Dose-response effect of an apple extract on postprandial glycaemia: a randomised controlled trial. The Glu-Pomme Study

NCT02940249

30/09/2016

Full Application Form

Filter Questions

1 Is your study considered research as defined in the guidance icon information? (●) Yes ( ) No

2 Does the research fall under the requirements of the HRA and therefore require ethical review by an NHS REC, Social Care REC or MoDREC? Please refer to the information in the guidance icon for further details. ( ) Yes (●) No

Data Collection

3 Select one category from the list below (categories are defined in the guidance icon).

My study involves:

- (●) a) Only primary data collection involving human subjects.
- ( ) b) Only further analysis of pre-existing data (originally obtained from human participants) which is identifiable and not in the public domain.
- ( ) c) Both primary data collection involving human subjects and further analysis of pre-existing data (originally obtained from human participants) which is identifiable and not in the public domain
- ( ) d) Data collection not involving any of the above but presenting sensitive issues
- ( ) e) None of the above

4 Select all that apply in order to determine the risk level of your application.

- [ ] a) Does the research involve participants who are particularly vulnerable or unable to give informed consent or in a dependent position?
- [ ] b) Will participants be asked to take part in the study without their consent or knowledge at the time or will deception of any sort be involved?
- [ ] c) Is there a risk that the research topic might lead to disclosures from the participant concerning their involvement in illegal activities or other activities that represent a threat to themselves or others?
- [ ] d) Could the study induce psychological stress or anxiety, or produce humiliation or cause harm or negative consequences beyond the risks encountered in a participant's usual everyday life?
- [ ] e) Does the study involve imaging techniques such as MRI scans or ultrasound?
- [ ] f) Does the study involve sources of non-ionising radiation (e.g. lasers)?
- [x] g) Does the study involve physically intrusive procedures, use of bodily materials, or DNA/RNA analysis? (including collection of human tissue)

You should only select the statement below if you have not selected any of the above. Your application will be invalid if you select the below statement in addition to any of the above.

- [ ] I have answered no to all questions in the risk checklist above and I believe that my research is low risk

Based on your answers to the above filter questions your research has been categorised as High Risk and upon submission will be subject to review at the next relevant Research Ethics Subcommittee meeting. You can now access an overview of the available sections of the application by selecting the navigate tile in the action panel on the left. Alternatively you can proceed through each section of the application by selecting the next tile.

Meeting dates and submission deadlines can be found here

Section A: General Information

A Applicant Details

Title	First Name	Surname
Miss	Emily	Prpa

Department Name	Diabetes & Nutritional Sciences
Address	4.46, Franklin-Wilkins Building
	150 Stamford Street
City	London
County	
Postcode	SE1 9NH
Telephone	+44 (0) 20 7848 4162
KCL Email	emily.prpa@kcl.ac.uk

A2 Applicant Status

MPhil / PhD/ Specialist Doctorate

A3 Faculty/Institute/School

Please refer to the information icon if you are unsure of your Faculty/Institute/School.

Life Sciences and Medicine

A4 Course/Qualification

PhD

A5 Supervisor Details

Title	First Name	Surname
Dr	Wendy	Hall

Position	Senior Lecturer
Department	Diabetes & Nutritional Sciences
Telephone	+44 (0) 20 7848 4197
Email	wendy.hall@kcl.ac.uk

A6 Do you have a medical supervisor?

- ○ Yes
- ◉ No

A8 Other Investigators/Collaborators

Title	First Name	Surname
Ms	Anne-Catherine	Perz

Organisation	King's College London
Address	Franklin Wilkins Building
	150 Stamford Street
Postcode	SE1 9NH
Telephone	
Email	anne-catherine.perz@kcl.ac.uk

What is the role of this investigator?

Formation and coding of test drinks. This allows for blinding of investigators during the trial.

Title	First Name	Surname
	N/A	N/A

Organisation	King's College London
Address	Franklin Wilkins Building
	150 Stamford Street
Postcode	SE1 9NH

Telephone

Email

What is the role of this investigator?

Intercalated BSc or MSc project students who may choose to work on this study for their dissertations (names not yet available, but the ethics committee will be notified)

Title	First Name	Surname
Prof	Kennedy	Cruickshank

Organisation: King's College London

Address: Room 4.22 Franklin Wilkins Building

150 Stamford Street

Postcode: SE1 9NH

Telephone: 020 7848 4270

Email: kennedy.cruickshank@kcl.ac.uk

What is the role of this investigator?

Medical supervisor

Section B: Project Information

B1 Project Title

Dose-response effect of an apple extract on postprandial glycaemia: a randomised controlled trial. The Glu-Pomme Study

B2 Proposed start date: 01/11/2016

B3 Expected completion date: 31/12/2017

B4 Is this a funded project?

- (•) Yes
- ( ) No

How is the project being funded?

Externally funded

Please state your Funder Reference Number:
If you are unaware of your Grant Award Reference or Contract's Funder Reference please contact your Award Management Division campus team leader who will provide the relevant information.

No reference number

B5 What are the aims and objectives of the project?

Provide the academic/scientific justification of the project as well as detailing and explaining the principal research question, objectives and hypotheses to be tested.

Please Note: Applications to the BDM and PNM RESC should include a full list of references/citations to back up the academic/scientific justification of the project.

This research project will determine the efficacy of an apple extract at different doses to inhibit postprandial glycaemia following a mixed carbohydrate meal. Postprandial hyperglycaemia has been associated with oxidative stress, glycation of functional proteins, pancreatic beta cell dysfunction and vascular damage (Cierello et al., 2008; Blaak et al., 2012). Thus, controlling postprandial hyperglycemia has been suggested as an important measure in the prevention and management of metabolic diseases, including type 2 diabetes mellitus (T2DM). Emerging evidence details that fruit polyphenols may have beneficial effects on metabolic health (Chong et al., 2010). Adding fruit-based polyphenol-rich extracts to food or beverages may modulate the rate of glucose absorption from a carbohydrate-containing meal and thus suppress sharp peaks in blood glucose concentrations. Previous research by the supervisor's group has demonstrated that 1200 mg of apple polyphenols (Appl'In™) inhibited the average incremental area under the curve (iAUC; T+0 to T+30 min) of plasma glucose by 54% and Cmax by 9% relative to placebo (matched drink and meal containing no apple extract) (Castro Acosta & Hall, presented at the ICPH conference, Tours, October 2015). In addition, Schulze et al. (2014) demonstrated that a polyphenol-rich apple extract diminished sodium-couple glucose transporter 1 (SGLT1) mediated glucose uptake in vitro and in vivo. The human study showed a significant reduction of glucose-dependent insulinotropic peptide (GIP) concentrations (and higher concentrations of glucagon-like peptide-1 (GLP-1)) after apple juice ingestion, suggesting a shift of the main site of glucose absorption to more distal regions of the gut (in which GLP-1 is secreted from L cells) (Schulze et al., 2014).

