PROTOCOL

The chemical structure of a lipid determines its effect on blood lipid profile and appetite regulation

Main Sponsor Imperial College London
Funders Malaysian Palm Oil Board
REC Ref:

Protocol authorised by

Professor Gary Frost,
Professor of Nutrition and Dietetics,
Department of Investigative Medicine,
Hammersmith Hospital Campus, Imperial College,
6th Floor, Commonwealth Building,
Du Cane Road, London,
W12 0NN

Dr. Edward Chambers
Research Associate
Department of Investigative Medicine,
Hammersmith Hospital Campus, Imperial College,
6th Floor, Commonwealth Building,
Du Cane Road, London,
W12 0NN

Milena Rundle
Research Postgraduate
Department of Investigative Medicine,
Hammersmith Hospital Campus, Imperial College,
6th Floor, Commonwealth Building,
Du Cane Road, London,
W12 0NN

Short title

Effect of palm olein intake on lipid profile and appetite regulation

Long title

The chemical structure of a lipid determines its effect on blood lipid profile and appetite regulation

Study Management Group

Chief Investigator	Professor Gary Frost
Co-investigators	Milena Rundle , Dr Edward Chambers
Study Management	Milena Rundle , Professor Gary Frost

Sponsor

Imperial College is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance Manager at:

Joint Research Compliance Office
Imperial College London and Imperial College Healthcare NHS Trust
Room 215, Level 2, Medical School Building
Norfolk Place
London
W2 1PG
http://www3.imperial.ac.uk/clinicalresearchgovernanceoffice

PROBLEMS RELATED TO THIS TRIAL SHOULD BE REFERRED TO PROFESSOR GARY FROST
g.frost@imperial.ac.uk

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1. INTRODUCTION

Obesity is now considered a major public health crisis worldwide with a quarter of adults and a third of children in the UK being obese with its direct cost to NHS rising from £479.3 million in 1998 to £4.2 billion in 2007 (1). Obesity has been linked to higher risks of cardiovascular disease, diabetes and many types of cancer. This multifactorial disease is closely connected to a sedentary lifestyle and a rise in the intake of energy dense foods reported in the last 50 years with fats accounting for its large portion. Over the past decade there has been a substantial rise in knowledge on different effects of various types of fats on health and well-being. WHO (2) recognised that categorising fats by simply the number of their double bonds: saturated, monounsaturated and polyunsaturated has many limitations and individual fatty acids within these groups have distinct biological properties and different effect on health.

Most recent NDNS survey (2011) showed that intake of saturated fat was over the recommended 11% for adults in the UK (12.6 %). Saturated fat has been previously linked to a rise in blood cholesterol and visceral adipose tissue compared to polyunsaturated fat (3). However it was reported by Reisler (4) that cocoa butter that is mostly saturated fat (67% saturated) has a neutral effect on plasma lipids due to its lower absorbability. Tomarelli (5) have previously shown that the positional distribution of fatty acids on the triglyceride backbone determines the physical characteristics of fat, which in turn impacts their digestibility, absorption and fat distribution on tissue. This may explain the neutral effects of some fats.

Palm oil is an edible vegetable oil extracted from the fruit of the palm oil tree. Palm oil extracted from the pulp (palm olein) is used in variety of food products like biscuits, margarine and breakfast cereals. Palm kernel oil extracted from the seeds is used in soaps and cosmetics. Consumption of the oil rose 10-fold since the 1980s and now stands at around 50 million tonnes per year. It accounts for 65% of all vegetable oils used globally and it is found in 50% of products on supermarket shelves. Despite the controversy surrounding the palm oil industry, it is still the most sustainable of all vegetable oils, with up to ten times more oil produced per hectare than other leading oilseed crops (6). With more research in the last two decades revealing detrimental effect of trans fats on health, most food companies have now removed them from their products and started using palm oil instead. Palm oil, being mostly saturated vegetable fat, has an opinion of negatively impacting cholesterol levels and contributing to increased adiposity that has been linked to type 2 diabetes. However, more research is needed to further investigate these issues (3).

