# Application Form for UREC Applications

## SECTION 1: APPLICATION DETAILS

1.1

**Project Title: The influence of Fat Perception on Satiety from Consumption of Reduced Fat Snacks**

Date of Submission: 2/2/2018 Proposed start date: 1/4/2018 Proposed End Date: 31 Dec 2018

1.24

**Principal Investigator: Dr Lisa Methven**

Office room number: 2-65b Internal telephone: 8714

Email address: l.methven@reading.ac.uk Alternative contact telephone: 07527 553161
(Please note that an undergraduate or postgraduate student cannot be a named principal investigator for research ethics purposes. The supervisor must be declared as Principal Investigator)

**Other applicants**

Name: Prof Julie Lovegrove Staff/: Institution/Department: Food and Nutritional Sciences. Email: j.a.lovegrove@reading.ac.uk

Name: Prof Martin Yeomans Staff: Institution/Department: School of Psychology, University of Sussex. Email: martin@sussex.ac.uk

Name: Dr Xirui Zhou Staff: Institution/Department: Food and Nutritional Sciences. Email: x.zhou@reading.ac.uk

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Name: Dr Anisha Wijeyesekera Staff: Institution/Department: Food and Nutritional Sciences. Email: a.wijeyesekera@reading.ac.uk

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1.3

**Project Submission Declaration**

I confirm that to the best of my knowledge I have made known all information relevant to the School Research Ethics Committee and I undertake to inform the Committee of any such information which subsequently becomes available whether before or after the research has begun.

I understand that it is a legal requirement that both staff and students undergo Criminal Records Checks when in a position of trust (i.e. when working with children or vulnerable adults).

I confirm that a list of the names and addresses of the subjects in this project will be compiled and that this, together with a copy of the Consent Form, will be retained within the School for a minimum of five years after the date that the project is completed.

Signed……L Mellver…… (Principal Investigator) Date: 15/2/2018……

…… (PDRA Xirui Zhou) Date:……

…… (CoI Prof Lovegrove) Date:……

… CML Williams…… (CoI Prof Claire Williams) Date:…02/02/18…

…… …… (CoI Dr Vasudevan …) Date:15/02/2018……

…… K Wijeyesekera …… (CoI Dr Wijeyesekera …) Date:……

1.4

**University Research Ethics Committee Applications**

Projects expected to require review by the University Research Ethics Committee must be reviewed by a member of the School research ethics committee and the Head of School before submission.

Signed…… (Chair/Deputy Chair of School Committee) Date:……

Signed…… (Head of Department) Date:……

Signed…… (School Ethics Administrator) Date:……

## SECTION 2: PROJECT DETAILS

2.1

Please provide a summary of the project in **non-specialist terms** that could be understood by **non-scientist members of the public**, which includes a description of the scientific background to the study (existing knowledge), the scientific questions the project will address and a justification of these. Please note that the description must be sufficient for the committee to take a reasonable view on the likely scientific rigour and value of the project

This study investigates the effect of fat level, fat type and fat structure, in a food emulsion, on satiety in subjects characterised by their reduced fat and mouthfeel perception (i.e., hypotasters). We propose that by modifying fat delivery, and level of free fatty acids (FFA) in a reformulated food we can reduce the gap between sensory expectation and gut/brain feedback signalling, leading to greater satisfaction (satiety and sustained hedonic response) and long term intake of low fat foods.

We have already submitted a school ethics application for the pilot study and received a favourable opinion to proceed (School of Chemistry, Food and Pharmacy, 14_17). This previous ethics application covered the recruitment of 80 participants for a series of studies and the experimental procedures for the first phase of the study. The first phase was conducted in a sub-group of 40 "hypotasters" (Group 1) using a food emulsion system (a mayonnaise). There were 3 stages in the pilot study.

- Stage 1, Screening and Characterising Volunteers
- Stage 2, Establish Sensory Tolerance in Expected Satiety of a fat reduced emulsion model (mayonnaise).
- Stage 3, Establish Mouth Gut Discordance of a fat reduced emulsion model (quantified as the discrepancy between expected satiety from sensory signals and measured satiety through a preload consumption study).

Specifically, we characterised participants' oral fat and mouthfeel sensitivity. Participants with reduced sensitivity (i.e "hypotasters") were invited to the second visit of the pilot study for detailed quantification of expected satiety and mouth-gut discordance, following consumption of the mayonnaise food model. Within the pilot study we asked permission from all participants to contact them again for this subsequent UREC approved study, too which they agreed.

This main study involves a different food matrix, a snack product (solid food model) rather than a mayonnaise.
In this proposed study, only newly recruited volunteers (N=~25) will undergo Stage 1 Screening **and** Characterisation, participants already recruited to pilot study will undergo Stage 1 **Screening** only'

**NOTE : All subjects will undergo screening for this study (ie whether they were part of the pilot study or not)**

Stages 2 and 3 of the main study are very similar to pilot study except that a snack product will be used instead of the reduced fat emulsion (mayonnaise) model. Finally, in stage 3, we will also take blood samples from the volunteers for measurement of satiety biomarkers that were not required in the pilot study.

(*This box may be expanded as required – **Word Limit Maximum 250***)

2.2

**Procedure**

Please describe concisely what the study will involve for your participants and the procedures and methodology to be undertaken (*you may expand this box as required*).

**Screening:**

For all volunteers that consent to have blood samples (approximately 22 ml, 6 times per visit day via cannulation) taken we will screen them for anaemia, normal liver and kidney function and exclude those with type 2 diabetes. Therefore, a small blood sample will be taken by venepuncture (by the research nurse or trained phlebotomist). The blood sample will be analysed for blood lipids, glucose, full blood count (including haemoglobin), and markers of kidney and liver function. If found to be eligible for the study, the participant will be contacted to confirm their agreement to participate in the study. If any of the screening results are found to be abnormal, these will also be reported to the participant and their GP (Appendix K).

Basic anthropometric measurements (height, weight, blood pressure and body composition (bio-impedance) will be taken for each volunteer.