This study aims to test the hypothesis that, following a mixed carbohydrate meal of starch and sucrose, the inhibitory effect of the aformentioned apple extract (Appl'In™) will be statistically and clinically significant at a lower dose. To test this hypothesis we will investigate the effects of increasing doses of apple polyphenol-rich extracts on postprandial glycaemia, insulinaemia and plasma concentrations of gut hormones, GIP and GLP-1 following a mixed carbohydrate (starch and sucrose) test meal in a randomised, controlled, cross-over, single-meal dietary intervention trial. We will also determine whether the apple extract is inhibiting postprandial glycaemia via delayed gastric emptying by including a paracetamol absorption test on a sub group of participants.

Outcome measures: The primary outcome variable will be iAUC T+0 to +30 min for plasma glucose concentrations since previous work by the supervisor's group suggests that apple and berry polyphenols inhibit the rate of glucose appearance in the blood in the early phase of postprandial glycaemia. Secondary outcome variables include iAUC T+0 to T+120 min , Cmax, Tmax changes from baseline up to T+240 min for plasma glucose concentrations, and iAUC T+0 to +30 min and T+0 to +120 min, Cmax, Tmax and changes from baseline up to T+240 min for plasma insulin, c-peptide, non-esterified fatty acids, GIP and GLP-1 concentrations. GIP, an incretin and inhibitor of gastric acid secretion, appears in the bloodstream immediately following entry of carbohydrate into the duodenum and its release is a sensitive indicator of SGLT1-mediated glucose uptake (Gorboulev et al., 2012). Other secondary outcomes include measures of vascular function (pulse wave velocity, augmentation index, blood pressure) from T+0 to +240 min. Urine will be collected for 24 h for analysis of urinary polyphenol metabolites and glucose concentrations. Paracetamol absorption rate will be measured in a sub-group of n=6 to assess effects on gastric emptying rate.

References

Blaak E, Antoine J, Benton D, Bjorck I, Bozzetto I, Bronus F, Diamant M, et al. Impact of postprandial glycaemia on health and prevention of disease 2012. Obesity reviews; 13, 923-984.
Chong MF, Macdonald R, Lovegrove JA. Fruit polyphenols and CVD risk: a review of human intervention studies. The British journal of nutrition 2010;104 Suppl 3:S28-39.
Ceriello A, Esposito K, Piconi L, Ihnat M. A, Thorpe J. E, Testa R, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes 2008; 57, 1349–1354.
Gorboulev V, Schürmann A, Vallon V, Kipp H, Jaschke A, Klessen D, et al. Na+-d-glucose Cotransporter SGLT1 is Pivotal for Intestinal Glucose Absorption and Glucose-Dependent Incretin Secretion. Diabetes 2012; 61,187-196.
Schulze C, Bangert A, Kottra G, Geillinger K, Schwanck B, Vollert H, et al. Inhibition of the intestinal sodium-coupled glucose transporter (SGLT1) by extracts and polyphenols from apple reduces postprandial blood glucose levels in mice and humans. Mol. Nutr. Food Res 2014; 00, 1-14.

B6 Where will the research be conducted? i.e in a facility within the college, in a private organisation, in a public place etc

Metabolic Research Unit, 4th Floor, Corridor A, Franklin Wilkins Building, King's College London, 150 Stamford Street, SE1 9NH.

B7 If outside of the UK, please state the country/countries in which data collection is expected to occur.

B8 Selection of methodology from list: (select each that applies)

- [x] Questionnaires
- [ ] Semi-structured interviews
- [ ] Unstructured Interviews
- [ ] Focus Groups
- [ ] Observation
- [x] Clinical Procedures or Interventions
- [ ] Non-clinical Procedures or Interventions
- [x] Randomised Controlled Trial
- [ ] Oral history
- [ ] Secondary analysis of pre-existing data from human participants
- [ ] Audio/video recording or photography in a public place
- [ ] Audio/video recording or photography in a private place
- [x] Administration of food substances
- [ ] Behavioural/Cognitive Testing
- [ ] Other

If you are using any standardised methods for any of the above selected methodologies, please provide an overview of any standardised documentation to be used. Please provide full names and references where appropriate.
Please note you are not required to submit any standardised forms as supporting documents.

B9 Give a summary of the project methodology.

Study population, recruitment and screening: Subjects will be recruited from the general adult population: healthy men and women aged 18-70 y. Individuals responding to advertisements will complete an initial telephone questionnaire, and then eligible respondents will be asked to attend King's College London for a screening visit. The screening visit will take place at least 2 weeks before the first study day. Firstly during the screening visit, the study protocol will be fully explained to participants. During this time, participants will have the opportunity to exclude themselves from the randomisation to the sub-group, if they believe they will not be able to tolerate the taste of the drink or tolerate the measurement procedures. Subjects will be consented before any measurements are taken. Fasting blood sample for assessment of liver function, haematology, blood lipids and glucose will be taken. Blood pressure, height, weight, waist and hip circumference, and % body fat (bioelectrical impedance) will be measured. Female participants will complete a women's health questionnaire on this visit to ensure study visits can be scheduled at similar times in their menstrual cycle (avoiding the week before and week of menses). Subjects accepted onto the study according to the inclusion and exclusion criteria will complete an EPIC 7-day food diary (used in previous studies by the research group) to record their habitual diet and then will be provided with dietary and lifestyle advice to follow during the study (e.g. avoiding high-polyphenol foods, high nitrate foods and oily fish for 48 h before each study day, avoiding strenuous exercise the day before a study visit). Volunteers who do not meet the study inclusion criteria will be contacted by email or letter and informed that they will not be asked to take part on this occasion, explaining the reason. Randomisation to treatment order will take place following acceptance on the study should the volunteer remain willing to take part. The participant will then attend 4 separate study days to consume each of the 4 treatments in random order for study, each study day lasting around 4-5 h.

