Reading Imperial Surrey Saturated fat Cholesterol Intervention (RISSCI) Study

Blood Cholesterol Response Study – PART 2 ('RISSCI-2') (PART 1: EC/2017/41/FHMS)

Study Protocol - Version 5, 25/11/19

Brief background to the study

The type of fat that we eat plays an important role in the development of heart disease. Diets high in saturated fats (found mostly in animal products such as meat and dairy foods) are related to greater levels of blood cholesterol (also known as Low Density Lipoprotein (LDL) cholesterol) which increase the risk of developing heart disease compared with diets high in unsaturated fats (found in vegetable oils). As a result, reducing intake of saturated fat to lower blood cholesterol levels has been a key dietary guideline to prevent heart disease for over 30 years. Studies have shown that there is a big variability in the lowering of blood cholesterol levels between individuals when changing from a diet high in saturated fats to one high in unsaturated fats. However, very little is known about the factors that determine this variability in blood cholesterol levels between individuals, and the different ways in which people process fat and cholesterol in the body. The main purpose of this study is to determine the factors that explain the differences in blood cholesterol response to different types of dietary fat.

In **PART 1** of this two-part collaborative project between the Universities of Reading and Surrey ('Blood Cholesterol Response Study' RISSCI-1; UEC/2017/41/FHMS): Reading Imperial Surrey Saturated fat Cholesterol Intervention ('RISSCI') Study the main aim was to measure the amount of variation in blood LDL-cholesterol in up to 150 healthy male participants in response to a high saturated fat diet consumed for 4 weeks followed by a low saturated fat diet meeting the level of saturated fat recommended by the government for the prevention of heart disease for 4 weeks. This allowed us to identify two subgroups of men who show either a high or low blood LDL-cholesterol response to a reduction in dietary saturated intake, as defined by the top and bottom 10% of responders/non-responders among the 150 male participants.

In **PART 2** of this project (**RISSCI-2**), we will study the characteristics of these two subgroups of men retained from RISSCI-1 in more depth, to investigate possible underlying causes for the variation in their blood LDL-cholesterol response. A total of 36 men (n=18 responders and n=18 non-responders) from RISSCI-1 will be provided with an opportunity to participate in this second follow-up study. Participants were made aware of the possibility that they might be invited onto this second, follow-up study when giving their informed consent for RISSCI-1, at which stage they either expressed their interest to take part or otherwise. In RISSCI-2, participants will be asked to repeat the same 8-week dietary interventions as in RISSCI-1, but will undergo more detailed measurements at visits 2 and 3 to increase our understanding of why some people's blood LDL-cholesterol is more sensitive to changes in dietary saturated fat intake. Findings from this study will enable us to tailor dietary advice to those who stand to gain the greatest health benefit by reducing their intake of saturated fat, thus overcoming the problems of setting dietary guidelines for whole populations. The University of Reading will submit a separate ethics application to their own committee.

Study Objectives

1. To invite two subgroups of participants from the RISSCI-1 study, whose blood LDL-cholesterol either responds ('Responders') or show little or no response ('Non-

responders') on changing from a high to a low saturated fatty acids (SFA) iso-energetic diet for participation in RISSCI-2, following an initial screening visit to ascertain their ongoing suitability to participate in a dietary intervention study.

2. Confirm informed consent from eligible participants for their participation in the RISSCI-2 Study.
3. Undertake a controlled dietary intervention study to examine the effects of two, 4-week isoenergetic diets that differ in their composition of dietary fats. The first diet ('Diet 1') will contain 18% of its total energy as SFA, while the second diet ('Diet 2') will contain 10% of its total energy as SFA, identical to RISSCI-1 study (UEC/2017/41/FHMS).
4. To undertake the following metabolic assessments immediately after Diets 1 and 2 to ascertain the mechanism underlying the variability in blood LDL-cholesterol changes in response to these diets of differing SFA content in the men classified as responders and non-responders:
    - Lipid profile (Total Cholesterol (TC), LDL-C, HDL-C and triacylglycerol (TAG)) and HDL particle composition
    - Markers of inflammation and endothelial function
    - Fat absorption (by feeding a manufactured fat that has been labelled with safe stable isotope tracer* that can be traced in the body, and can be used to measure how much is excreted in stool)
    - Gut permeability (by measuring the urinary recovery of ingested carbohydrates provided as part of a drink)
    - LDL-receptor expression measured in peripheral blood mononuclear cells (PBMC)
    - Gut microbiota composition measured in stool samples
    - Endogenous cholesterol synthesis as measured by serum phytosterols, a biomarker of this process
    - Serum deconjugated bile acids
    - Short chain fatty acids measured in serum and stool samples
5. Identify 'metabolic fingerprints' (known as metabolomics) in serum and urine that can be used as simple biomarkers of the blood LDL-cholesterol sensitivity to changes in the levels of dietary saturated fat intake.