**Inclusion criteria:**

Men and women

Aged 18-70 years

BMI: 23-32 kg/m2

Fasting glucose < 7 mmol/l

Fasting total cholesterol < 7.5 mmol/L

Fasting triglycerides < 2.3 mmol/L

Weight stable in the last three months

**Exclusion criteria:**

Diagnosed with diabetes or cardiovascular disease (e.g. stroke or heart attack), gastrointestinal (e.g. IBS, inflammatory conditions, gastroenteritis), endocrine or renal diseases

Smoker

Taking prescribed medications that could influence study outcomes (e.g. lipid lowering medications, anti-depressants, anticoagulants)

Food allergies (e.g. gluten, dairy) and intolerances (e.g. lactose)

Drug abuse

Anaemia (men: haemoglobin<130 g/L and women <115 g/L)

Hypertension (systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg)

Planning or currently on a weight reducing programme

Pregnancy, planned pregnancy in the next year or lactating

Currently taking part or participation in other research studies within the last three months

There are 3 stages to the study:

**Stage 1, Characterising Volunteers**

**Note : this has already been completed for the majority of volunteers through the pilot study as discussed above. Therefore this stage 1 is needed to recruit a further 25 volunteers for the main study. This wording for Stage 1 is identical to the study already proceeding under school ethics (study 14_17).**

Volunteers will be characterised on their ability to taste fatty acids. Fat is perceived through three sensory modalities;

mouthfeel, taste ("oleogustus") and odour. Humans vary in their perception of fat across all three sensory modalities. Historically, focus has been on mouthfeel differences. Response to fatty mouthfeel is through trigeminal receptors which are predominantly located around the papillae structures on the tongue. Therefore, papillae density has been found to be correlated to fat-mouthfeel perception. However, there is also inter-individual variation in sensitivity to the oleogustus response. Evidence from several studies suggests that subjects that have low oral sensitivity to free fatty acids (hypo-sensitive) have a higher intake of fatty-foods.

We hypothesise that differences in oral sensitivity to FFA may be related to variation in post ingestion feedback as it is the FFAs that are signalling as metabolites further down the GI tract. Subsequent studies will incorporate hyper- and hypo-sensitive subjects; however in this first study we will focus on hypo-sensitive subjects as we need to establish the differences in fat content that lead to a change in satiety; if we did this with a hyper-sensitive group we may have selected a fat level difference that is not noticeable to hypo-sensitive subjects.

Aim : the overall aim was to recruit 80 subjects and identify 40 subjects that have hypo-oleogustus sensitivity and 40 that are hypersensitive. We have recruited 55 subjects to date. In this main study we require a further 25 volunteers.

**Methods:**

1. Fatty Acid Taste Sensitivity. We use a range of concentrations of oleic acid (a monounsaturated fat found in oils such as olive oil) in milk. Food grade thickener (starch and gum blend) and liquid paraffin are used at low levels to mask texture and lubricity. We use a discrimination forced choice test (a 3-AFC test) where the participant is asked to state the odd sample out of a set. Participants expectorate (spit) the samples into a provided spittoon. Testing is done under red light to mask the visual difference. Participants wear noses clips to avoid the olfactory sensation. (This test has been used successfully in a previous study UREC 12/04). Approximate time to complete: 10 min.
2. Mouthfeel Sensitivity. We will test participants for mouthfeel sensitivity but participants are not screened on this basis. A mouthfeel discrimination test will be carried out using savoury biscuits which are constant in overall fat content but vary in mouthfeel characteristics. Five samples will be prepared varying in mouthfeel and participants will be asked to taste the samples and then rate them for a small number of mouthfeel attributes (e.g. Crunchiness, Hardness, Greasiness). Approximate time to complete: 10 min.
3. Tactile sensitivity testing using Von Frey filaments. These monofilaments have previously been used to evaluate tactile sensitivity on the tongue, and to relate this to taste sensitivity. Participants will be asked to wear a blindfold, the middle of their tongue is then either stimulated or not stimulated with two Von Frey filaments of different sizes. The participant responds to say whether they have felt the stimulation and how sure they are (signal sure, signal not sure, no signal sure, no signal not sure). This is repeated 30 times in rapid succession. Responses are analysed using an R-index (%) value which is standard for a signal-noise detection test. Approximate time to complete: 10 min.
4. Mouth behaviour test (Appendix F). Consumers differ in their mouth behaviour; their preferred method of oral processing. A simple short questionnaire will be used, which has been validated in the USA, to categorise people as Crunchers, Chewers, "Suckers" and "Smooshers". Whereas chewers tend to chew foods to a fine particle size before swallowing, crunchers rapidly crunch and swallow. Such differences not only effect texture perception and preference, they may also effect fat release and fat perception. Approximate time to complete: 5 min.
5. Fungiform papillae (FP) density. In order to determine FP density on the tongue, a digital camera will be used to record an image of the number of FP in two one $cm^2$ areas of the tongue. A small area of the participant's tongue will be temporarily dyed blue using food colour applied by a cotton wool bud. The tongue will then be blotted dry to remove excess moisture prior to recording a digital image. The blue colour will fade after approximately 1 hour and the extent of the colouration is similar to eating certain coloured sweets (e.g. blue Smarties). FPD will be measured as it relates to both mouthfeel sensitivity, as the trigeminal are wrapped around papillae, and taste sensitivity (the taste buds are held within papillae. Approximate time to complete: 5 min.
6. Genotyping. We will investigate genes related to sensory perception and food intake.

   Genes related to sensory perception will include, but are not limited to, *CD36, CA6* and *TAS2R38*. *CD36* is a fatty acid translocase and variation in single nucleotide polymorphisms (SNPs) for *CD36* have been related to

differences in fat perception and fat liking in previous studies. *CA6* is related to production of papillae and taste cells and, therefore, SNPs of *CA6* have been related to differences in mouthfeel and taste sensitivity. The gene most frequently associated with Taste sensitivity is *TAS2R38* and although this is primarily linked to bitter taste, it will be included in order to compare results from this study to previous literature.

Key target genes related to food intake via either gut hormone release or reward will also be genotyped (key genes here will include, but are not limited to, FTO, CNR1 and DAT1). *FTO* gene, the gene that encodes the fat mass and obesity-associated protein (also known as alpha-ketoglutarate-dependent dioxygenase) is associated with energy intake but not reward. *CNR1* gene encodes the Cannabinoid Receptor type 1 ($CB_1$) which facilitates the release of the gut hormone ghrelin, causing appetite stimulation. *DAT1* is the dopamine transporter gene encoding for dopamine release and influencing reward mechanisms, polymorphisms in *DAT1* have been associated with overeating. Only genes related to sensory perception, gut hormone release and food reward mechanisms will be investigated.

Buccal cell will be collected from the inside of the participants' mouth using proprietary swabs. The swabs will be used for subsequent DNA extraction. Characterisation of all the taste receptors' genotypes will be determined by SNP genotyping using reverse transcription-polymerase chain reaction (RT-PCR). Volunteers will be given a Gene Fact Sheet (see Appendix E). Approximate time to complete: 5 min.