Study design: This study will follow the same protocol as used in previous studies all approved by the College REC, ref BDM/11/12-88 and BDM/14/15-10. A randomised, controlled, double-blind, 4-arm, cross-over study will be conducted. Subjects will receive apple extracts (delivering 600, 900 and 1200 mg apple polyphenols), or a matched placebo with no apple extract, in the form of a drink, in a random order at 4 separate study visits immediately before a high-carbohydrate meal. In a sub group of participants (n=6), the drink will also contain 1.5 g dissolved paracetamol to determine the effect on gastric emptying rate (this follows the standard paracetamol absorption test procedure, of which has been used in a previous study conducted by the supervisor (REF BDM/14/15-26)). Randomisation to treatment order (and to sub group) will take place following acceptance on the study. Seven days wash-out period will be required between study days, and participants will be advised to avoid high-polyphenol foods (e.g. berries, tea, red wine) for 2 days before each study visit and high-nitrate foods (green leafy vegetables, beetroot) and oily fish one day before each visit. Standardised diet and exercise advice will be given prior to the visit. Between the screening visit and first study visit, participants will complete an EPIC 7-day food diary (used in previous studies by the research group). Subjects will arrive on each study visit between 08.00 and 10.00 h, after a 12 h overnight fast. Subjects weight will be recorded (Tanita bioimpedance scales). Subjects will rest in a supine position for 10 min. An Arteriograph cuff will be fitted to the upper arm and the measurement taken (blood pressure [BP], pulse wave velocity [PWV], augmentation index [Aix]). A cannula will be inserted into the participant's forearm vein (opposite arm to Arteriograph) and baseline fasting blood glucose samples will be taken in duplicate. All test drinks will be blended to equal in volume, macronutrient, and energy content, and flavourings will be used to maintain a similar appearance and taste as much as possible. Following consumption of the test drink, the high carbohydrate-meal (75 g carbohydrate as starch and sucrose) will be served (white bread + a low polyphenol apricot jam). Both the drink and meal must be consumed within 8 minutes. Blood samples will be collected at baseline (T+0 min, before the test meal), T+10, 20, 30, 45, 60, 75, 90 and 120, 150, 180 and 240 min for plasma glucose and NEFA analyses by colorimetric kit (Instrumentation Laboratories, Warrington, UK), and plasma insulin, C-peptide, GIP and GLP-1 analyses by immunoassay (Siemens Medical Solutions Diagnositics Europe Ltd; Millipore Corporation, USA). For the sub-group of participants (whose test drink contains paracetamol), all postprandial blood samples will be analysed for paracetamol by a colorimetric assay. The Arteriograph will be inflated at T+65, 125, 185, 245. During study days, participants will additionally collect 24 h urine (collected over 2 g boric acid), fractioned into different collection periods of 0-4 h (collected during study visit), 4-8 h, and 8-24 h. Participants will be provided with the two urine collection containers at the end of each study visit, which they will return to KCL after the collection period has ended (i.e. the next day). A cool bag will be provided for storing and transporting these containers. Analysis of urinary polyphenol metabolites and glucose concentrations will be conducted using LCMS and colorimetric kit, respectively.

Data Analysis: All results will be expressed as mean ± 95% confidence intervals, or if the data is not normally distributed, as medians with IQR. For each parameter over time the change from baseline and the iAUC from 0-30 min (when the difference in the rate of appearance of glucose in the blood is expected) and from 0-120 min (to determine longer lasting differences in glycaemia) will be calculated. Each iAUC and change from baseline at each time point will be analysed with a mixed model ANCOVA, with fixed effects for treatment group and study period, and age, BMI, sex and period baseline values as covariates. SPSS software will be used to carry out statistical tests.

If the summary of your methodology would be supported by a flowchart please attach this here (an editable flowchart can be found via the link in the guidance icon)

Type	Name	File Name	Date	Version	Size
Other	Methodology Flowchart	flow chart study procedures.docx	22/09/2016 12:00:00 AM	1	29.5 KB

B10 I confirm that the researcher who will be administering all tests and/or procedures is competent in the methods.

- (•) Yes
- ( ) No

Section C(I): Participants

C1 Detail your projected number of participants and provide justification for this sample size.

Study population, recruitment and screening: Healthy men and postmenopausal women 18-70 y will be recruited. Power calculations were calculated for plasma glucose concentrations obtained from a previous postprandial glycaemia study conducted by the supervisor using 1200 mg apple polyphenols (REF BDM/14/15-10). A sample size of 30 subjects has 80% power to detect a difference between average mean incremental area under the curve values T+0 to +30 min of 10 mmol/L.min between test drinks with a significance level of α=0.01 (two-tailed), calculated from a 16 mmol/L.min SD of the mean difference. The supervisor's previous study found a mean difference between 1200 mg apple polyphenols and placebo of 25 mmol/L.min, so the study is powered to detect any differences following lower doses that are 40% of this effect size.

Power calculations for the sub-group were calculated for plasma paracetamol concentrations obtained from a previous postprandial glycaemia study conducted by the supervisor. A sample size of 6 has 80% power to detect a difference between the Cmax of test drinks of 0.054 mmol/L with a significance level of α=0.05 (two-tailed), calculated from 0.048 mmol/L SD and correlation between paired observations of 0.6. These values were obtained from a previous study conducted by the supervisor (REF BDM/14/15-26)

C2a What are the Inclusion Criteria? Where appropriate explain how you will screen your participants. (the selection criteria should be clearly defined for multiple participant groups)

Individuals responding to advertisements will complete an initial telephone questionnaire; eligible respondents will be asked to attend King's College London for a screening visit at least 2 weeks before the first study day. For the screening visit, subjects arrive after a 12 h fast and a fasting blood sample is taken for assessment of liver function, haematology, blood lipids and glucose. Blood pressure, height, weight, waist and hip circumference, and % body fat (bioelectrical impedance) will be measured.