* Some elements are composed of atoms that are chemically identical but have a different mass due to different numbers of neutrons. These are termed isotopes. In the biological environment, the most abundant isotopes of the major elements, i.e. hydrogen (H), carbon (C), nitrogen (N) and oxygen (O), are 1 H, 12C, 14N and 16O. These are stable, but there are also other stable isotopes of these elements which are much less common (ranging from 0.02 to 1.1%). These are 2 H (deuterium), 13C, 15N, 17O and 18O. In this research project we will be using 13C. When one of these less abundant isotopes is substituted for the common form in a molecule, this is known as 'labelling' and creates a 'tracer'. The original molecule is known as the tracee. The tracer and tracee have the same chemical properties. The amount of tracer relative to the amount of tracee is expressed as the tracer:tracee ratio (TTR). TTR corrected for the background TTR is referred to as enrichment. The different mass of stable isotopes enables them to be detected and accurately measured by mass spectrometry. Measurement of isotopic enrichment with this tracer permits us to trace the fate of molecules of interest (saturated fat in this instance) through the body. Labelling molecules with stable isotopes to create tracers has become a gold-standard method to study the metabolism of lipids and

lipoproteins in humans (http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1000.2052&rep=rep1&type=pdf).

Hypothesis

We predict that men who show a greater change in blood LDL-cholesterol response ('responders') to a diet low in saturated fatty acids (SFA) will show a greater reduction in the absorption of dietary fat in their gut than low responders. We believe that this effect may be, at least partly, explained by changes in the permeability of the gut cell lining which may be increased by eating SFA thus allowing the passage of unwanted bacterial components from the gut lumen into the bloodstream. Responders will also show a greater upregulation of the numbers of receptors on the liver involved in the clearance of blood LDL cholesterol from the circulation (LDL-receptor) when changing from a high to a low SFA diet which will be related to the reduction in circulating LDL-cholesterol levels.

Criteria for the selection of volunteers

Inclusion criteria: Hyper- and hypo-responsive males, defined as the participants (derived from the first stage of the project, RISSCI-1) exhibiting changes in serum LDL-cholesterol in response to a lowering of SFA intake in the top and bottom 10%* of a larger cohort (n=150). The cohort will consist of middle-aged men (30-65 y) with a BMI of 19-32 kg/m^2; fasting serum total cholesterol < 7.5 mmol/l and TAG < 2.3 mmol/l. Selected participants from RISSCI-1 will be re-screened prior to commencing RISSCI-2 to ensure their on-going health and eligibility for the study.

* this percentage may be greater depending on final sample size achieved on RISSCI-1 in order to attain desired number of participants for RISSCI-2

Recruitment onto study: Participants will be invited to take part in RISSCI-2 via email (please find invitation email draft attached to submission).

Exclusion criteria: smokers, medical history of MI or stroke in the past 12 months; diabetes (defined as fasting glucose > 7.0 mmol/l) or other endocrine disorders; medication for hyperlipidaemia (e.g. statins) or prescribed antibiotics within the last three months; drinking in excess of 14 units of alcohol per week, anaemia (<130 g/L haemoglobin), or planning a weight-reducing regime or taking any dietary supplements known to influence lipids/gut microbiota (e.g. plant stanols, fish oil, phytochemicals, natural laxatives, probiotics and prebiotics); unwilling to regularly consume study intervention products (butter/spreads, oils, dairy, snacks); or any other unusual medical history or diet and lifestyle habits or practices that would preclude participants from participating in a dietary intervention metabolic study.