7. Screening for eating behaviour disorders and restrained eating. We will use the SCOFF questionnaire to screen out any participants with eating behaviour disorders and the 3-Factor Eating Questionnaire to categorise participants by restrained eating (Appendices I and J). Although the former is a screening question we will still do this at the end of visit 1 (stage 1) as we do not want the answering of these questions to influence behavioural responses. We will use the personality trait questionnaire (Big Five Inventory) (Appendix I) as personality may influence eating behaviour, but also to ensure that we do not have a bias of personality types between volunteers that consenting to taking of blood samples and those that opt-out of blood sampling.

**Stage 2, Establish Sensory Tolerance in Expected Satiety of a fat reduced snack model.**

**Note: the methodology proposed here is based on the stage 2 of the pilot study (SCFP 14_17), except that it is testing a fat reduced snack model.**

Reduced fat products are typically reformulated to match the perceived texture and mouthfeel of the original product. Changing fat droplet size in emulsions can alter both expected and actual satiety. This stage aims to quantify sensory tolerance to fat reduction by using emulsion technology developed at the Quadram Institute, Norwich. In this study emulsions will be applied to an expanded snack product (extruded corn snack) in a similar manner to the coating of a commercial product where oil is normally applied to yield a fat content of approximately 32% (w/w) on the final product. As in the pilot study, an emulsification process will be used to incorporate water or gel droplets into the oil phase to reduce the fat content of the product whilst aiming to mimic the physical properties of the oil normally applied. The type of droplets (e.g. Gel in Oil versus Water in Oil droplets) and the proportion of droplets will be varied to create a range of emulsion models of varying reduced fat content. Direct addition of free fatty acid (oleic acid) at levels slightly above the threshold level of the hypertasters will also be included in one of the reduced fat emulsion models.
These snack products will be supplied by Pepsico. Findings will be generic and will also apply to other systems.

Aim: To define sensory tolerance; the maximum amount fat can be lowered by without leading to significant changes in expected satiety, fatty perception and/or consumer liking using standardized measures. Learnings from the pilot emulsion study will be used to estimate the maximum fat reduction, so only a limited number of fat levels will be trialled in the snack system, but the influence of free fatty acids and emulsion type can also be tested for effects on perception and expected satiety within the snack model.

**Method:**

1. The emulsion snack models (maximum of 6 small snack samples) will be presented to subjects in a monadic sequential manner (i.e. one at a time) in a balanced order. After tasting each sample, participants will rate a variety of measures including: hedonic liking, fat perception ("how fatty do you think this product is"), mouthfeel perception (e.g. mouthcoating). Questions that are not relevant to the study hypothesis will also be asked to try to

prevent biasing the participants through focusing them on fat perception (e.g. "how salty" "how strong is the flavour"). Following tasting of each sample, they will also be shown an image of a full portion of the sample (approximately 40 g) and asked to rate expected satiety (e.g. "If you were to consume a full portion of this product (show picture), how full do you think you would feel?" "How long do you think it would be before you felt hungry again?"). Unstructured line scales (visual analogue scales, VAS) will be used, participants will received training in scale use (see example in Appendix G).

2. For three of the samples, the high fat control, low fat control, and maximum-reduced fat emulsion product, we will examine breakdown of the product in the oral cavity by asking the volunteers to provide expectorated boluses. In each case they will be asked to chew a set amount of snack (e.g. 10 g) for a set amount of time (e.g. 20 s) and then to spit out the sample.

Output: These tests will determine the maximum level to which fat can be reduced in snacks without causing a significant difference in expected satiety, fatty perception or liking. This will determine whether the emulsion type and / or the addition of a low level of free fatty acids influences expected satiety, fatty perception or liking. The expectorated boluses will be used to determine the fat distribution and snack breakdown characteristics between the high fat control and reduced fat emulsions as these characteristics may help to explain differences in perception,

**Stage 3, Establish Mouth Gut Discordance of a fat reduced snack model (quantified as the discrepancy between expected satiety from sensory signals and measured satiety through a preload consumption study).**

In the pilot study, we aim to have established mouth-gut discordance by showing that the reduction in delivery of fat in a mouthfeel matched emulsion model leads to rebound hunger and consequent over-eating. The aim now is to demonstrate that the reduced fat snack utilising the emulsion technology and a minor addition of free fatty acid can bridge the discordance gap and not lead to rebound hunger / overeating.

**Method:**

1. Using a standard preload study design, and the same fat-emulsion snack model from stage 2, we will contrast effects of 3 test samples in a balanced cross-over design. The 3 samples are: (i) Positive control with 100% of the applied spray being oil (approximately 13g fat per 40 g snack portion); (ii) Low fat snack model, where the same amount of spray is applied to the snack fat but as the reduced fat emulsion determined in stage 2, and (iii) Negative control with the same % fat reduction as in sample (ii) achieved through lowering the amount of oil sprayed onto the snack base.

   As it is a 3-way cross over design, participants need to attend 3 visits. Pre-menopausal women differ in hunger at different stages of the menstrual cycle and will be asked to avoid the week prior to their menstrual cycle. Menstruating women have two phases: follicular and luteal with ovulation occurring at the end of the follicular phase. A number of studies have reported that women in the luteal phase (low estrogen and high progesterone) have higher caloric intake and consume more carbohydrates than women in the follicular phase (high estrogen but low progesterone); hence we aim to avoid the luteal phase.

2. Participants will attend the Food and Nutritional Sciences(FNS) sensory science centre following an overnight fast (approximately 08:50, volunteers staggered by a few minutes) and given a standard study breakfast of cereal, milk and orange juice. They will be asked to clean their teeth and use a mouthwash (provided by the study team) between 7 and 8 am; this is to standardise mouth care procedures as oral microflora can contribute to oral lipase activity and the breakdown of dietary fats. They will be given the test snack (pre-load study food, approximately 50 g) to consume at +2 hours (approx. 11 am) on each study day. We anticipate that the low-fat snack model with matched sensory expected satiety will have a fat reduction of over 25 % but that this may reach a 60% reduction.

3. Primary outcomes: Hunger and satiety will be measured (by VAS, see example in Appendix H) before breakfast, between breakfast and the pre-load study food, immediately after consumption, and every 30 minutes up to 1 hours post pre-load (approx. 12:00). Three hours after breakfast and 1h post the preload study food participants will be given an ad-libitum pasta meal. An excessive portion size will be given to each participant and they will be asked to eat until full. This is achieved by providing a 500 g portion on one plate and replacing it after 5 minutes

with a second 500g portion plate. Weight of pasta consumed will be measured.