Inclusion Criteria:
a) Age: 18-70 y
b) Male and female
c) Healthy (free of diagnosed diseases listed in the exclusion criteria)
d) Body Mass Index 18-35 kg/m2
e) Able to understand the information sheet and willing to comply with study protocol
f) Able to give informed written consent

C2b What are the Exclusion Criteria? Where appropriate explain how you will screen your participants. (the selection criteria should be clearly defined for multiple participant groups)

Individuals responding to advertisements will complete an initial telephone questionnaire (Attached); eligible respondents will be asked to attend King's College London for a screening visit at least 2 weeks before the first study day. For the screening visit, subjects arrive after a 12 h fast and a fasting blood sample is taken for

assessment of liver function, haematology, blood lipids and glucose. Blood pressure, height, weight, waist and hip circumference, and % body fat (bioelectrical impedance) will be measured. Volunteers who do not meet the study inclusion criteria will be contacted by email or letter and informed that they will not be asked to take part on this occasion, explaining the reason.

Exclusion criteria
a) Those diagnosed with Phenylketonuria (PKU)
b) Those with known or suspected food and/or paracetamol intolerances, allergies or hypersensitivity
c) Women who are known to be pregnant or who are intending to become pregnant over the course of the study
d) Women who are breast feeding
e) Participation in another clinical trial
f) Those who have donated blood within 3 months of the screening visit and participants for whom participation in this study would result in having donated more than 1500 millilitres of blood in the previous 12 months.
g) Full Blood Counts and Liver Function test results outside of the normal range.
i) Current smokers, or reported giving up smoking within the last 6 months
j) History of substance abuse or alcoholism
k) Reported history of Cardiovascular disease, diabetes (or fasting glucose ≥ 7.1 mmol/L), cancer, kidney, liver or bowel disease, gastrointestinal disorder or use of drug likely to alter gastrointestinal function
l) Unwilling to restrict consumption of specified high polyphenol foods for 48 h before the study
m) Weight change >3kg in preceding 2 months and body mass index <18 or >35 kg/m2
n) Blood pressure ≥160/100 mmHg
o) Total cholesterol ≥ 7.5 mmol/L; fasting triacylglycerol concentrations ≥ 5.0 mmol/L
p) Medications that may interfere with the study: alpha-glucosidase inhibitors (acarbose: Glucobay), insulin-sensitising drugs (metformin: Glucophage, Glucophage SR, Eucreas, Janumet; thiazolidinediones: Actos, Competact), sulfonylureas (Daonil, Diamicron, Diamicron MR, Glibenese, Minodiab, Amaryl Tolbutamide), and lipid-lowering drugs (statins, nicotinic acid, colestyramine anhydrous, ezetimibe, fibrates); and medications that may react unpredictably with paracetamol: ketoconazole, metoclopramide, carbamazepine, phenobarbital, phenytoin, primidone, warfarin and other products containing paracetamol. Other medications should be reviewed by medical representative from KCL on a case by case basis.
q) Nutritional supplements that may interfere with the study: higher dose vitamins/minerals (>200% Recommend Nutrient Intake), B vitamins, Vitamin C, calcium, copper, chromium, iodine, iron, magnesium, manganese, phosphorus, potassium and zinc. Subjects already taking vitamin or minerals at a dose around 100% or less up to 200% of the RNI, or evening primrose/algal/fish oil supplements will be asked to maintain habitual intake patterns, ensuring that they take them every day and not sporadically. They will be advised not to stop taking supplements or start taking new supplements during the course of the study.

C3 What are the upper and lower age limits? Provide justification for these where appropriate.

The lower age limit is 18 y. The upper age limit is set at 70 y in order to reduce inter-individual variation in postprandial glycaemic responses to a high-carbohydrate meal. There is an increased likelihood of a more pronounced glycaemia following high-carbohdyrate meals in people over the age of 70 y due to the greater prevalence of glucose intolerance, and large variations between individuals may obscure any subtle effects of the intervention itself.

C4 How will potential participants be identified and approached?

Volunteers responding to advertisements (posters among KCL, internal email circulars, social media [facebook, twitter] and external advertising including Metro advertisement) will complete an initial telephone questionnaire, and then eligible respondents will be asked to attend a screening visit at King's College London. Following discussion between the subject and the researcher about the requirements of the study, voluntary written informed consent will be provided by all subjects prior to any study procedures being performed, including fasting before the screening visit. Those subjects who are deemed to be eligible initially following completion of the telephone questionnaire, and who consent to the study, will be entered onto a screening log. The subject will be screened by the researcher and the outcome of the screening activities will be recorded into a personal file. All inclusion/exclusion criteria must be completed at the screening visit or prior to attendance of the subject at the study site for baseline testing.

C5 If any participants are under 16 will you seek additional consent from parents or carers? ○ Yes ○ No ◉ N/A

C6 Please specify any incentives being offered and a justification for their use.

Participants will receive £100 in compensation for the time dedicated to the study (£25 per study visit). If the participant is withdrawn from the study after completion of 1 or 2 study visits only due to events beyond their control (e.g. illness, no longer eligible, unable to tolerate study procedures) they will receive a pro rata honorarium.

No honorarium will be given for attendance at the screening visit, but they will receive breakfast and a full health screening including blood pressure, body composition, full blood count, full lipid profile, liver function and glucose tests.

Informed Consent

C7 Will informed consent be sought? (•) Yes ( ) No

C7a How will this be sought? Who will take consent and how will it be recorded?
Note: Justification must be provided for not gaining written consent

Interested volunteers will be sent an information sheet by post or email, and given an oral explanation over the phone. If they are eligible according to the initial telephone screening questionnaire they will be invited to attend a screening session at KCL. At the screening visit it will be the responsibility of the researcher to obtain written (signed and dated by the participant and researcher) informed consent from each individual participating in the study after adequate explanation of the aims, methods, objectives and potential hazards of the study. The researcher will also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time and volunteers will have the opportunity to ask questions. The participant will be provided with a copy of their signed and dated consent form and any other written information which they should be instructed to retain. Screening results will only be sent to the volunteer's GP if results are outside the normal range and the volunteer wishes it.