Number of volunteers, study setting and method for taking informed consent: The change in serum LDL-C in the top and bottom 10% of responders and non-responders in our previous study investigating the impact of high and low saturated fat dietary interventions ('DIVAS'), provides a maximum sample size of 30 (15/group) at 90% power in a 2-sided (P<0.05). This concurs with the sample size from the change in the endogenous cholesterol synthesis in healthy men as measured by 13C-labelled acetate (Alphonse and Jones (2015) Lipids, 51, 519-36). With a 20% drop-out rate, we aim to recruit 36 participants for the Metabolic Study across the two sites – University of Surrey & University of Reading. This sample size would also be sufficient to detect significant differences in serum bile acids

between SFA intervention groups (n=16/group, power of 80%) (Eelderink C et al (2015) Food Func 6, 3236-48).

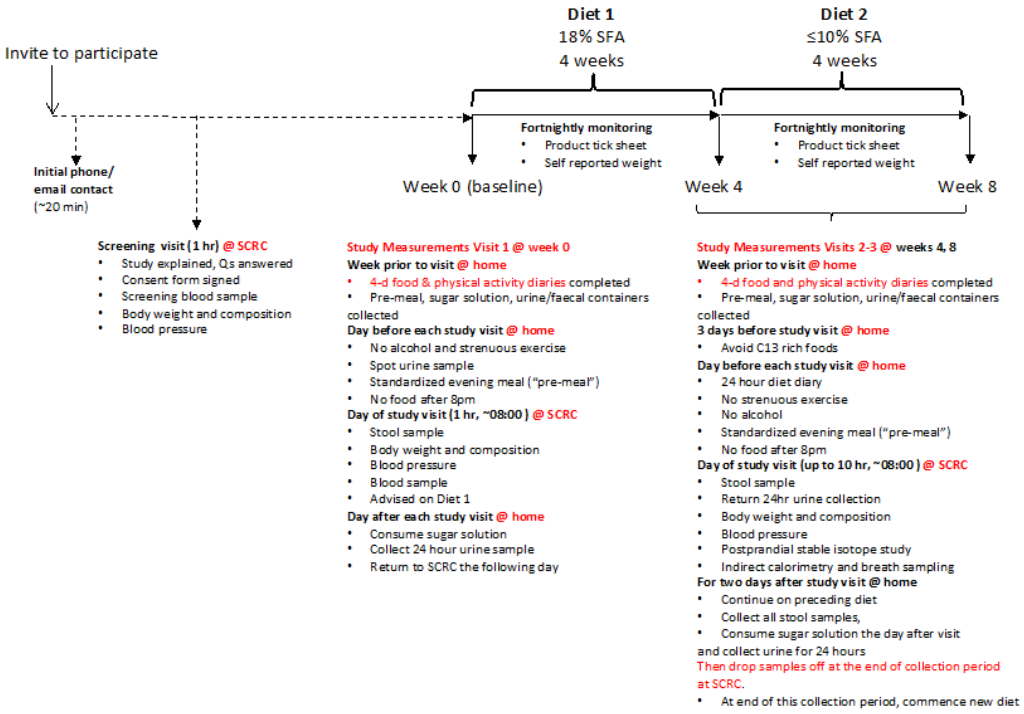
Study setting: All aspects of this study that involve the participation of human volunteers (screening, pre and post dietary intervention visits), will be undertaken within the Surrey Clinical Research Centre at UoS which is fully staffed with study physicians and research nurses.

Method of taking informed consent: Selected/potential volunteers invited to attend a screening visit will be sent the *'Participant Information Sheet'* either by post or e-mail. This will provide a brief description of the study and its purpose, and explain why they have been invited to volunteer. It will also explain the obligations and commitments of the participant throughout the study, and what will happen if they wish to withdraw. It will inform the participant of what happens to the data and samples, of any benefits and disadvantages of taking part in the study, what happens when the study stops, and details about remuneration for their participation in the study. Finally, it will provide the contact details of persons they can contact if they have any questions, complaints or concerns about the study. If the volunteer accepts the invitation to attend the screening visit after reading the *Participant Information Sheet*, we will arrange the visit and go through the document with them and answer any queries. If they are happy to proceed, they will be invited to read and sign a '*Consent Form'*. The volunteer will sign the *Consent Form* in the presence of the study researcher, who will date and counter sign the Volunteer's signature. A copy of the *Consent Form* will then be taken and given to the volunteer.

