Reading Imperial* Surrey Saturated fat Cholesterol Intervention (RISSCI) Study

RISSCI-1 Blood Cholesterol Response Study

Study Protocol - Version 8, 16/7/18

Brief background to the study

Raised blood cholesterol (also referred to as blood LDL-cholesterol) is a major risk factor for developing heart disease. Dietary saturated fat is recognised as the main dietary component responsible for raising blood LDL-cholesterol, and reducing its intake has been the mainstay of dietary guidelines for the prevention of heart disease for over 30 years. However, there is very little evidence for a direct link between the intake of saturated fat and risk of dying from heart disease. One explanation for this, is that the link between saturated fat intake and heart disease is not a direct one, but relies heavily on the ability of saturated fat to raise blood LDL-cholesterol levels. This LDL cholesterol-raising effect of saturated fat is complex, and highly variable between individuals because of differences in the metabolism of dietary fat and cholesterol between people. While these differences in metabolism make it difficult to study how dietary saturated fat influences LDL-cholesterol in large numbers of people, they can be measured in the laboratory and used as biological markers to distinguish between people who respond well from those who will respond less well to moderate-fat diets which are lower in saturated fat. The main aim of this study is to measure the amount of variation in blood LDL-cholesterol in 150 healthy volunteers (75 at the University of Surrey and 75 at the University of Reading) in response to lowering the amount of saturated fat in their diet to the level recommended by the government for the prevention of heart disease. This collaborative project between the Universities of Reading and Surrey (**'RISSCI-1 Blood Cholesterol Response Study')**, will allow us to investigate possible underlying causes for this variation in the blood cholesterol response in the whole group, and to identify two subgroups of men who show either a high or low LDL-cholesterol response to a reduction in dietary saturated intake. These men, will be provided with an opportunity to participate in a similar follow-up study ('RISSCI 2') that will require 36 men, 18 at the University of Surrey and18 at the University of Reading). In this follow-up study, the participants will be asked to repeat a similar study protocol as for RISSCI-1, and undergo more detailed measurements to determine how saturated fat is metabolised in the body (see section on 'Retention of Participants'). The results of the RISSCI-1 study will be used to overcome the problems of setting dietary guidelines for whole populations, which are frequently inappropriate for some subgroups of people within that population. This will be achieved by the tailoring of dietary advice to those at higher risk of developing heart disease, and who stand to gain the greatest benefit to their health. **Note: Imperial College London is not involved in RISSCI-1.*

Study Objectives

1. To recruit 75 middle-aged men from the local population in Surrey who meet our inclusion and exclusion criteria, using Surrey Clinical Research Centre's (SCRC) data base of human volunteers.

2. Invite eligible volunteers to a screening visit to ascertain their suitability to participate in a dietary intervention study (NB: >75 prospective volunteers will need to be screened until the target sample size of 75 is attained).

3. Obtain informed consent from eligible volunteers for their participation in the RISSCI-1 Blood Cholesterol Response Study, and permission to contact them regarding participation in the follow-up study (see point 6 below).

4. Undertake a dietary intervention study to examine the effects of two, 4 week diets that differ in their composition of fatty acids. The first diet **('Diet 1')** will contain 18% of its total energy as saturated fatty acids (SFA), while the second diet **('Diet 2')** will contain 10% of its total energy as SFA. Blood and stool samples taken at the beginning (week 0) and end of Diet 1 (week 4), and end of Diet 2 (week 8), will be analysed to measure blood LDL-cholesterol, LDL-receptor gene expression (from isolated peripheral blood mononuclear cells) and other relevant blood and faecal* metabolites (* optional sample). The white blood cell buffy coat will also be isolated from the blood samples collected at the baseline visit to enable genotyping of relevant genes involved in the absorption and metabolism of dietary fat.

5. To examine the data for evidence of associations between the changes in blood LDL-cholesterol, and the physical and biochemical characteristics of the participants as possible determinants of the variation in serum cholesterol response across the whole cohort (n=150). This will include measurement of a common genetic polymorphism in *APOLIPOPROTEIN E*, as an established determinant of variation in blood cholesterol in response to dietary SFA.

6. To identify two subgroups of individuals whose blood LDL-cholesterol either responds ('Responders') or show little or no response ('Non-responders') on changing from Diet 1 to Diet 2, for participation in the follow-up study (RISSCI-2), which will be conducted at the Universities of Surrey (n=18), Reading (n=18) and Imperial College London. A separate ethics application will be submitted for RISSCI-2.