4. Secondary outcomes; Circulating biomarkers of satiety and hunger: Participants will be asked to consent to provide biofluid samples for the determination of biomarkers of satiety and hunger. The study is powered for the preload study differences in ad-libitum intake and not on the circulating biomarkers; therefore, if volunteers expressed a preference to not provide blood samples they will not be excluded from the study, but continue with the behavioural study only. The circulating biomarker data we obtain will help to explain mechanisms behind the study behavioural outcomes and provide data on which future studies can be powered. The following samples will be taken after informed consent (appendix C) is given:

    a. Blood sample collection: Volunteers who consent to providing blood samples will have a cannula inserted (by the research nurse) in a forearm vein on arrival to the Hugh Sinclair Unit of Human Nutrition each study day. Two fasting samples will be taken (-10 and 0 min respectively) before the test breakfast is given. Blood samples will then be drawn at the following time intervals: Post breakfast, (+15 min, approx. 09:15), before the pre-load snack intervention (+1h45 min, approx. 10:45), post pre-load intervention (+2h5min, approx. 11:05), 30 min post preload (approx. 11:35), prelunch (approx. 12:00), post lunch (approx. 12:30). Hunger and satiety hormones (gastrointestinal hormones) and steroid hormones in the prepared plasma will be measured; these will include but are not limited to ghrelin, leptin, glucagon-like peptide (GLP-1), gastric inhibitory peptide (GIP), cholecystokinin (CCK) and peptide YY (PYY). Estrogen and progesterone will be measured; their ratio can affect caloric intake, but the effect of this ratio on satiety and intake of fat-snacks is not yet known. Endocannabinoids (anandamide and 2-arachidonoylglycerol), known to be lipid mediators, will also be quantified alongside lipidomics using HPLC/MS/MS. Such lipidomics can detect low-abundance 'endocannabinoid-like' (eCBL) bioactive signalling lipids. Plasma triacylglycerol (TAG), non-esterifed fatty acids (NEFA) and insulin will also be measured. Nuclear Magnetic Resonance (NMR) Spectroscopy-based metabolomics will be used to profile metabolites in the blood.

    b. Saliva samples: Non-stimulated saliva samples (1 ml) will be collected at the same 6 time points as for the blood samples. Two types of analysis will be performed; gastrointestinal hormones and metabolomics using NMR. Hormone measurements in saliva are more difficult to quantify than in blood, hence the preference for blood samples; however we aim to compare measurements from the two sample types to provide evidence for future studies and to be able to incorporate data from any volunteers that do not consent to providing bloods. Saliva NMR metabolic profiles provide information on metabolic outputs taking place in the system at a given moment in time. We will use these to investigate whether there is a metabolomics signature that can be determined following consumption of the low and high fat snacks.

    c. Urine samples: volunteers will be asked to provide one urine sample at home following an overnight fast, and bring into the unit, and one 3.5 hour urine sample to be collected during each visit. (They will be asked to void their bladder at 8.50 and then collect all urine from 08:50 to 11:50 (i.e. pre study lunch) whilst at the Unit). These samples will be also analysed by NMR since urinary NMR metabolic profiles provide information relating to by-products of endogenous metabolic pathways as well as products of microbe-host co-metabolism, thus will aid in identifying potential mechanisms post consumption of low versus high fat snacks.

(Note: All questionnaires or interviews should be appended to this application)

2.3

Where will the project take place? Sensory Science Centre and Hugh Sinclair Unit of Human Nutrition and The Chemical Analysis Facility NMR Laboratory

If the project is to take place in Hugh Sinclair Unit of Human Nutrition, projects must be reviewed and approved by the Hugh Sinclair Manager (Mrs Sarah Hargreaves s.e.hargreaves@reading.ac.uk

Signed……. (Hugh Sinclair Unit Manager) Date:…….

2.4

**Funding**

Is the research supported by funding from a research council or other *external* sources (e.g. charities, business)? Yes/ (please delete)

**If Yes, please give details:** BBSRC, Unilever and Pepsico.

Please note that *all* projects (except those considered as low risk, which would be the decision of the School's internal review committee and require Head of Department approval) require approval from the University Research Ethics Committee.

2.5

**Ethical Issues**

Could this research lead to any risk of harm or distress to the researcher, participant or immediate others? Please explain why this is necessary and how any risk will be managed.

All samples used in the study that have been produced at Pepsico will be manufactured under the Good Manufacturing Guidelines and will have undergone microbiological clearance testing. The levels of fat and saturated fat we are giving to participants is not excessive, however we will screen out participants with hypercholesterolaemia (high cholesterol) determined at screening, known to have cardio-vascular problems, or that are on a low fat diet (see Appendix C).

Venepuncture and cannulation may cause a small bruise at the site of needle penetration; it will be performed by the research nurses. Clinical cover (research nurse) will be available at all times during the 4 h study visits. However, in case of an emergency (e.g. fainting, blood spillage) researchers will take immediate action according to the Department of Food & Nutritional Sciences relevant SOP that describes in detail the actions that need to be taken by the researchers. Also, a Departmental First Aider will be available in the building when blood samples are taken both at screening and during the study visits. In case of emergency, the research staff will call the emergency services (999).

(*this box may be expanded as required*)

2.6

**Deception**

Will the research involve any element of intentional deception at any stage (i.e. providing false or misleading information about the study, or omitting information)?
[If so, this should be justified. You should also consider including debriefing materials for participants, which outline the nature and the justification of the deception used]

Yes: the study will inform the participants that we are interested in their appetite, liking and perception, however we cannot tell them that we are specifically interested in fatty perception alone because this will bias their responses. Therefore, we will ask questions relating to their perception of attributes other than fat

in Stage 2. In Stage 3 we will not draw attention to the snack (mid-morning) component of the visit (i.e. the pre-load samples), although participants may realise that it is this component we are investigating as they will have scored expected satiety of this component in Stage 2.

2.7

**Payment**

Will you be paying your participants for their involvement in the study? Yes/No (delete)
If yes, please specify and justify the amount paid

Participants will be reimbursed for their time as follows:
Stage 1 (screening and subject characterisation): participants will be reimbursed £10 for the one visit (approximately 1 h).
Stage 2: participants will be reimbursed £10 for the one visit (approximately 1 h).
Stage 3: participants will be reimbursed £60 for the three visits (three half days) if they are not providing blood, urine and saliva samples; however, they will be reimbursed to total of £140 for the three visits if they are also providing blood, urine and saliva samples.

Note: excessive payment may be considered coercive and therefore unethical. Travel expenses need not to be declared.

2.8

**Data protection and confidentiality**

What steps will be taken to ensure participant confidentiality? How will the data be stored?

Volunteers will be given a participant number, and throughout data collecting will be referred to by this number. Data linking the name of the participant to their participant number will be kept in a locked filing cabinet in a locked office in the Department of Food and Nutritional Sciences. The Department of Food & Nutritional Sciences, where the study will be performed, is fully licensed under the Human Tissue Act 2004 and as such will adhere to the guidelines necessary for the storage of saliva and blood samples.