Screening visit: Participants invited to be screened for RISSCI-2 study will be asked to fast for 12 hours and avoid drinking alcohol the evening before attending their screening visit at the Surrey Clinical Research Centre (SCRC). On the morning of their visit, (this screening visit will be approximately 50 minutes in duration and can be performed any time between 08:00-10:00 am), they will sign the Consent Form and have their body weight measured for calculation of their body mass index (BMI). After measurement of their body composition and blood pressure, they will provide a sample of venous blood (7 ml including deadspace) for the measurement of serum cholesterol, triacylglycerol (TAG) glucose, haemoglobin, full blood count, kidney function tests and liver function tests. If participants are found to be suitable for the study on the basis of these screening results they will be informed; if they are willing to participate their participation will be confirmed. Results of the blood test are important to ensure that the participants are not in ill health and that they still meet the eligibility criteria for the study, which may have changed since they were screened in Part 1. If screening results come back as abnormal, the results will be sent to both the participant and their GP for information. The GP notification letter will state that some of the participant's values fall out of the reference range; it will then be up to the GP to make a clinical decision as to whether any medical intervention is required. Participants will be notified of their eligibility via phone contact or email within a month of their screening appointment.

Experimental design and methods to be used in 2-RISSCI

Study design



The RISSCI-2 study has an identical design and dietary intervention as RISSCI-1 (UEC/2017/41/FHMS); a study diagram is provided above. In brief these studies have a sequential design in which diets will be consumed in the following order: Diet 1 (high SFA, 18% of total energy intake) for 4 weeks, followed by Diet 2 (low SFA, 10% of total energy intake) for a further 4 weeks. Study measurements will be taken during three visits to the SCRC at UoS: at the beginning (week 1) and end of Diet 1 (week 4 +/- 3 d), and at the end of Diet 2 (week 8 +/- 3 d). Visit 1 will be a short 1 hr visit during which basic measurements will be taken (e.g. a fasted blood sample, anthropometrics, blood pressure). Visits 2 and 3 will be longer (approximately 10 hours) fat absorption study visits and take place immediately after each 4-week diet. Up to a week prior to visits 1, 2 and 3, participants will need to come to the SCRC to collect evening meals, sugar solutions and sample pots; these visits should last no more than 20 minutes. They will also need to attend the SCRC for 2 consecutive days after Visits 2 and 3 to return stool samples. This comes up to a total of 11 visits (including a screening visit).

Intervention diets: The intervention diets are identical to those given in RISSCI-1. In brief, to comply with current dietary recommendations, Diets 1 and 2 will contain 35% energy from total fat, with Diets 1 and 2 providing 18% and 10% total energy of SFA, respectively. The SFA-replacement fats will be mixture of poly (PUFA) and mono (MUFA)-unsaturated fatty acids. Diets 1 and 2 will provide 4%/11% and 10%/14% of total energy as PUFA/MUFA respectively. These diets will be consumed within the homes of free-living participants, by the substitution of 40g of habitual fat, with either SFA-rich or MUFA/PUFA-rich cooking oils, spreads, dairy products and snack foods*, while maintaining their habitual diet (consistent intake of protein and carbohydrates, including dietary fibre). Replacement foods for the dietary exchange will be collected from the SCRC, and dietary compliance to the study protocol will be monitored every two weeks using tick sheets completed daily. Since this dietary exchange model will be identical to that used in RISSCI-1, the participants will be familiar with what the dietary intervention entails.

Procedure: After the screening visit, eligible participants will be contacted by telephone or email and given the opportunity to ask any further questions about the study and to arrange the date of the first intervention study visit (referred to as the baseline study visit or week 1). They will also need to arrange a pre-visit to collect from the SCRC, the 4-day diary, a study evening meal and the sample pots (stool/urine).

* commercially available spreads, oils and snacks will be provided by the researchers free of charge. Participants will be advised on the types of other dairy foods (milk, yogurt, cheese) to opt for, but will have to purchase these themselves.