Several clinical studies have investigated the effect of palm oil on blood lipids and lipoproteins when compared to other saturated fats as well as mono- and polyunsaturated. Ng et al (7) compared diets enriched in coconut oil with palm olein, in both study groups coconut oil intake resulted in increased TC and LDL compared with palm olein. In another study Ng et al (8) has shown that there was no difference in serum lipids when compared to olive oil leading to assumption that palm oil would be a safe substitute in humans with healthy cholesterol levels; these finding were further backed up by Choudri et al (9).

The report of WHO, an Expert Consultation on Fats and Fatty Acids in Human Nutrition (2) has noted that *"there is possible evidence to suggest that the total cholesterol and low-density-lipoprotein cholesterol raising effects of saturated palmitic acid are lower for vegetable than animal sources because it is present predominantly in the sn-1 and sn-3 position of the triglyceride backbone as opposed to animal fats such as lard"*. As such, palm oil, like other vegetable oils, oleic acid (a monounsaturated fatty acid) is predominantly situated at the sn-2 position, while in animals fats it is predominantly palmitic acid (chain lengths of C16:0) that is situated there. Even though palm olein and lard have similar proportions of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA), they differ significantly in their positional distribution on the tricylglycerol (TAG) molecule. Palm olein TAG contains only 7-11% palmitic acid at the sn-2 position while about 87% is unsaturated fatty acids (oleic acid and linoleic acid). Lard has the highest amount of palmitic acid in the sn-2 position at

70%. This helps to explain why even ~40% saturated palm olein behaves similarly as monounsaturated fat in affecting cholesterol level.

The most recent study reported by Gouk et al (10) on the effects of saturated fatty acid (SFA) situated at sn-1,3 positions on fat deposition in mice. The findings were in line with the sn-2 hypothesis where i) Long chain SFA at the sn-1,3 positions of TAG reduce body fat deposition and ii) stearic acid at the sn-1,3 positions of TAG reduce more body fat deposition compared with that of palmitic and oleic acids. However, these observations need to be confirmed in humans.

Peptide YY (PYY) and glucogon like peptide-1 (GLP-1) are anorectic gut hormones involved in appetite regulation. Lipids are known to stimulate the release of these hormones through binding to receptors in the small intestine. Little research exists as to whether fatty acid composition influences PYY or GLP-1 release and thus influences appetite regulation. Current evidence suggests that PUFA and SFA may exert a greater PYY response than MUFA, however the mechanism for this is unknown (12). Therefore this study will also consider whether the positional distribution of palmitic acid on the TAG molecule influences the production of PYY or GLP-1.

Hypothesis:
The position of saturated fatty acids in palm oil will have no effect on appetite regulation and blood lipid.

Aims of the study:
i) saturated palmitic acid at the sn-1,3 positions of the fat molecules have less contribution to obesity compared with sn-1,3 polyunsaturated fatty acids rich fat.
ii) unsaturated fatty acids present at the sn-2 position of the fat molecules do not increase blood cholesterol levels

2. Study Design: Acute effect of saturated fatty acids on blood lipid profile and appetite regulation

Study Methodology: A randomized, single blind, cross over study

Participants: 12 healthy males and females aged 18 to 60 years with body mass index (BMI) of 18.5-29.9kg/m^2

Support of number of volunteers: This is a pilot study; therefore a power calculation is not possible. However, in a recent study by Mandoe (2015), 12 volunteers were recruited for 4 separate study visits to detect acute effect of different fatty acids on postprandial lipid profile.

Recruitment: Existing volunteer databases will be searched, such as the Healthy Volunteer Panel at the NIHR Wellcome Trust Clinical Research Facility. After a short telephone interview to explain the study, participants will be sent the participant information sheet and be given at least 24 hours to consider participating in the study. After that arrangements will be made for a more in depth explanation and discussion of the study with one of the research team. If participants wish to proceed and give signed consent, a more formal screening interview regarding medical and family history and assessment of eligibility will be conducted.