C7b How long will participants be given to decide if they wish to participate?

Potential participants will be able to discuss the study and read the information leaflet for at least 3 days prior to a screening appointment, booked at the participant's convenience. They will be asked to sign the consent form and return it at the screening visit. They will be informed over the phone, prior to the screening visit, that they are free to withdraw from the study at any point and they are not obliged to give a reason.

C8 Could your past or present relationship with potential participants give rise to a perceived pressure to participate? If so, what steps will you take to mitigate this issue?

In the event that a potential participant is known to one of the study investigators (for example, a student colleague), the study team will ensure that the informed consent procedures will be conducted by an alternative investigator who has no relationship with the volunteer.

C9 Detail the process by which participants may withdraw from the research both during the research and after it has been completed. A final withdrawal date should also be provided, after which participants may no longer withdraw their data from the study.

They will be informed over the phone, prior to the screening visit, that they are free to withdraw from the study at any point and they are not obliged to give a reason. Withdrawal from the study will be dealt with face-to-face or over the telephone if possible (otherwise by email or letter) so that the participant has the opportunity to give their reasons for withdrawal and express their wishes concerning any data that has been collected and any further contact. If they withdraw following screening but before attending the first study visit, their data will be not be used in the study. If they withdraw once the study has started their data will be used in the final report unless the participant requests withdrawal of their data. Participants will be informed that their data cannot be withdrawn once the study has been published in a journal or compiled in a report (01/01/18), and that any data that is collected after the point of randomisation will be anonymised. Should a subject decide to withdraw all efforts will be made to complete and report the observations as thoroughly as possible. If a participant does withdraw, an explanation of why the subject is withdrawing from the study will be recorded. If the reason for removal of a subject from the study is an adverse event or an abnormal laboratory test result, the principal specific event or test will also be recorded on the personal file. Any adverse events ongoing at the final visit which are considered in any way related to the study treatment or study regime will be followed up until resolved, the conditions stabilises or is otherwise explained. Any adverse events will be recorded separately from records of withdrawals.

Subject Replacement: Subjects will not be replaced on the study in the event of subject withdrawal unless the study has not yet commenced or is in its very early stages. In order to avoid loss of statistical power through subject withdrawal, an adequate sample size will be conserved by initially recruiting 10% more subjects than required (n=33, to complete 30 subjects; n=7 allocated to sub-group).

Section D: High Risk Research

D1g Risk Identified: The study involves physically intrusive procedures, use of bodily material or DNA/RNA analysis? Please fully explain how the risk will be mitigated.

Moderately intrusive procedures include anthropometry (height, weight, etc.), Arteriograph measurement, women's health questionnaire and urine collection. The women's health questionnaire will be completed at the screening visit and it will be explained that the information provided is to ensure that their study visits are not allocated on the week before and the week of menses. For urine collection, standard operating procedures previously used by this research department will be followed (e.g. providing a jug) and all participants will have health and safety considerations regarding the acid (inside urine collection containers) verbally explained to them. Invasive procedures include venepuncture at screening to extract 16.5 ml (~3 tsp) for analysis of lipids, full blood count, liver function and glucose concentrations, and cannulation of the antecubital vein on study days for analysis of study outcomes in blood plasma (142 ml/ 29 tsp in one study day, 584.5 ml/ 120 tsp in total including the screening visit). Venepuncture and blood pressure measurements may cause brief discomfort and there is a risk of bruising afterwards. Researcher will ensure participants have not recently donated blood (within the last 3 months) and that their participation in the study would not result in the donation of more than 1500 mL of blood in the previous 12 months. Food substances will be administered as part of this study: the apple extract will be incorporated into a low-sugar drink. This will be a commercially available, standardised fruit extract already used in supplements and food products.

These procedures have been used many times in previous studies within the Nutritional Sciences Division, cause no risk to health and wellbeing, and have been found to be acceptable to volunteers. The supervisor, Dr Wendy Hall (BA MSc PhD RNutr), is an experienced researcher with considerable expertise in running randomised controlled dietary intervention studies involving all the procedures to be used in the current study in the field of diet and CVD risk. The applicant will be closely supervised by Dr Hall and fully trained in all procedures. The applicant has also had experience of running a dietary intervention trial in her Nutrition MSc at KCL. Medics will be used to carry out cannulation and phlebotomy on study days. Research division technicians will also be available to supervise daily tasks such as sample handling and use of the Metabolic Research Unit kitchen. Dr Hall has a food hygiene certificate and will make sure that appropriate hygienic practices are followed when foodstuffs are prepared and provided to participants.

In the occurrence of any incident or adverse reaction during the blood sampling and study day, the Department Safety Manager, Rosie Calokatsia will be contact and if necessary participant will be conducted to the First Aid Room (4.178), 4th Floor, Franklin Wilkins Building, King's College London, 150 Stamford Street, SE1 9NH.

D2 What are the potential risks and burdens to the participant?

Urine collection containers will contain <2g boric acid for preservation. Boric acid is low in toxicity if it contacts the skin and all participants will be made aware of the health and safety precautions required to minimise risk of contact. Blood pressure measurement may cause bruising through cuff inflation and may temporarily cause minor discomfort. Efforts will be made to minimise this risk by appropriate training of research students. Venepuncture can cause brief discomfort and there is a risk of bruising. Participants may experience dizziness during and/or after the study's blood sampling. Once the study's blood sampling is finished, snacks will be provided in order to prevent dizziness or discomfort at end point. All participants will be questioned about any potential food allergies before the dietary interventions so the risk of allergic reaction is minimised. It is possible that participants may be inconvenienced or may have to change their diet and lifestyle by having to attend for screening and study visits (inlcuding returning urine samples), being contacted by the researcher and avoiding certain foods before study visits. Additionally, completing the 7 day food diary may be an inconvenience for participants. To minimise this effect, the requirements of the study will be extensively explained both orally and in writing in order to preselect a sample of participants who would not find this inconvenient. This is a necessary element of a dietary intervention study and we do not anticipate that participants will be adversely affected by this as they will have already given informed consent once they have understood what will be asked of them. Should participants find the study inconvenient then they will be free to withdraw from the study without needing to explain their reason for doing so. Equally, all participants during the screening visit will have the opportunity to exclude themselves from the randomisation to the sub-group, if they believe they will not be able to tolerate the taste of the drink.