Hypothesis

In accordance with the variation in blood LDL-cholesterol response, that we and many others have reported previously following substitution of dietary saturated with unsaturated fats (*RISCK*[2], *DIVAS*[1]), we hypothesise that consuming Diet 1 (a high saturated fat diet) for 4 weeks followed by diet 2 (a low saturated fat/high unsaturated fat diet) for a further 4 weeks, will: **1)** produce a spread of blood LDL-cholesterol responses that enables us to study associations between the participants' baseline characteristics as possible determinants of the observed variation in blood LDL-cholesterol response, and **2)** identify two distinct subgroups of individuals who either respond or show little or no response in their blood LDL-cholesterol. These distinct groups will be defined by the top and bottom 10% of change in the concentration of blood LDL-cholesterol in the subject group of 150.

Criteria for the selection of participants

Inclusion criteria: To remove the confounding effects of significant variance in blood cholesterol metabolism introduced by gender effects in a small cohort, the study will be restricted to 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. No ethnicity restrictions will be imposed

however participants will be required to complete an ethnicity questionnaire, as it is known that this can influence lipid metabolism.

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 on 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 volunteers from participating in a dietary intervention and metabolic study.

Number of participants, recruitment methods and method for taking informed consent: The total sample size for the RISSCI-1 Blood Cholesterol Response Study is 150 men; 75 men will be recruited at both the Universities of Surrey (UoS) and Reading (UoR), the latter of which will conduct an identical study to that performed at UoS. UoR will seek a favourable ethical opinion from its own University Research Ethics Committee. The sample size of 150 was chosen to provide the required number of responders and non-responders on changing from Diet 1 to Diet 2, to provide significant differences in our metabolic outcome variables between each of these subgroups in the follow-up study (RISSCI-2). On the basis of the highest and lowest 10% of change in blood LDL-cholesterol in a previous human intervention trial 'DIVAS'[1], the screening of 150 men will provide subgroups of 15 hyper-responders and 15 hypo-responders with 20% drop-out rate, requiring 36 men in total), which corresponds to the predicted differences in outcome variables in our RISSCI-1 follow-up study. From our previous trials, we predict that the distribution of blood LDL-cholesterol response will be similar at UoS and UoR, and thus provide equal numbers of high and low LDL-cholesterol responders at each site.

Recruitment methods: All aspects of this study that involve the participation of human volunteers (screening, pre and post dietary intervention visits), will be undertaken within the SCRC at UoS. This is a world class research facility with MHRA Phase I Standard and Supplementary Accreditation to run clinical trials. It is fully staffed with study physicians, research nurses, and recruitment managers, and holds a large database of human volunteers who have registered as being willing to participate in clinical trials. Many of these volunteers have already been participants on previous studies, so are familiar with the SCRC, and taking part in intervention studies. After completion of the Medical & Lifestyle questionnaire and/or a brief telephone interview, volunteers who meet the inclusion and exclusion criteria for the study will be invited to attend the screening visit. Recruitment materials used for the purpose of recruitment from the SCRC database will be based on a standard template used by the unit. Separate recruitment material (a shorter email & alternative poster) will be used by members of the RISSCI research team for the purpose of advertising to staff and the wider community. Posters will be placed on designated poster boards, and HoD approval will be sought prior to email distribution.

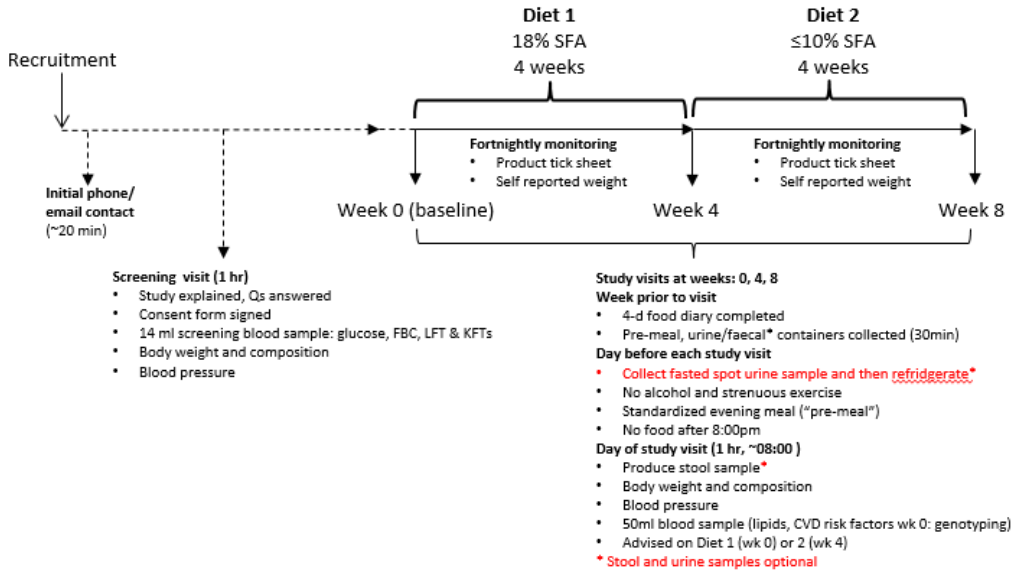
Method of taking informed consent: Potential volunteers invited to attend a screening visit will be sent the *'Participant Information Sheet'* either by post or e-mail. This will explain the purpose of, and provide a brief description of the study, and explain why they have been