Visit 1- Acclimatisation study

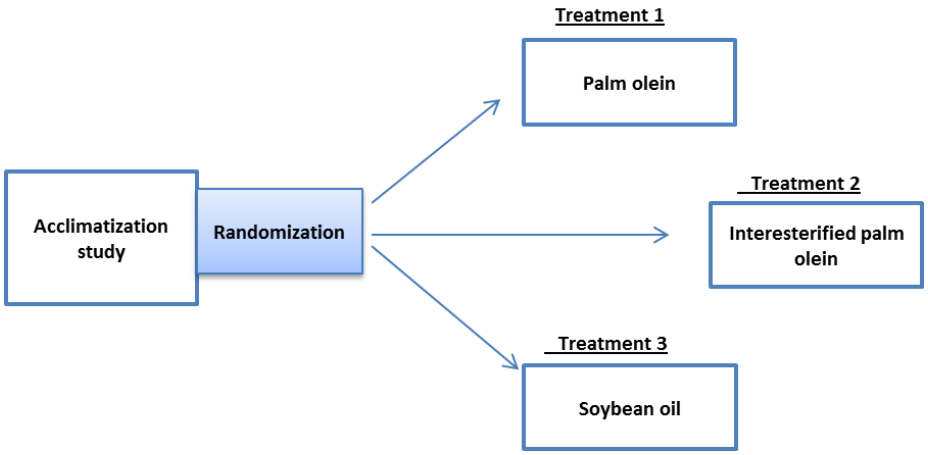
The day prior to the study visit, participants will be requested to refrain from strenuous exercise and alcohol. Participants will then be requested to fast overnight (they are allowed to drink water) and will come to the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital the following morning. Anthropometric measurements of height, weight and body fat percentage using bioelectrical impedance will be collected. Fasting blood samples will be taken through an intravenous peripheral cannula at -15 and -5 min. Participants will then receive a standardised study drink (milkshake-like drink consisting of whey protein, glucose powder, palm olein and water) to consume over 5 minutes at 0 min. Paracetamol (1g) dissolved in water will be given with the drink to test gastric emptying. This method has been validated by Medhus et al. (13). Postprandial blood samples will be taken for the next 8 hours at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480 min.. At every time point during the acclimatization visit, blood will be drawn into the cannula (no blood will be drawn into syringe and discarded) then flushed back into the vein using a prepacked saline posiflush (BD). This was a patient can experience the sensation of blood being drawn and cannula being flushed. The importance of undertaking an acclimatization visit in appetite studies has been observed in our research group where there was a significant difference in response to a study meal between visit 1 and visit 2. It was also documents in literature, Chandarana et at 2009 (Gastroenterology 2009; 136:2115–2126), observed higher cortisol levels (stress hormone) during first study visit which correlated positively with levels of peptide YY (PYY). They recommend that for appetite specific study, methodology of acclimatization visit is in place to minimize the bias and differences between treatment weeks.

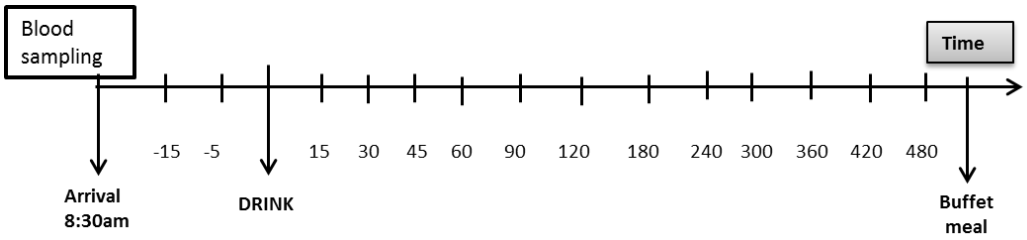
Appetite and satiety will be assessed by a visual analogue scale, a small questionnaire to assess level of hunger at every timepoint . After 480 minutes a buffet meal will be served as another measure of appetite (leftover food will be weighed out and compared between weeks).