2.9

**Consent**

Please describe the process by which participants will be informed about the nature of the study and the process by which you will obtain consent

Participants will be given a consent and screening form (appendix C) as well as the participant information sheet which will be explained to the volunteers in order that they have the opportunity to ask any questions. These questions will be suitably addressed before they are asked to sign the consent form. No testing will commence before a consent form is signed.
Participants that have already consented to the pilot study, and signed to say they can be contacted again, will be contacted directly about this main study, however they will be asked to sign the consent form for this subsequent main study in addition to their pilot study consent form.

2.10

**Genotyping**

Are you intending to genotype the participants? Which genotypes will be determined?

Yes. Genes related to sensory perception will include, but are not limited to, *CD36, CA6* and *TAS2R38.* Key target genes related to food intake via either gut hormone release or reward will also be genotyped (key genes here will include, but are not limited to, FTO, CNR1 and DAT1). (See Gene fact sheet as part of the Participant Information Sheet, Appendix E). Only genes related to sensory perception, gut hormone release and food reward mechanisms will be investigated.

Please note that a copy of all information sheets on the implications of determining the specific genotype(s) to be undertaken must be appended to this application.

SECTION 3: PARTICIPANT DETAILS

3.1

**Sample Size**

How many participants do you plan to recruit? Please provide a suitable power calculation demonstrating how the sample size has been arrived at or a suitable justification explaining why this is not possible/appropriate for the study.

We aim to recruit 80 participants; 40 hypo- and 40 hyper- fatty acids tasters.
Power is estimated from a combination of the expected effects of both the sensory and energy manipulations. For the sensory effect, work has contrasted intake after equicaloric emulsion preloads varying in oil particle size and consequently perceived creaminess. A significant 12% reduction in meal size occurred using a within-participant design with 34 male participants in the small droplet-size (more creamy) preload (Lett et al., 2016).
In addition, we have based our calculation on the paper of Woodend 2001 where based on their results of the influence of a 836 kJ preload of safflower oil (of which the energy is similar to the 35g snack with 32% fat), a minimum sample size of 38 would be required to detect an intake change of 20%.

3.2

Will the research involve children or vulnerable adults (e.g. adults with mental health problems or neurological conditions)? No

If yes, how will you ensure these participants fully understand the study and the nature of their involvement in it and freely consent to participate?
(Please append letters and, if relevant, consent forms, for parents, guardians or carers). Please note: information letters must be supplied for all participants wherever possible, including children. Written consent should be obtained from children wherever possible in addition to that required from parents.

3.3

Will your research involve children under the age of 18 years? No
Will your research involve children under the age of 5 years? No

3.4

Will your research involve NHS patients, Clients of Social Services or will GP or NHS databases be used for recruitment purposes? No

Please note that if your research involves NHS patients or Clients of Social Services your application will have to be reviewed by the University Research Ethics Committee and by an NHS research ethics committee.

3.5

**Recruitment**

Please describe the recruitment process and append all advertising and letters of recruitment.

Students and staff at the university will be contacted via email (either directly if they have already signed up to volunteer databases; or via generic email circulation lists). Non-university volunteers will also be recruited directly via email where they have already signed up to volunteer databases. Volunteers will also be recruited via social media sites and via posters in the Reading area. We may also recruit volunteers with the help of Sensory Dimensions (Sensory and Market research company on Whiteknights Campus). The poster that will be used can be seen in Appendix D.
Participants that have already consented to the pilot study, and signed to say they can be contacted again, will be contacted directly about this main study.
Staff and students from the Department of Food and Nutritional Sciences will not be invited to participate in the study.

**Important Notes**

1. The Principal Investigator must complete the Checklist in Appendix A to ensure that all the relevant steps and have been taken and all the appropriate documentation has been appended.

2. If you expect that your application will need to be reviewed by the University Research Ethics Committee you must also complete the Form in Appendix B.

3. For template consent forms, please see Appendices C.

# Appendix A: Application checklist

**This must be completed by an academic staff member (e.g. supervisor)**

Please tick to confirm that the following information has been included and is correct.
Indicate (N/A) if not applicable:

**Information Sheet**

| | |
|---|---|
| Is on headed notepaper | ✓☐ |
| Includes Investigator's name and email / telephone number | ✓☐ |
| Includes Supervisor's name and email / telephone number | ✓☐ |
| Statement that participation is voluntary | ✓☐ |
| Statement that participants are free to withdraw their co-operation | ✓☐ |
| Reference to the ethical process | ✓☐ |
| Reference to Disclosure | ☐ N/A ☐✓ |
| Reference to confidentiality, storage and disposal of personal information collected | ✓☐ |
| **Consent form(s)** | ✓☐ |

**Other relevant material**

| | |
|---|---|
| Questionnaires | ✓☐ N/A ☐ |
| Advertisement/leaflets | ✓☐ N/A ☐ |
| Letters | ☐ N/A ☐✓ |
| Other (please specify) | ☐ N/A ☐✓ |

**Expected duration of the project** (months) 12

**Name (print) ……Dr Lisa Methven…… Signature ……**

## Appendix B

# Project Submission Form

Note All sections of this form should be completed. Please continue on separate sheets if necessary.

Principal Investigator: Dr Lisa Methven

School: Chemistry, Food and Pharmacy

Title of Project: **The influence of Fat Perception on Satiety from consumption of Reduced Fat Snacks**

Proposed starting date: 1/4/2018

Brief description of Project:

This study investigates the effect of fat level, fat type and fat structure, in a snack product, on satiety in subjects characterised in their fat and mouthfeel perception. We propose that by modifying fat delivery and level of free fatty acids (FFA) in a reformulated food we can reduce the gap between sensory expectation and gut/brain feedback signalling, leading to greater satisfaction (satiety and sustained hedonic response) and long term intake of low fat foods.

I confirm that to the best of my knowledge I have made known all information relevant to the School Ethics Committee and I undertake to inform the Committee of any such information which subsequently becomes available whether before or after the research has begun.

I confirm that a list of the names and addresses of the subjects in this project will be compiled and that this, together with a copy of the Consent Form, will be retained within the School for a minimum of five years after the date that the project is completed.