D3 What are the potential benefits to the participant?

Participants will be screened for clinical biochemistry indicators (full blood count, liver function) of general health and cardiovascular risk factors (fasting lipids, glucose, blood pressure, waist circumference and BMI). Any results deemed to be of clinical significance will be reported to the participant. A letter to their GP will be provided to the participant so that they may then make the choice of whether to pass this on to their GP or not. Participants will be given honoraria of £25 per study day and will be reimbursed for their food and travel costs (train, tube or bus fares) where necessary. They will also receive breakfast at the screening visit and brunch at the 4 study visits. They may in future benefit from the outcome of this research study in that our understanding of dietary prevention of diabetes will be improved and will be provided with a copy of the final report on request.

D4 If you have guaranteed participant anonymity in the final report, confirm how this will be ensured.

Volunteers will be assigned a subject code. Following screening and acceptance onto the study, all further records, databases and reports will refer to each subject by their code, not by their name. All records, databases and reports will be kept in a safe place and only the researcher and supervisor will have access to them. The final report will contain only summary data, not individual data.

Section E(I): Data Handling, Protection And Storage

E1 Will the identification of potential participants involve the review of identifiable personal information?

- (●) Yes
- ( ) No

E1a If yes please give details and how this will be handled.

The recruitment (eligibility) questionnaire will be used to record non-anonymised data such as names, addresses, email and telephone number details, which will be on the same document used to record medical history, lifestyle, body measurements, and reproductive status. If volunteers are not eligible or not interested in participating and do not proceed to the screening visit, these questionnaires will be shredded and disposed of (see attached recruitment questionnaire). Eligible volunteers will be assigned a subject code for the screening visit. At the screening visit all further records, databases and reports will refer to each subject by their code, not by their name. Recruitment questionnaires containing identifiable information will be kept securely in a locked cupboard in a locked office

(Room 4.46A, Corridor B, 4th floor, Franklin−Wilkins Building, King's College London, 150 Stamford Street, SE1 9NH) during the study. Personal data held within computers, will be password protected and stored on encrypted memory sticks or restricted server access when not in use. Access to such data will be granted only to appropriate members of the research team. On completion of the study, the research project's paper records will be archived in a filing cabinet in Dr Hall's locked office.

E2 Who will have access to participants personal data?

Dr Wendy Hall, Ms Emily Prpa, and any intercalated BSc or MSc project students who may choose to work on this study for their dissertations (names not yet available, but the ethics committee will be notified). Personal information will not be disclosed to anyone outside the study team.

E3 How will you ensure the confidentiality of personal data?

Volunteers will be assigned a subject code. Following screening and acceptance onto the study, all further records, databases and reports will refer to each subject by their code, not by their name. All records, databases and reports will be kept in a safe place and only the reseacher and supervisor will have access to them. Medical results will not be disclosed to any other party than the participant. The research records will be held securely at King's College London, according to the Data Protection Act 1988, and in accordance with the College Guidelines. The researcher and supervisor will have access to the anonymised data. Participants' personal details will be kept securely in the supervisor's office (Room 4.108, Diabetes & Nutritional Sciences Division, Franklin−Wilkins Building, King's College London, 150 Stamford Street, SE1 9NH). Personal data stored in filing cabinets, cupboards and/or rooms will be kept in a locked room when not in use. Personal data held within computers, will be password protected and stored on encrypted memory sticks or restricted server access when not in use. Access to such data will be granted only to appropriate members of the research team. On completion of the study, the research project's paper records will be stored in a secure environment (filing cabinet in a locked office) that enables continued access to the required records by the researcher, supervisor and authorised members.

E4 Will any of the following activities be undertaken?: (select each that applies)

E4a Use of personal details such as address, phone number, email etc.	◉ Yes	○ No
E4b Sharing personal data with external sources.	○ Yes	◉ No
E4c Publication of quotes attributed directly to participants.	○ Yes	◉ No
E4d Publication of data from which participants could be identified.	○ Yes	◉ No
E4e Use of audio and/or visual recording devices.	○ Yes	◉ No
E4f Electronic storage of personal data (encrypted, password protected).	◉ Yes	○ No

E5 Where will data be stored during and after the study?

In a password-protected shared folder accessible via researcher' and supervisor' laptops/PCs, and in paper form in the locked offices of study researcher and supervisor and the Metabolic Research Unit, 4th floor, Corridor A, Franklin-Wilkins Building, Kings College London, 150 Stamford Street, London, SE1 9NH.

The address of the supervisor's office is:
FWB Room 4.108
Diabetes and Nutritional Sciences Division
King's College London
Franklin-Wilkins Building
150 Stamford Street
London
SE1 9NH

E6 Who will have ownership and responsibility for data storage during and after the study?

Dr Wendy Hall and Ms Emily Prpa.

E7 How long will personal data be stored for after the study is completed?

Personal contact details, consent forms and other administrative records will be stored for no longer than 12 months and will be stored in paper form in a locked office in the Franklin-Wilkins Building (room 4.108, 4th floor).

E8 How long will research data be stored for after the study is completed?

Anonymised data from the study will be stored for at least 10 y as they can be used for purposes such as calculation of statistical power for future studies or for teaching purposes.

E9a Will data be archived for further use? ◉ Yes ○ No

E9b If yes, will the data be identifiable? ○ Yes ◉ No

E10 How will results be disseminated?

- ☑ Internal report (thesis)
- ☑ Journals
- ☑ Conference
- ☐ Other

Section G: Human Tissue

G1 Does the study involve the use or collection of bodily materials or tissue from a human being? ◉ Yes ○ No

G1a Where biological samples are to be taken, I confirm that the amount taken or the size of the sample is minimised to avoid the collection of excess material

- ◉ Yes
- ○ No
- ○ N/A

G1b Is the tissue used in this study defined as "relevant material" as defined by the Human Tissue Act 2004?

