protocol.

King’s College London non-staff claim forms will be completed and the honorarium paid directly to the participant.
6. Data Collection and Analysis

6.1 Subject Population for Analysis
This is a single time point cross-sectional comparison of two populations: people of white European origin (WEO) and people of black West African origin (BWAO). All analysis will be to compare primary and secondary outcomes between these two groups.

6.2 Statistical Methods
Summary statistics will be presented as means and standard deviations (skewed data will be transformed prior to analysis). Transformed data will be presented as unadjusted and adjusted means, and confidence intervals.

<table>
<thead>
<tr>
<th>Data Collected</th>
<th>Analysis to be Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics data</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>Insulin secretory reserve (ISR)</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>Whole-body insulin sensitivity (M)</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>Hepatic insulin sensitivity</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>Peripheral insulin sensitivity</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>1st phase insulin response</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>Fasting beta-cell responsiveness (M₀)</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>Post-prandial beta-cell responsiveness (M₁)</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>GLP-1 iAUC</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>GIP iAUC</td>
<td>Descriptive statistics; comparison of means between independent groups; p &lt; 0.05</td>
</tr>
<tr>
<td>Intramyocellular</td>
<td>Descriptive statistics; comparison of means</td>
</tr>
</tbody>
</table>
Intrahepatocellular lipid storage | Descriptive statistics; comparison of means between independent groups; p < 0.05
---|---
Intrapancreatic lipid storage | Descriptive statistics; comparison of means between independent groups; p < 0.05
Visceral fat volume | Descriptive statistics; comparison of means between independent groups; p < 0.05
Waist circumference, BMI | Descriptive statistics; comparison of means between independent groups; p < 0.05
Fasting lipids, blood pressure | Descriptive statistics; comparison of means between independent groups; p < 0.05

Further exploratory analysis using multiple regression will look at potential confounding factors namely anthropometric, biomedical and clinical variables.

7. Safety and Adverse Events

7.1 Definitions

Adverse Event (AE)
An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries will be regarded as adverse events. Abnormal results of laboratory or diagnostic procedures are considered to be adverse events if the abnormality:
- Results in study withdrawal
- Is associated with a serious adverse event
- Is associated with clinical signs or symptoms
- Leads to additional treatment or to further diagnostic tests
- Is considered by the Investigator to be of clinical significance.

Serious Adverse Event (SAE)
A serious adverse event (SAE) is any adverse event that is:
- Fatal
- Life-threatening
- Requires or prolongs a hospital stay
- Results in persistent or significant disability or incapacity
- A congenital anomaly or birth defect

Important medical events are events that may not be immediately life-threatening, but are clearly of major clinical significance and may be SAEs. They may jeopardize the subject, and may require intervention to prevent one or the other serious outcomes noted above.
Hospitalization
Hospitalization shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse effect; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse effect (e.g., for a preexisting condition not associated with a new adverse effect or with a worsening of the preexisting condition; admission for a protocol-specified procedure) is not, in itself, a serious adverse effect.

Unanticipated Problems Involving Risk To Subjects or Others (UPIRTSO)
An adverse event that in the opinion of the Principal Investigator is unexpected, related to the device, and serious.

7.2 Safety Monitoring Plan
Emergency Medical Safety Contact:
Dr Cynthia Mohandas
Telephone: 07871 922423

7.2.1 Anticipated Risks / Risk Mitigation

<table>
<thead>
<tr>
<th>Study Procedure</th>
<th>Anticipated Risks</th>
<th>Risk Mitigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veneupuncture &amp; intravenous cannulation</td>
<td>Discomfort, slight bruising, fainting</td>
<td>Trained phlebotomists will perform all blood draws. Strict aseptic technique and use of local anaesthetic.</td>
</tr>
<tr>
<td>Health screening</td>
<td>Possibility of detecting undiagnosed dyslipidaemia, anaemia, abnormal renal or liver function</td>
<td>Participant’s GP will be informed of results and early treatment can be initiated.</td>
</tr>
<tr>
<td>MRI scanning</td>
<td>Possibility of detecting abnormalities</td>
<td>A trained radiographer will inspect scans and a referral for further investigation will be made if any abnormalities are detected.</td>
</tr>
<tr>
<td>Fasting blood testing and procedures (e.g. clamp)</td>
<td>Possibility of hypoglycaemia</td>
<td>Regular monitoring of blood glucose concentrations and provision of a meal at the end of each fasting procedure before the participant departs</td>
</tr>
<tr>
<td>Isotope tracers</td>
<td>Possible fear of preparation standards</td>
<td>Prepared in a manufacturing unit which is part of a specials manufacturing unit (MS11387). This means that the unit has been inspected by the Medicine and Healthcare Products Regulatory Agency (MHRA)</td>
</tr>
</tbody>
</table>
and has been found to comply with the requirements of Good Manufacturing Practice (GMP). Extensive experience in using these for clamp studies by the research group members.