Visit 2-4 – Supplementation visit

Following visit 1, participants will be randomized into different orders of treatment (see chart below). They will attend 3 study visits separated by a week. One of the three test fats used in long term intervention: palm olein, interesterified palm olein and soybean oil will be incorporated into the study drink at each visit. Study day protocol will be the same as visit 1 with exception of amount of blood taken as this will increase to 10 ml at each time point to allow the analysis of hormones and biomarkers. A visual analogue scale will be collected at every timepoint to assess hunger.

Blood will be analysed for serum insulin and plasma glucose, lipids, as well as appetite hormones PYY and GLP-1, markers of obesity and inflammation.





4. PARTICIPANT ENTRY

PRE-RANDOMISATION EVALUATIONS

Screening (1h)

Participants will be asked to attend the NIHR/Wellcome Imperial CRF having fasted for 12 hours. They will be interviewed by one of the researchers and asked questions about past medical history and current health. A venous blood sample will be taken to assess full blood count, urea, electrolytes, liver function, lipid profile, glucose and HbA1c. Height, body weight, blood pressure, waist circumference and body fat will be measured. Electrocardiogram will be performed to assess cardiac function (ECG). Women of childbearing age will be asked to provide a urine sample for pregnancy test. Screening should take no more than 1 hour. A standard letter will be send to the GP informing about volunteer participation in the study. If any of the health screening checks or blood tests reveals any medical problems, GP will be informed so that they can coordinate further care, arrange any further tests, and refer volunteers on if necessary.

INCLUSION CRITERIA

- 12 Healthy and overweight male and female volunteers of all ethnicities (body mass index (BMI) of 18.5-29.9 kg/m^2)
- Age between 18-60 years (inclusive)

EXCLUSION CRITERIA

- Abnormal liver function test (elevated transaminases- ALT, AST)/ abnormal kidney function test (elevated plasma creatinine)
- History of type 2 diabetes mellitus, cancer, stomach ulcers, drug abuse or alcoholism, gastrointestinal disorders like Crohn's disease
- Smokers
- On lipid/blood pressure- lowering medication/supplements
- Blood pressure>140/90 mm Hg
- Fasting TC > 6.2 mmol/L
- Fasting TAG > 2.0 mmol/L
- Subject must not be allergic to intervention
- Pregnancy and breastfeeding (pregnancy test will be undertaken at the screening visit)
- Subjects taking nutritional supplements or on any weight-loss programs
- Subjects who gained or lost ≥ 3kg weight in the past three months
- Subjects with history of hypo- and hyperthyroidism
- Subjects who are anaemic and those who donated blood within three months of the study

Subjects with the above conditions would have an altered pattern of hormones and inflammatory molecules because of their disease process and would therefore give us confounding or misleading results.

WITHDRAWAL CRITERIA

The safety of the study participants takes priority. Any significant adverse event (as assessed by the researchers) will halt the study and the ethics committee and sponsor will be informed as per standard protocol. All adverse events will be recorded and investigators will review each adverse event as it arises. In addition, participants will be free to withdraw at any time and are not required to give a reason.

5. Adverse Events

Adverse Event (AE): Any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): Any untoward and unexpected medical occurrence that:

- results in death
- is life- threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- requires hospitalisation, or prolongation of existing inpatients' hospitalisation.
- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Non-serious AEs

All such events, whether expected or not, should be recorded.

Serious AEs (SEAs)

An SAE form should be completed and faxed to the Chief Investigator within 24 h. However, relapse, death and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the Research Ethics Committee where in the opinion of the Chief Investigator the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence.

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form.

Local investigators should report any SAEs to the sponsor and their Local Research Ethics Committee and/ or Research and Development Office.