Week prior to Visits 1-3:

< 7 days prior to study visits

Participants will meet with the study investigator (pre-visit) to receive a 4-day food diary, a 7-day physical activity questionnaire, a carbohydrate drink and an evening meal to consume the day before the visit and urine/faecal collection containers. This pre-visit will last about 20 minutes.

Day before visit

Participants will be asked to abstain from consuming alcohol, oily fish (e.g. salmon, mackerel, sardines), vigorous exercise and caffeine (after 4pm), which might otherwise affect study outcomes.

Participants will be asked to provide morning / pre-breakfast mid-stream spot urine sample.

Participants will be asked to consume the standard evening meal (a can of soup) provided by researchers no later than 8pm to ensure a 12 hour fasting interval, with only water permitted during this time frame.

Visit 1 (Baseline)

Participants will attend the SCRC for baseline measurements including body weight, body composition (waist/hip circumferences via tape measure, percentage body fat and muscle mass using bioelectrical impedance), and blood pressure. Stool (from that morning) and urine (from previous day) to be returned together with the completed 4-day diary and physical activity questionnaire. Participants will then provide a venous blood sample (44ml including deadspace, equivalent to 3 tablespoons) for the measurement of blood lipid profile and HDL particle composition, other markers of heart disease (e.g. inflammatory and endothelial function markers) and for the isolation of peripheral blood mononuclear cells to measure LDL-receptors gene expression. Participants will then be offered with a light breakfast and will be supplied with their first set of foods for Diet 1, and instructed on how to incorporate these foods into their habitual diet. They will also be informed that they can collect foods from the SCRC whenever necessary and reminded on how to record their compliance to the study foods. Participants will be also asked to complete a Medication diary throughout the study.

Day after baseline visit – urine collection for gut permeability assessment

Participants will be provided with a carbohydrate drink to consume first thing in the morning containing low levels of two types of natural sugars (lactulose 10g, mannitol 5g). They will then be asked to collect their urine for 24 hours and return the sample the following day.

Visits 2 and 3 (stable isotope studies):

< 7 days prior to visits

Participants will meet with the study investigator for a pre-visit, to receive the same study items as they received before study visit 1. Prior to study visits 2 and 3, participants will also be provided with a carbohydrate drink (containing low levels of lactulose, mannitol; 10 and 5g, respectively) designed to test gut permeability.

3 days prior to study visit

Participants will be asked not to consume foods that are naturally rich in the stable isotope carbon-13 (13-C) (e.g. corn (maize) and corn based products e.g. sweetcorn, cornflakes, corn

tortillas and exotic fruits), in order to reduce variation in background enrichment of natural 13-C when measuring this isotope in breath and stool samples.

1 day prior to study visit

Participants will also be asked not to consume alcohol, oily fish (e.g. salmon, mackerel, sardines), no caffeine (e.g. tea, coffee, energy drinks) after 4 pm, or perform vigorous exercise, all of which might interfere with study measurements.

Participants will be asked to consume their last meal of the day (a provided standardised can of soup) no later than 8pm to ensure a fasting period of 12 hours. Participants will be told that only water can be consumed during this period.

Day of study visit

Participants will be asked to arrive at the SCRC at 08:00 in a fasted state, with their faecal and urine samples. On arrival at the SCRC, anthropometrics will be measured (as described above) following which participants will be asked to rest for a period of 15 minutes; blood pressure will be measured and then indirect calorimetry will be performed over 20 minutes (using a ventilated hood) to non-invasively measure gaseous exchange of O_2 and CO_2 required for the stable isotope calculations. The ventilated hood is a clear plastic dome shaped structure and canopy (to prevent air leaks) which will be placed over the participant's head and used to collect inspired/expired air. A cannula will be inserted into the antecubital vein of the forearm to allow blood samples to be taken during the 9-10 h study day. A baseline 40ml blood sample (44ml including deadspace) will be taken (t -20). Also, a baseline breath sample will be collected by blowing into a glass 'Exetainer' tube via a straw. A further 11ml (including deadspace) fasting blood sample will be taken, and then a test meal with the same macronutrient content as the respective diets will be given at time 0. The test meal will include an emulsified chocolate drink (200 mg [U-13C]-palmitic acid, milk, chocolate flavouring), toast and jam. The meal will be prepared and served by researchers who have passed their relevant Food Hygiene Certificate (Level 2). The meal will be prepared in the SCRC kitchen which is maintained at a high standard of hygiene with fridge checks completed daily by staff. Researchers at the UoR will also have the relevant hygeine training and will prepare food at their site. The identical meal will be provided again 330 min after the start of the first meal. Blood (14x 11ml samples /time point total day volume including deadspace, 209 ml total including baseline) and breath will be sampled regularly at the following time points with T0 denoting the time that the first test meal started to be consumed: T 15, T30, T45, T60, T90, T129, T180, T240, T300, T330 (lunch), T360, T390, T420, T480. These samples will be used to measure serum biochemistry and stable isotope (U-13C) analyses. Participants will provide 10 min indirect calorimetry readings every two hours. At the end of the study visit, the cannula will be removed, and subjects will be provided with a light snack before they leave the SCRC.