○ Yes
◉ No

G1c Does the study involve the collection of new, relevant material? ○ Yes ◉ No

G1d Detail how this material falls under the review remit of CREC.

The blood samples collected will be processed to render them acellular on the day of collection and then frozen to store before analysis. Analysis will take place within 6 months of the end of the study and then the remaining plasma sample will be disposed of. Therefore whole blood and infranatant of whole blood (containing red cells, platelets and white cells) will be disposed of on the day of the study.

24 hour urine samples will be collected over 2 g boric acid, to avoid bacterial degradation, and stored in cool bags during the entire collection period and centrifuged, acellular samples will be stored at -80 until further analysis.

G1e Explain how the consent arrangements for your study meet the requirements for "appropriate consent" as per the HTA "Code of Practice on consent."

The participants will be healthy competent adults and therefore consent will be in writing.

G2 Does the study involve DNA or RNA analysis of any kind? ○ Yes ◉ No

G3 Are substances or products to be administered? ◉ Yes ○ No

G3a Are substances to be administered which are classified as medicinal products? ◉ Yes ○ No

If yes detail the following: Name of the product, Dose, Number of doses to be administered, How the product will be stored/dispensed and Route of administration

1.5 g of paracetamol (non-soluble form) will be administered into each test drink for six participants, administered on the 4 separate study visits (a total dose of 6 g paracetamol over the course of the study). Paracetamol will be crushed in a pestle and mortar and mixed into the test drink for oral consumption.

Paracetamol is a pharmacological tracer applied for measurement of the rate of gastric emptying. The paracetamol absorption test is well tolerated by patients (Medhus et al., 2001) and has also been used in a previous study by the supervisor (REF BDM/14/15-26).

G3b Are substances to be administered which are not classified as medicinal products? ◉ Yes ○ No

If yes detail the following: Name of the substance(s), Amounts to be administered, Number of times substance will be administered, How the product will be obtained and/or stored, prepared (if appropriate) and dispensed/distributed and Route of administration

The test drink will be administered at each of the four study visits. Each test drink will consist of a “no added sugar” fruit drink (~40ml) with increasing doses of apple extract (Appl'In™, 600 mg, 900 mg, 1200 mg apple polyphenols) added to it and ~200 ml water. The placebo drink will contain no apple extract. Sucrose (~6g) may be added to disguise difference in taste between drinks. Each drink will be delivered in opaque bottles and consumed with a straw within 2 minutes. The "no added sugar" fruit drink will be labelled with GLU-Pomme trial and date opened (to be used within 1 month). Clean utensils and sanitise work surface will be used for preparation of test drinks.
The test meal will be administered on 4 separate study visits. The meal consists of: ~100g soft white bread and ~30g fruit jam. The jam will be refrigerated and labelled with date opened and GLU-Pomme Trial label. This will be used within 6 weeks, once opened. The bread will be bought and frozen on day of purchase to prevent mould growth on less busy weeks. The bread will be defrosted in time for breakfast. The bread packaging will be labelled with GLU-Pomme trial and

date. If frozen, bread will be used within 3 months.Clean utensils and sanitise work surface will be used for preparation of the test meal. Bread will be weighed on a plate and then jam will be spread evenly over the bread. Participants will consume the test meal immediately after finishing the test drink, within 5 minutes.

G4 Does the study involve only moderately invasive/intrusive procedures? (•) Yes ( ) No

G4a Please provide details of moderately invasive/intrusive procedures

Blood sampling will take place in the Metabolic Research Unit. At the screening visit a volume of 16.5 ml/3 tsp will be taken for glucose, liver function, haematology, lipids and plasma fatty acids. For glucose, full blood count (FBC), full lipid count (FLIP) and liver function test (LFT), 1 ml of serum + 1 ml of plasma + 4 ml of blood will be needed to send to Kings College Hospital for analysis where the whole blood will be disposed of within 4 days following FBC analysis. One aliquot of plasma (1ml) + one aliquot of serum (1ml) will be stored in a -40 freezer located in the ILAB, room 4.14, 4th Floor, Diabetes & Nutritional Sciences Division, King's College London, Franklin Wilkins Building, 150 Stamford St, London SE1 9NH. Per study day a total of 142 ml/ 29 tsp of blood will be taken, in total 584.5 ml per study including screening blood sample. All samples collected will be centrifuged to obtain plasma or serum, two aliquots of plasma + two aliquots of serum will be sent to King's College Hospital for analysis of: plasma glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1); and serum insulin and C-peptide, respectively. Two aliquots of plasma and one aliquot of serum will be stored at -40 or -80 respectively, in a locked laboratory (ILAB) for later glucose analysis and conservation as spare. Volunteers will be randomised and assigned a subject code. Following acceptance onto the study, all sample labels, records, databases and reports will be identifiable by study and subject code; no names or abbreviations that could identify the participant will be used. All samples, records, databases and reports will be kept in a safe place and only investigators will have access to them. A trained phlebotomist will be used for venepuncture.

Subjects will be asked to collect 24 h urine samples at each study visit. Standard operating procedures previously used by this research department will be followed (e.g. providing a jug) to allow for ease of collection for participants. Cool bags will be provided for transporting and storing urine collection flasks.

G5 Does the study involve other invasive/intrusive procedures? ( ) Yes (•) No

Tissue Sites

G6 Name the location or sites where the human tissue will be handled, analysed and stored.

Organisation	King's College London
Address	Metabolic Research Unit, Corridor A, 4th Floor
	Franklin Wilkins Building, 150 Stamford St
City	London
County	
Postcode	SE1 9NH
Telephone	N/A
Email	N/A

G7 Give details of the investigators experience or training which qualifies them to conduct the required procedures.

A venepunctured trained person (researcher, staff) will take the blood samples during screening visits.
A trained phlebotomist will be used for venepuncture and cannulation during study days.
Phlebotomists will be employed on a day-by-day basis from a pool of people working at King's College London (based at Waterloo campus and St Thomas' hospital) and from a directory of junior doctors and paramedics compiled from their participation in previous studies.
Prof Kennedy Cruickshank will act as medical supervisor.
The supervisor, Dr Wendy Hall, has wide experience in conducting similar clinical trials.