Contact details for reporting SAEs
Fax 020 838 33142, attention Professor Gary Frost
Please send SAE forms to Professor Gary Frost
Tel: 020 838 33242 (Mon to Fri 09.00- 17.00)

6. STATISTICS AND DATA ANALYSIS

SAMPLE STORAGE AND ANALYSIS

Coded samples will be stored in the laboratories of the Department of Investigative Medicine, Hammersmith Hospital. They will be available only to the study researchers.

Following a screening visit, volunteers will be given a study number and randomisation will be carried out. An independent researcher (i.e. not linked to the study) will be given the task of randomisation, which will be by sealed envelopes (www.sealedenvelope.com). Data will be analysed by the study research team at Imperial College. Sample analysis will be done in the labs of Imperial College; some analysis might be undertaken by other institutions where appropriate. All samples will always be anonymous and labelled appropriately with only volunteer ID. Statistical analysis will be performed by the research study team.

7. REGULATORY ISSUES

ETHICS APPROVAL

This study is awaiting ethical approval from the Wales Research Ethics Committee 3. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

The study must be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study.

CONSENT

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered, and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In such cases, the participants remain within the study for the purposes of follow-up and data analyses. All participants are free to withdraw at any time from the study without giving reasons and without prejudicing further treatment.

CONFIDENTIALITY

Subjects will be given a personal study code number which will be used throughout the study and in the analysis of data. Samples will be coded using study acronym and participants number. Only the researchers working on the study will have access to the randomisation records which link the code to the volunteer. The randomisation records will be kept on the Trust database.

The Chief Investigator will preserve the confidentiality of participants in the study and is registered under the Data Protection Act. All staff involved in the study are aware of the requirements of the Data Protection Act and all data will be treated as confidential. All trust and University policies for data protection and confidentiality will be followed.

INDEMNITY

Imperial College holds negligent harm and non-negligent harm insurance policies, which apply to this study.

SPONSOR

Imperial College London will act as the main sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

FUNDING

This research project is part of a programme grant funded by Malaysian Palm Oil Board.

Participants will be reimbursed for their time. Total of £140 will be awarded for completion of investigation, this includes travel expenses.

If a participant does not complete the study, for any reason, they will be reimbursed a proportion of the total amount based on the proportion of the study they participated in.

Travel costs to and from screening will not be covered for both eligible and non-eligible volunteers.

AUDITS AND INSPECTIONS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

8. PUBLICATION POLICY

The findings of the research will be published in an open-access, peer-reviewed journal. In addition we will be collaborating with patient groups and professional groups to disseminate the findings via multiple media channels such as patient association publications, print and broadcast media.

9. REFERENCES

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Imperial College London

Department of Investigative Medicine
Hammersmith Hospital Campus
Imperial College London
6th Floor, Commonwealth Building
Du Cane Road
W120NN
Tel 020 838 33242
Fax 020 838 33142

CONSENT FORM

Acute effect of palm olein intake on lipid profile and appetite regulation

Please initial boxes

I have been given the opportunity to ask questions and to discuss the study ☐

I have received satisfactory answers to all my questions ☐

I have received enough information about the study ☐

I confirm that I have read and understand the **Information Sheet for Research Participants Version 4 – 12th August 2016** for the above study. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

I agree to be registered as a patient with Hammersmith Hospital and have a paper copy of medical records, with only authorized staff having access. ☐

I understand that my study results/research notes may be looked at by responsible individuals from Imperial College and/or Imperial College NHS Healthcare Trust and regulatory authorities for audit purposes where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

I give permission for my non-clinical images to be used for research by responsible individuals from Imperial College and/or Imperial College NHS Healthcare Trust so long as they do not contain identifying personal information. ☐

I agree to have my tissue samples and all other data collected in this study to be stored and used in future ethically approved studies **Yes** ☐ **No** ☐

I agree to be contacted again by the investigators to be invited to participate in future research (delete as appropriate) **Yes** ☐ **No** ☐

I agree to take part in this study ☐

Name of Participant:	**Signature:**	**Date:**
_____	_____	_____/_____/_____
Name of Researcher:	**Signature:**	**Date:**

_____ _____ ____/____/_____