Signed…….(Investigator) Date……

…….(Head of Department) Date……

…….(Student) Date……
(Where applicable)

**Checklist**

1. This form is signed by my Head of Department ☐✓

2. The Consent form includes a statement to the effect that the project has been subject to ethical review, according to the procedures specified by the University Research Ethics Committee, and has been allowed to proceed ✓

3. I have made, and explained within this application, arrangements for any confidential material generated by the research to be stored securely within the University and, where appropriate, subsequently disposed of securely. ✓

4. I have made arrangements for expenses to be paid to participants in the research, if any, OR, if not, I have explained why not. ✓

5. Tick EITHER **(a) OR (b) - Head of School to sign if (b) ticked**

   (a) The proposed research does **NOT** involve the taking of blood samples; ☐

   **OR**

   (b) For anyone whose proximity to the blood samples brings a risk of Hepatitis B, documentary evidence of protection prior to the risk of exposure will be retained by the Head of School. ✓

   Signed…….(Head of Department) Date……

6. Tick **EITHER (a) OR (b)**

   (a) The proposed research does **NOT** involve the storage of human tissue, as defined by the Human Tissue Act 2004; ☐

   **OR**

   (b) I have explained within the application how the requirements of the Human Tissue Act 2004 will be met. ✓

7. Tick **EITHER (a), (b) OR (c)**

   (a) The proposed research will not generate any information about the health of participants; ☐

   **OR**

(b) In the circumstance that any test reveals an abnormal result, I will inform the participant and, with the participant's consent, also inform their GP, providing a copy of those results to each; ☐

**OR**

(c) I have explained within the application why (b) above is not appropriate. ☐

8. Tick **EITHER (a) OR (b) - Head of School to sign if (b) ticked**

(a) the proposed research does not involve children under the age of 5; ☑

**OR**

(b) My Head of School has given details of the proposed research to the University's insurance officer, and the research will not proceed until I have confirmation that insurance cover is in place. ☐

Signed……. (Head of Department) Date……

This form and further relevant information (see Sections 5 (b)-(e) of the Notes for Guidance) should be returned to, School Ethics Administrator. You will be notified of the Committee's decision as quickly as possible, and you should not proceed with the project until then.

# Appendix C

## Title- A Study of Sensory Perception and Satiety following breakfast, snack and lunch

**Screening and Consent Form for Sensory and Consumer Trials**

**Investigator:**

Dr Lisa Methven : email : l.methven@reading.ac.uk, telephone 0118 3788714

Dept.of Food & Nutritional Sciences, University of Reading, Whiteknights, Reading, RG6 6AP.

Xirui Zhou, Researcher, xirui.zhou@reading.ac.uk

**Please INITIAL the box**

| | | |
|---|---|---|
| 1. | I have had explained to me the purposes of the project and what will be required of me, and any questions I have had have been answered to my satisfaction. I agree to the arrangements described in the Information Sheet in so far as they relate to my participation. | |
| 2. | I understand that participation is entirely voluntary and that I have the right to withdraw from the project any time. | |
| 3. | I have received a copy of this Consent Form and of the accompanying Information Sheet. | |
| 4. | I have read the Information Sheet and been told the reasons why a buccal cell sample is required. I consent to a buccal cell sample being taken for taste, appetite and reward genotyping (as in gene factsheet). | yes □ no □ |
| 6. | I consent to answering demographic information | yes □ no □ |
| 7. | I consent to having blood samples taken, through venipuncture (needle) and the screening appointment and through a cannula during the study days. | yes □ no □ |
| 8. | I consent to providing urine samples | yes □ no □ |
| 9. | I consent to providing saliva samples | yes □ no □ |

I have had explained to me that consent for my contact details and personal information to be added to the Hugh Sinclair Unit of Human Nutrition Volunteer Database is entirely voluntary. Accordingly I consent as indicated below:

| | | |
|---|---|---|
| 10. | I consent to my contact details being stored on the Nutrition Unit Volunteer Database. | yes □ no □ |
| 11. | I consent to my screening information (including date of birth, height, weight, blood pressure, smoking status, long-term use of medication, one bitter taste receptor genotype [TAS2R38,], and blood test results, such as level of cholesterol, triacylglycerol, and glucose) being stored on the Nutrition Unit Volunteer Database | yes □ no □ |

I understand that the genotypes being tested in this study are for genes related to sensory perception, gut hormone release and food reward mechanisms. Although some of these may be

indirectly linked to diet related diseases (for example they may be related to food choice and intake), the relationships are not strong and there are no known direct implications not courses of action that can be recommended. However, if I would like to receive my genotype results I can request this here:

| | | |
|---|---|---|
| 12. | I would like to receive the results of genotyping from my buccal cell sample. | yes □ no □ |

I understand that this application has been reviewed by the School of Chemistry, Food and Pharmacy School Research Ethics Committee and has been given a favourable ethical opinion for conduct.

**Name ……Date of Birth: ……..**

**Signed ……**

**Date ……**

**Address of Participant:**

(Please add if you wish to receive the overall results of the study, and/or you consent to be part of the Hugh Sinclair Unit of Human Nutrition Volunteer Database)

**Telephone number:…….**

**General Practitioner (GP) details**

**Name:…….**

**Address:**

**Telephone: ……..**

**Witnessed by**

**Name of researcher taking consent:**

**Signature: Date:**

## Medical and Lifestyle Screening Questionnaire

| Name: | | Title: |
|---|---|---|
| Address: | | Date of Birth: |
| | | Sex: |
| Daytime Telephone: | Evening Telephone: | Best time to call: |
| Weight (kg): | Height (m): | BMI (kg/m$^2$): |
| Blood pressure Right arm / left arm (circle)<br>1. _____ 2. _____ 3. _____ | | |
| E-mail:<br>Do you use emails on a regular basis? YES/NO | | |

**Study requirements**

- Are you able to spend 3 half days (8:50 to 12:30) in the sensory centre YES/NO (do not continue if "No")
- Are you a member of staff or a student of Food and Nutritional Sciences? YES/NO (do not continue if "Yes")

**Dietary and Screening Questions**

In order to ensure the food and / or beverage products you will be presented with are safe for you to consume, **please answer the following questions :**

1. **Do you have any food allergies or intolerances?** (For examples nuts, wheat etc). If YES, please specify (eg Wheat, Gluten, Corn, Nuts, Milk etc).

**YES / NO**

Type of allergy / intolerance.....

2. **Are there any foods / food types / ingredients that you do not consume for other reasons** (personal, cultural, religious etc) ? If YES, please specify (eg Pork, All Meat, Alcohol etc).

**YES / NO**

Foods / Drinks NOT consumed.....

3. **Do you have any medical condition(s) which may affect your food intake?** (For example, on a salt/sodium controlled diet, on a low fat diet etc)

**YES / NO**

If YES, please specify……..