G8 Provide details of the human tissue licence, where applicable.

A Human Tissue Authority licence is held by Prof Kennedy Cruickshank for the Franklin Wilkins Building.
HTA Licence No 12523.
Room FWB 4.14. Nutritional Sciences.

Section H: Insurance, Risks and Ethical Issues

H1 I confirm that if my study involves any of the Risk Assessment criteria outlined in the information icon guidance, I will ensure that a Risk Assessment form is completed and signed by my Department before data collection commences.

(●) Yes ( ) No ( ) N/A

H2 I confirm that I have read the exclusion criteria for the College's Clinical Trials and Research Projects Involving Human Subjects Insurance Policy, detailed in the guidance icon, and that:

- (●) a) This project meets the inclusion criteria of the policy
- ( ) b) This project falls under the exclusion criteria and I have gained approval from the Finance Department, as instructed in the guidance icon
- ( ) c) This project falls under the exclusion criteria but approval has not been granted by the Finance Department

H3 I confirm that my travel insurance arrangements are as follows:

- ( ) a) I will secure College travel insurance (see guidance icon for further details)
- ( ) b) I will secure personal travel insurance
- (●) c) I do not require travel insurance as I will conduct the research in my country of legal residence
- ( ) d) I will not secure travel insurance

H4 I confirm that if Disclosure & Barring Service clearance is required for my study, this will be obtained prior to the commencement of data collection.

( ) Yes ( ) No (●) N/A

H5 I confirm that the No Fault Compensation Scheme will be offered to all UK based participants.

(●) Yes ( ) No ( ) N/A

H6 Give the details of any other review body approvals or permissions obtained (including gatekeepers, other Ethics Committees, peer review, R&D permission).

None

H7 Give details of any other ethical issues which have not been addressed elsewhere in the application and explain how you will mitigate these risks.

None

Section I: Supporting Documents

I1 Participant Information Sheet

Type	Name	File Name	Date	Version	Size
Participant Information Sheet	Participant information sheet	Participant information sheet.docx	29/09/2016 12:00:00 AM	Final	7.5 MB

Consent form (if applicable)

I2 Consent form

Type	Name	File Name	Date	Version	Size
Consent Form	CONSENT FORM	CONSENT FORM.docx	29/09/2016 12:00:00 AM	Final	175.3 KB

Questionnaires/Surveys (if applicable)

I4 Questionnaires/Surveys

Type	Name	File Name	Date	Version	Size
Questionnaires	Telephone questionnaire	Telephone questionnaire.docx	29/09/2016 12:00:00 AM	Final	182.8 KB
Questionnaires	Womens health questionnaire	Womens health questionnaire.docx	29/09/2016 12:00:00 AM	Final	43.5 KB

Indicative questions, topic guides etc (if applicable)

I5 Indicative questions, topic guides etc

Evidence of any other approvals or permissions (includes gatekeeper, R&D, other ethical approvals) (if applicable)

I6 Evidence of any other approvals or permissions (includes gatekeeper, R&D, other ethical approvals)

Approach letters to gatekeeper organisations (if applicable)

I7 Approach letters to gatekeeper organisations

Advertisement document (email, poster, flyer etc) (if applicable)

I8 Advertisement document (email, poster, flyer etc)

Type	Name	File Name	Date	Version	Size
Advertisement Document	E CIRCULAR email	E CIRCULAR email.docx	29/09/2016 12:00:00 AM	Final	14.2 KB
Advertisement Document	POSTER 1 with strips	POSTER 1 with strips.docx	29/09/2016 12:00:00 AM	Final	1.3 MB
Advertisement Document	POSTER 2	POSTER 2.docx	29/09/2016 12:00:00 AM	Final	1.3 MB
Advertisement Document	POSTER 3	POSTER 3.docx	29/09/2016 12:00:00 AM	Final	1.3 MB
Advertisement Document	POSTER 4 with strips	POSTER 4 with strips.docx	29/09/2016 12:00:00 AM	Final	1.3 MB

Cover Letter (for amendments and modifications) (if applicable)

I9 Cover Letter (for amendments and modifications)

Other (if applicable)

I10 Other

Type	Name	File Name	Date	Version	Size
Other	Food Diary Example	Food Diary Example.jpg	29/09/2016 12:00:00 AM	1	88.5 KB

Researcher/Applicant

J1 Researcher/Applicant Signature

I undertake to abide by accepted ethical principles and appropriate code(s) of practice in carrying out this study. The information supplied above is to the best of my knowledge accurate. I have read the Application Guidelines and clearly understand my obligations and the rights of participants, particularly as regards obtaining valid consent. I understand that I must not commence research with human participants until I have received full approval from the ethics committee.

Please note that in order to authorise your application you must sign off using your KCL email address i.e. joe.bloggs@kcl.ac.uk and your KCL password.

Signed: This form was signed by Emily Prpa (emily.prpa@kcl.ac.uk) on 30/09/2016 13:20

Supervisor authorisation for student projects (including PhD)

J2 Supervisor Signature
I confirm that I have read this application and will be acting as the student researcher's supervisor for this project. The proposal is viable and the student has appropriate skills to undertake the research. Participant selection and recruitment procedures, including the Information Sheet(s) to be provided and the manner of obtaining informed consent, are appropriate and the ethical issues arising from the project have been addressed in the application. I understand that research with human participants must not commence without full approval from the ethics committee.

I understand that by authorising this application I am confirming that the student has read an appropriate professional code of ethical practice and completed a risk assessment form where appropriate.

Note to applicant: In order for your named supervisor to authorise your application they must have an activated REMAS account. If they have not yet activated their account prior to you requesting their authorisation, they will need to do this by logging into the system.

Supervisor Authorisation For Student Projects (Including PhD)

Supervisors should authorise by entering their full KCL email i.e. joe.bloggs@kcl.ac.uk and KCL password

Signed: This form was signed by Wendy Hall (wendy.hall@kcl.ac.uk) on 30/09/2016 13:32