Urine sample for gut permeability assessment

The day after the study visits participants will be asked to consume the carbohydrate solution as the first ingested liquid/food in the morning, and will then start collecting their urine in 3 L

urine bottles for the next 24 hours. Participants will also be asked to complete a food diary over this 24 hour period.

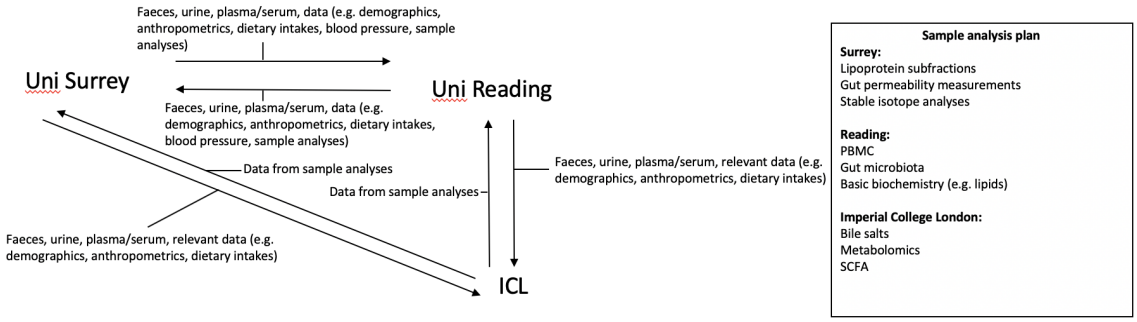
Stool samples

Participants will be asked to complete a 48 h hour faecal collection, where they will collect all stool passed (including whilst at the SCRC) and will need to return to the CRC for two consecutive days after each of these study visits to provide stool samples (from the preceding 24 hours). Containers will be provided for these collections.

At visit 2, participants will be instructed on how to incorporate the foods for Diet 2 into their daily diet for the following 4 weeks, and will be informed that they can collect the foods from the SCRC at any time, as before.

Information on the collection retention, use and disposal of research data and measures in place to ensure confidentiality of personal data: Blood samples will be taken at screening, with blood, urine and faecal samples collected at the baseline, week 4 and week 8 visits. Faecal samples, urine samples and plasma/serum samples may be transported to other research sites (UoR and ICL) for storage/analysis.

Data/sample sharing diagram



Ethical issues: There are no risks or reported side effects associated with the study foods in the intervention diets, as they are all normal components of the diet and commercially available supermarket foods. While we do not anticipate any adverse reactions to the study foods, food sensitivity and/or allergy will be recorded and avoided accordingly. All procedures will be performed by trained researchers; this includes venepuncture which will be performed according to SRC Standing Operating Procedure (SOP Ref. CPA-SOP-2). However, in case of an emergency (e.g. fainting, blood spillage) SCRC staff are trained in life support as appropriate to their roles. In case of emergency SRC SOPs will be followed.

Consideration for working with other institutions (e.g. data protection):

The RISSCI-2 Blood Cholesterol Response Study will be undertaken at two sites, Hugh Sinclair Unit of Human Nutrition at UoR and SCRC at UoS, at which the study protocols are identical (with the exception of a difference in staffing). UoS will obtain a favourable ethical opinion from its own University Research Ethics Committee. Blood serum, urine and stool samples collected at pre and/or post-dietary visits (baseline, weeks 4 and 8), PBMC for gene expression from the pre-diet (baseline) visit only, will be anonymised with a study code to ensure confidentiality of personal data.