4. Do you smoke? **YES/NO**

If 'YES', please give details:
- Type (e.g. cigarettes, pipe, E-cigarettes)
- Frequency (e.g. daily, social occasions only)

If 'NO', have you ever smoked? YES/NO
Please tell us when you stopped:

## Medical Questions:

1. Have you been diagnosed as having any of the following?

a) High blood cholesterol YES/NO

b) High blood pressure YES/NO

c) Thyroid disorder YES/NO

d) Diabetes or other endocrine disorders YES/NO

e) Heart problems, stroke or any vascular disease in the past 12 months YES/NO

f) Inflammatory diseases (e.g. rheumatoid arthritis) YES/NO

h) Renal, gastrointestinal, respiratory or liver disease/disorder YES/NO

i) Cancer YES/NO

2. Have you been diagnosed as suffering from any other illness? YES/NO
   *If 'YES', please give details*

   …….

3. Within the past 3 months, have you taken any medication (prescription or non-prescription)? YES/NO
   *If 'YES', please provide:*
   - *Name of medication(s)*
   - *Reason(s) for use*
   - *Dosage*
   - *Frequency of use, e.g. daily, once every 2 weeks.*

4. Have you been diagnosed with an infectious disease, e.g. hepatitis B? YES/NO
   *If 'YES', please give details*

5. Have you had any surgery within the past 3 months or do you have surgery planned? YES/NO
   *If 'YES', please give details*

6. Have you ever suffered from a pulmonary embolism, deep vein thrombosis, blood clots or had a blood transfusion? YES/NO

*If 'YES', please give details*

7. Do you have a pacemaker? YES/NO

8. This question is **only to female** participants.

a) Are you:

| | | |
|---|---|---|
| Premenopausal | ☐ | Premenopausal |
| Perimenopausal | ☐ | Perimenopausal |
| Postmenopausal | ☐ | Postmenopausal |

*If you are premenopausal:*

b) Are you using contraception? YES/NO

*If 'YES', please give details (including the name of the contraceptive pill or device)*

……

c) Do you have regular menstrual cycles? YES/NO

d) Are you pregnant, lactating or planning a pregnancy in the next year? YES/NO

*If you are postmenopausal:*

e) Do you remember when your final menstrual cycle was?

| | |
|---|---|
| Less than 1 year ago | ☐ |
| 1-2 years ago | ☐ |
| 2-5 years ago | ☐ |
| More than 5 years ago | ☐ |
| Can't remember | ☐ |

f) Are you using hormone replacement therapy (HRT)? YES/NO

*If 'YES', how long have you been on HRT?*

## Lifestyle questions

9. Are you currently taking part in or been involved in a clinical trial/research study within the last 3 months either here or elsewhere? YES/NO

If 'YES', please give details:

10. Have you been screened or contacted recently about taking part in a study here or elsewhere? YES/NO

If 'YES', please give details

11. Are you a blood donor? YES/NO

If 'YES', when was the last time you donated blood?

If you are eligible to participate in the study, are you willing to postpone further blood donations until 3 months after your final study visit? YES/NO

15. Are you following or planning to start a restricted diet, e.g. to lose weight? YES/NO

If 'YES', would you be willing to postpone this until after your final study visit? YES/NO

17. Has your weight been stable (less than 3 kg weight loss/gain) in the last 3 months? YES/NO

18. Do you drink alcohol? YES/NO

If 'YES', approximately how many units do you drink per week? _____Units

One unit of alcohol is half a pint of beer/lager, a single pub measure of spirits e.g. gin/vodka, or a small glass of wine (125 ml).

19. Do you exercise more than three times a week, including walking? YES/NO

If 'YES', please specify the type of exercise, frequency and intensity

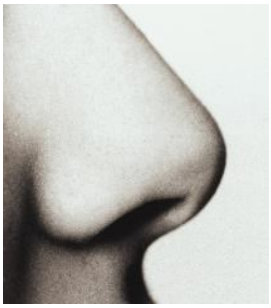
20. Do you have any holidays/trips planned within the next three months? YES/NO

If 'YES', please give details (e.g. dates and duration):

This is the end of the questionnaire - thank you for your time.
The information you have provided will remain confidential at all times.

**Appendix Di: Recruitment Poster**

# A Study into Sensory Perception & Appetite

## *TASTERS REQUIRED !*

We are looking for consumers that are willing to take part in tasting and appetite studies, varying in time from 1 hour to 4 hours, on 4 visits over an 18 month period.

**REQUIREMENTS:**

- 18-70 years of age
- Non-Smokers
- Not allergic / intolerant to Dairy Products or Gluten
- Not a staff member or student of Food and Nutritional Sciences

**You will be reimbursed for your time ☺**

If you are Interested in taking part in this study please contact Sherrie at supertaster@reading.ac.uk or on 0788 5972287

***Tear off strips at bottom of poster will say:***
Tasting and Appetite Study
Contact Sherrie at supertaster@reading.ac.uk; or on 0788 5972287

## Appendix Dii: Recruitment Email

**Dear Potential Participant,**
We are looking for consumers who are willing to take part in tasting and appetite studies, varying in time from 1 hour to 4 hours over an 18 month period.
**You will be reimbursed for your time ☺**

**Our main criteria:**18-70 years of age, Non-Smokers, Not allergic / intolerant to Dairy Products or Gluten. Not a staff member or student of Food and Nutritional Sciences.

If you are Interested in taking part in this study please contact Sherrie at supertaster@reading.ac.uk or on 0788 5972287

## Appendix Diii: Recruitment via Social Media

Do you have a little time to spare to taste foods and enjoy a few meals “on us” ? We are looking for consumers that are willing to take part in tasting and appetite studies, varying in time from 1 hour to 4 hours over an 18 month period.
**You will be reimbursed for your time ☺**
If you are Interested in taking part in this study please contact Sherrie at supertaster@reading.ac.uk or on 0788 5972287

# Appendix E

**Department of Food and Nutritional Sciences,**
**Whiteknights, Reading RG6 6AP**
***Researcher (principal)*: Dr Lisa Methven**

*Email*: l.methven@reading.ac.uk
*Phone*: 0118 9264792

## Participant Information Sheet: Sensory Perception and Satiety following breakfast, snack and lunch

### Background :

Sensory specific satiety is a well-known phenomena; where the sensory characteristics of foods (taste, aroma etc.) affect whether we want to eat or stop eating a particular food. However, people vary in their sensitivity to sensory signals, like aroma and taste. In this study, we will investigate whether such differences in sensitivity influence appetite.

### Who would we like to participate in the study?

We are looking to recruit healthy, non-smoking volunteers age 18-70, that are not staff or students of the Department of Food and Nutritional Sciences.

### Do I have to take part?

Your participation is entirely voluntary and you are free to withdraw at any time without giving any reason.

### What will be involved if you take part?

Screening appointment

You will be invited to come for an initial screening visit in the morning in an unfed state (fasted, without having eaten breakfast or eating or drinking anything except water for 12 hours). During this screening visit you will be encouraged to ask any question regarding the study and we will ask you to complete a Medical and Lifestyle Questionnaire if you have not completed this for us beforehand. Your consent for participating in this study will be taken and you will be given a photocopy of the signed consent form to keep. After that, your weight, height and blood pressure will be measured.