### 7.2.2 Medical Monitoring for Participant Safety

The Principal Investigator will oversee the safety of the study, including careful assessment and appropriate reporting of adverse events. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

### 7.3 Adverse Event Reporting

All Adverse Events occurring during the study period must be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that study treatment or participation is not the cause.

The Sponsor-Investigator will promptly review documented adverse effects and abnormal test findings to determine
1) if the abnormal test finding should be classified as an adverse effect;
2) if there is a reasonable possibility that the adverse effect was caused by the study protocol/procedures
3) if the adverse effect meets the criteria for a serious adverse effect.

If the Sponsor-Investigator’s final determination of causality is “unknown and of questionable relationship to the study protocol”, the adverse effect will be classified as associated with study protocol for reporting purposes. If the investigator-sponsor’s final determination of causality is “unknown but not related to the study protocol”, this determination and the rationale for the determination will be documented in the respective subject’s case history.

#### 7.3.1 Adverse Events

All observed or volunteered adverse effects and abnormal test findings will be recorded in the subjects’ case histories. For all adverse effects, sufficient information will be pursued and/or obtained so as to permit
1) an adequate determination of the outcome of the effect (i.e., whether the effect should be classified as a serious adverse effect) and;
2) an assessment of the casual relationship between the adverse effect and the procedure

Adverse effects or abnormal test findings felt to be associated with the investigational procedures will be followed until the effect (or its sequelae) or the
abnormal test finding resolves or stabilizes at a level acceptable to the Sponsor-Investigator.

Adverse Events that do not qualify as Serious Adverse Events, or as Unanticipated Adverse Device Effects will be reported to the Sponsor at designate interval determined by the Sponsor.

Adverse Events that do not qualify as Serious Adverse Events, or as Unanticipated Adverse Device Effects will be reported to the Ethics Committee with the continuing review progress report.

7.3.2 Serious Adverse Events
Investigators must report serious adverse events to the Study Sponsor within 24 hours of learning of the event. A serious adverse event form must be completed by the Investigator and faxed to the Study Sponsor within 24 hours. Study Sponsor contact information for Serious Adverse Event Notification:
  Keith Brennan
  0207 848 6960 (Telephone)
  0207 848 6394 (Fax)

At the time of the initial report, the following information should be provided:
  Study Identifier    Reason the event is classified as serious
  Study Center       Investigator assessment of
  Subject Number     association between event
  Event Description  and study device
  Date of Onset      Current Status

Serious Adverse Events that are at least possibly related must be reported to the Ethics Committee within 10 working days.

7.3.3 UPIRTSO Events
Investigators are required to submit a report of UPIRTSO events to the Ethics Committee within 10 working days of first learning of the event.

8. Data Handling and Record Keeping

8.1 Confidentiality
Information about study subjects will be kept confidential.
8.2 **Source Documents**

*Source Data* are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the CRF shall match the *Source Data* recorded on the *Source Documents*.

8.3 **Case Report Forms**

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”. All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialled and dated. **DO NOT ERASE OR WHITE OUT ERRORS.** For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

A Case Report Form will be completed for each subject enrolled into the clinical study. The investigator-sponsor will review, approve and sign/date each completed CRF; the investigator-sponsor’s signature serving as attestation of the investigator-sponsor’s responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.

8.4 **Clinical Reports**

An annual progress report will be submitted to Diabetes UK. Investigators will submit a final report of the clinical study to the sponsor and Diabetes UK within 6 weeks of termination or completion of the clinical study or the Investigator’s part of the clinical study. The principal investigator is responsible for submitting these reports.

9. **Study Monitoring, Auditing, and Inspecting**

9.1 **Auditing and Inspections**

The investigator will permit study-related monitoring, audits, and inspections by the Ethics Committee, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).
Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10. Ethical Considerations
This study is to be conducted according to the Declaration of Helsinki (1996) and the principles of Good Clinical Practice, applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a Research Ethics Committee and the Research and Development committees of King’s College Hospital NHS Foundation Trust and St Thomas’ and Guy’s Hospital NHS Foundation Trust for formal approval of the study conduct. The decision of the REC concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

11. Study Finances
This study is financed through a project grant from Diabetes UK.

12. Publication Plan
The results of the study will be reported at conferences and in peer-reviewed journals. Dr Louise Goff and Professor Stephanie Amiel have the primary responsibility for publication of any results of the study and are required to provide approval before any information can be used or passed on to a third party.

13. References


14. Attachments

1. Covering letter
2. CV for Chief Investigator (CI)
3. CV for supervisor (student research)
4. CV for student
5. CV for 2 research nurses
6. Participant information sheet (PIS)
7. Consent form
8. Copies of advertisement materials for research participants
9. Letters of invitation to participant (L1)
10. Letters of eligibility and non-eligibility (L2, L3)
11. GP/consultant information sheets or letters (L4, L5)
12. Screening Questionnaire (S1)
13. Evidence of Sponsor insurance or indemnity
14. Letter from funder
15. Diabetes UK funding application reviewer feedback
16. Non-NHS Site Specific Information (SSI) Form (signed/authorised)