Personal data used for research purposes will be used in accordance with the General Data Protection Regulation (GDPR) 2016 and Data Protection Act (DPA) 2018. Participants will be informed about how their research data will be used, handled and stored at the screening visit and in the Participant Information Sheet.

The participant's medical & lifestyle questionnaire and signed consent form (both collected on paper) will be stored in a locked filing cabinet (one at each research site, UoS and UoR) with restricted access to UoS/UoR study staff only (i.e. UoR staff will not have access to UoS consent or medical forms or vice versa). Participant information will be inputted into the study database by the PhD student, PDRA or Research Assistant. Consent forms will also be digitalised and stored with the participant information in a restricted folder, accessible by only the UoS PI, PDRA and PhD student, within a collaborative fileshare/folder for the human study on the UoS University network (UoR will not have access to UoSs consent forms and vice versa). Between UoR, UoS and ICL (who are analysing some of the biological samples), coded/pseudo-anonymised data such as study outcomes (biochemistry, anthropometrics, dietary intakes etc) will be shared securely between sites using a Teams/SharePoint collaborative drive which offers a mitigation against security risks and is in accordance with the safeguards for research data processing, as documented within the GDPR (Art 89). Access to this drive will be limited and restricted, permissions will be kept up to date, and information is reviewed and deleted when no longer required. Access to Teams is will be via log in and strong password only. UoR and UoS will act as Data controllers for their own study sites, and personal / identifiable information from one site will not be made available to the other sites.

Participants will be assigned a unique study code at screening which will be recorded in a restricted folder on the collaborative fileshare against the participant personal information. For the duration of the study, participants will be identified by the unique study code only. Results of laboratory tests and measurements performed in the clinical unit will be recorded in an Excel spreadsheet against the participant unique study code and saved to an area of the collaborative fileshare accessible to all members of the research team. Publications and reports will not include individual identifiable data.

Samples will be stored at -20-80°C until transportation at this temperature by accredited courier to UoR / Imperial for analysis and processing (see diagram for flow of samples). Samples will be placed in sealed containers, double contained and labelled with its content, hazard information, contact details, project details and ethics number as per HTA requirements. Samples will be stored in locked, alarmed freezers and HTA logs will be maintained. All of the study intervention visit samples will be processed at each of the study sites according to the agreed study protocols, and stored at the appropriate temperature until sample transfer and analysis on completion of the study. A Material Transfer Agreement (MTA) is in place between the UoR and UoS, in accordance with the Human Tissue Act 2004. All data produced from these samples will be stored on a password-protected central data base held on the secure University servers at UoR/UoS, and handled in accordance with GDPR. Data between sites (UoR

All project data related to the administration of the project, (e.g. consent form) will be held for at least 6 years and all research data (e.g. study outcomes, samples provided) for at least 10 years in accordance with University policy. This is because samples collected for future analysis may be studied as part of a follow-on project or the study results may be used for statistical purposes relating to the study intervention (dietary fat).

In a collaborative research project, where personal (including pseudonymised) data is being shared between Universities that both have research purposes that may be both joint and independent, both parties are acting as independent Data Controllers, and that data is being shared between those parties.

Data policies for other sites

Reading and Imperial: Data and samples will be kept beyond the duration of the study (for a maximum period of 10 years) for scientific research purposes, as samples collected for future analysis may be studied as part of a follow-on project or the study results may be used for statistical purposes relating to the study intervention (dietary fat).

Study evaluation and statistical analysis

Dietary compliance to the study protocol will be evaluated on a fortnightly basis in person or over the telephone by using product tick-sheets completed by the volunteers daily and monitoring of body weight. Volunteers will be asked to self-report their body weight, measured on home scales on day one and half way through of each four-week dietary intervention period. A change in body weight in excess of 1 kg will be reviewed by the study researchers and further dietary advice will be given to the volunteers as required. Data from the metabolic study will be checked for normality and analysed using a mixed factor ANOVA with repeated measures to determine the impact of the dietary interventions on our outcome measures. The study will be registered as a clinical trial on the ISRCTN trial registration website prior to the recruitment of the volunteers for the RISSCI-2 Blood Cholesterol Response Study.

References

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