invited to volunteer. It will also explain what will happen if they choose to withdraw, and what the obligations and commitments of the participant are throughout the study. 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 over 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 an independent witness, 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.

Retention of participants for the follow-up study (RISSCI-2): The *Consent Form* contains a question that asks participants if they would be willing to be considered to take-part in a similar follow-up study. The *Participant Information Sheet* will explain that in the RISSCI-2 study volunteers will be asked to repeat exactly the same two diets as in the RISSCI-1 study (Diet 1 followed by Diet 2) each for 4 weeks, and attend two short (~0.5-1 h) and two longer visits (~ 9 h) at the end of each diet, for more detailed measurements to determine how saturated fat is metabolised in the body. The longer visits will involve the volunteers being cannulated to obtain small, timed blood samples (~1ml) over 8 h, and the collection of urine and stool samples as in the RISSCI-1 study. It will be made clear to participants that they are under no obligation to take-part in this follow-up study, and that the stool and urine samples on this second study would not be optional.

Experimental design and methods to be used in RISSCI-1

RISSCI-1 Blood Cholesterol Response Study Design



Screening visit: Volunteers who meet the study inclusion and exclusion criteria (as determined by the Medical & Lifestyle questionnaire sent by e-mail or performed during the telephone interview), and have accepted the invitation to attend a screening visit, will be asked to fast for 12 hours and avoid drinking alcohol the evening before attending their visit to the SCRC. On the morning of their visit, *(all appointments for visits to the SCRC will be made between 7.00-10.00am and be approximately 1 hour in duration)*, they will sign the *Consent Form* (as mentioned above), and have their body weight and height measured for their BMI, and following the measurement of their blood pressure, provide a sample of venous blood (14 ml) for the measurement of serum cholesterol, triacylglycerol (TAG) glucose, kidney and liver function tests (9 ml) and determination of the haemoglobin level and full blood count (5 ml). Suitable individuals will be invited to participate in the RISSCI-1 Blood Cholesterol Response Study. Participants' GPs will be notified regarding research participation and will be provided a copy of the screening blood test results;; it will therefore be up to the GP's clinical judgement as to whether any action is required. The study has a sequential design in which diets will be consumed in the following order: Diet 1 for 4 weeks followed by Diet 2 for a further 4 weeks. This sequential intervention study design has been adopted by several studies, including our own Wellcome Trust funded 'SATgene' study (n=90)[3], and FSA-sponsored 'FLAVURS' fruit and vegetable intervention (n=180)[4]. A double blind, placebo-controlled cross-over study cannot be adopted in this case, primarily because of the variation introduced by the manipulation of dietary fat, and returning subjects to their habitual diet during the wash-out period. Visits to the SCRC at UoS, will be made at the beginning (week 0) and end of Diet 1 (week 4) and Diet 2 (week 8).

Intervention diets: 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. 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 and snack foods, while maintaining their habitual diet (consistent intake of protein and carbohydrates, including dietary fibre). This will be achieved using a dietary exchange model developed for the '*DIVAS*' study[1], which replaced 9% total energy as SFA with MUFA/PUFA. This strategy has been successful in implementing large, complex dietary interventions at UoR and UoS (eg. *DIVAS*[1], *RISCK*[2], *SATgene*[3], *OPTILIP*[5]). 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, either in person or over the telephone by the post-doctoral research dietitian (Dr Rona Antoni).

Procedure: After the screening visit, eligible volunteers will be asked to complete a 4-day weighed dietary intake. In this pre-study period, participants will be contacted by telephone and given the opportunity to ask any further questions about the study and to arrange the date of the first intervention visit (referred to as the baseline visit or week 0). They will also need to collect from the SCRC, the study meal to consume the evening before the baseline visit, containers for the collection of the urine* and faecal* samples (*optional) and be given instructions on how to collect these samples. On the day prior to each study visit, they will be instructed to avoid alcohol, vigorous exercise and to consume the standard meal provided by the researchers before fasting overnight for 12 h (only drinking water during this time). Participants will be asked to bring along a fasted urine spot sample (optional, collection the morning before study day, stored in cool bag and ice packs) and a stool sample (optional,

collected from the morning of the study day) to the study visit and will have their body weight, body composition (waist/hip circumferences via tape measure, percentage body fat and muscle mass using bioelectrical impedance), and blood pressure measured. Participants will also be asked to provide a brief 24-hour dietary recall, and to complete a physical activity questionnaire (Appendix XIV). Participants will also complete an ethnicity questionnaire on this initial baseline visit (Appendix XV). Participants will then provide a venous blood sample (50ml, equivalent to 3 tablespoons) for the measurement of blood total cholesterol, triacylglycerol, HDL-C (concentration/composition) and LDL-cholesterol, other markers of cardiovascular disease (e.g. inflammatory markers), for the isolation of white blood cell (WBC) buffy coat for the recovery of DNA for genotyping, and for the isolation peripheral blood mononuclear cells to measure LDL-receptors gene expression. Participants will then be supplied with their first set of foods for Diet 1, and instructed on how to incorporate these foods into their own diet. They will also be informed that they can collect foods from the SCRC when necessary and how to record their compliance to the study foods. After 4 weeks on this diet, participants will complete a 4-day weighed dietary intake before attending the SCRC for their second study visit, the procedure for which will be identical to the 'baseline' visit. Individuals will then be instructed on how to include the foods for Diet 2 in their daily diet for the next 4 weeks, and will be informed that they can collect the foods from the SCRC as before. They will also be provided with the standard meal and collection pots for the optional urine sample and stool sample to collect prior to the third study visit (week 8). At this final visit, the procedure will be identical to the study visit performed at weeks 0 and 4 of the study. Individuals identified within the top and bottom 10% of change in fasting blood (LDL) cholesterol (week 8 minus week 4), will then be invited to participate in the RISSCI-2 follow-up study. Participants will be asked to complete an expenses form to enable them to be compensated for their travel, time and inconvenience (£120, plus an additional £20 for stool samples and £10 for urine samples; max total £150), following full completion of the RISSCI-1 Baseline Cholesterol Response Study. No payment will be provided to participants who withdraw from the study.

It was decided to make the urine and faecal samples optional in order to minimise participant burden.

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 and faecal samples collected at the baseline, week 4 and week 8 visits. Samples will be transported and analysed at the UoR.

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 in the supermarket. 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 a Standing Operating Procedure (SOP Ref. CPA-SOP-2). However, in case of an emergency (e.g. fainting, blood spillage) SCRC staff are trained in all relevant SOPs that describe in detail the actions that need to be taken by the researchers. Also, first aid is available, and physicians on-call in the SCRC when blood is being taken. In case of emergency, the research scientists will notify the SCRC staff who will provide first aid and, if necessary, contact University security to call the emergency services (999).

Consideration for working with other institutions (e.g. data protection): The RISSCI-1 Blood Cholesterol Response Study will be undertaken at two sites, SCRC at UoS and Hugh Sinclair Unit of Human Nutrition at UoR, at which the study protocols are identical, with the exception of a difference in recruitment strategies and staffing. UoR will obtain a favourable ethical opinion from its own University Research Ethics Committee. Blood serum and stool samples collected at pre and post-dietary visits (baseline, weeks 4 and 8), PBMC and WBC buffy coats for isolation of DNA from the pre-diet (baseline) visit only, will be anonymised with a study code to ensure confidentiality of personal data. The names and codes of participants at each research site will be stored securely on the University server at both the UoR and UoS, access to which will be password protected and only accessible at each site by the respective Principle Investigators (Professors Griffin & Lovegrove) and post-doctoral research assistants (Drs Antoni & Koutsos). After collection of the screening samples at UoS, samples will be stored briefly at room temperature or 4^{o}C (<30 min) prior to centrifugation (1700 g x 15 min) and aliquotting of samples into appropriately labelled tubes. Samples will be stored at -20°C until transportation at this temperature by courier to UoR for analysis. 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) will be in place for the transfer of samples between UoS and UoR, 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 UoS/UoR, and handled in accordance with the Data Protections Act 1998. After analysis, all samples will be stored frozen (-20^{o}C or -80 ^{o}C) for a maximum period of 36 months, and then destroyed.

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 and monitoring of body weight. Participants will be asked to self-report their weight, measured on home scales on day one and half way through of each four-week dietary intervention period. Any changes in body weight of at least 1 kg will be reviewed by the study researchers and further dietary advice will be given to the participants 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 'Clinical Trials.gov' prior to the recruitment of the participants for the RISSCI-1 Blood Cholesterol Response Study.

References

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