If you have consented to proving blood then a small blood sample (~10 ml, volume equivalent to less than one tablespoon) will be taken. After this screening procedure, you will be provided with a light breakfast before you leave.

You will be informed of the results of your blood tests on blood glucose, blood fat and haemoglobin levels. If you are found suitable for the study and are willing to participate, we will confirm with you that you agree to participate in the study. If you have any abnormal screening results, these will be reported to both you and your GP.

There are then 3 stages to this study.

**NOTE : If you have already participated in our pilot study (SCFP study number 14/17) then you will not be asked to take part in Stage 1, you will go directly to stage 2.**

**Stage 1, Sensory Sensitivity Testing (a 1 hour visit)**

Volunteers that are suitable for the study (see screening questionnaire) will be invited for one visit to the Sensory Science Centre, lasting approximately 1 hour. We will ask you to take part in a series of tests as follows:

1. A taste sensitivity test. You will be asked to taste a series of solutions and in every set of 3 samples you will be asked to identify which you think is the odd one out. You will be asked to wear a nose-clip during this test and it takes up to 10 minutes.
2. A mouthfeel sensitivity test. You will be asked to taste a small range of biscuits and to score them in order of increasing mouthfeel attributes (e.g. “thickness”, “crunchiness” “greasiness”).
3. A touch sensitivity test. This uses very fine fibres and we ask to say when you can feel the fibre.
4. To allow us to place a very small amount of blue food colour on your tongue using a cotton wool bud and for us to take a photographic image of your tongue.
5. To allow us to take a swab from the inside of your mouth; this swab will contain buccal cells. This allows us to extract DNA from your cells and to genotype this DNA for specific taste related genes (see separate Gene Fact Sheet).
6. To complete a short questionnaire which will ask you some demographic questions as well as some questions about how you prefer to eat certain foods.

**Stage 2, Tasting and Scoring a Small Range of Foods ( a 1 hour visit)**

You will be asked to taste up to 6 snacks. After tasting each sample you will be asked to score some sensory attributes relating to the sample. You will also be asked to rate how full you think you would feel if you ate a full portion of each sample. For selected samples we will also ask you to provide an expectorated sample (to spit out the sample) after a set chewing time as we need to investigate the breakdown of the product on chewing.

**Stage 3, Appetite Study Days (3 Separate Visit Days, each of 4 to 5 hours)**

On each of these days you will be asked to not eat anything from 10pm the evening before, to clean your teeth and use a mouthwash between 7 and 8am (we will provide these) and then to arrive at the University at approximately 08:50 am, whereupon you will be given a breakfast. At approximately11 am you will be given a snack, and 1 hour later we will provide your lunch. We will ask you to score your hunger / fullness repeatedly on simple lines scales from before breakfast to after lunch at regular time intervals (15-30 minutes). We will ask you to eat all the breaksfast and the snack, and then as much of the lunch as you want to consume. There will be at least a 1 week period between each of these study days. Premenstrual women will be asked to void the week before their menstrual cycle.

In addition, we will ask if you are willing to have blood, urine and saliva samples taken (see consent form) in order that we can measure your uptake of the nutrients from the meals you have consumed, as well as the levels of appetite and satiety hormones released. The sampling will be as follows:

**Blood samples**: On arrival each study day the nurse will fit a small flexible cannula into a vein in your arm, and this will remain in place with minimal discomfort to allow us to

take blood samples during the study day (you will be able move your arm, but we will ask you to not to bend it too much during the study session). Two small fasting blood samples (up to 22 ml equivalent to 1.5 tablespoons) will be taken before you are provided with breakfast. We will ask you to consume the breakfast within 15 minutes. Six small blood samples (up to 22 ml,) will then be collected at regular intervals over the next 3.5 hours. You will be asked to remain in the nutrition and sensory units for the duration of the study visit where you will have free access to water and facilities to watch TV, DVDs, work, read and use the internet. After the last blood sample the nurse will remove the cannula before you go home.

**Urine sample**: On arrival for each study day we will provide you with a container in which to collect your urine. We will ask you to void your bladder at approximately 8.50 (do not collect this sample) and then collect all urine from 08:50 until before lunch at 11:50 in the container provided.

**Saliva samples:** We will ask you to collect saliva samples (approximately 1 ml) by spitting into a tube at six occasions during each study visit.

**Consent**

The study is entirely voluntary, and only those who give informed consent will be allowed to take part in the study. You can decide not to participate at any point and all participants are free to withdraw at any time with any detriment.

**Confidentiality**

- Your name will be recorded in order to allocate you a participant number.
- This is a linked-anonymised study; the list linking names and participant numbers will be kept in a locked cabinet, along with the consent form, only assessable to the study researchers.
- Your identifiable data (consent form) will be destroyed after 5 years.

**Are there any adverse consequences to your health as a result of being a volunteer on this study?**

There are no health risks associated with taking part in this study. All products and ingredients to be tested are safe for human consumption, and any pure compounds used are of food or pharmaceutical grade. Blood samples will be taken by a qualified research nurse, who is trained in venepuncture and cannulation and will provide 'on-call' medical cover throughout the study. The procedure for taking blood directly from the small flexible cannula is routine. The volume of blood collected during each study visit is 132 ml (equivalent to approximately 9 tablespoons), will cause no adverse consequences and is usually painless, although occasionally cannulation may cause a small bruise at the site of blood collection.

**What are the potential benefits of the study?**

This study would help gain a better understanding of how sensory perception influences appetite. There is little direct benefit to you.

**What data is being collected and what will be done with this data?**

All of the stage 1 sensory sensitivity testing will be reveal how well each participants perceived mouthfeel and taste. This includes genotype data from buccal swabs, details of this are on the fact sheet below. There are two aim of the genotype data; firstly to see is to determine whether differences in taste and

mouthfeel sensitivity relate to genotype and to product liking, secondly whether they relate to satiety. The data from stage 2 (product tasting) will be used to relate consumers scores of the products to differences in product composition and differences in sensory sensitivity between consumers. There are two types of data from the stage 3 visits; satiety data (scores of hunger and fullness as well as how much lunch is eaten) and biological data from the blood, urine and saliva samples. In the blood, urine and saliva samples we will measure satiety hormones (and relate this to the satiety data) and metabolites (to see how the different products led to release of nutrients). The aim of all of this data is to create a model to understand how sensory perception of foods varying in fat content relates to satiety.
After the study this data, entirely anonymised, may be shared with other partners inside and outside of the UK, it may also be combined with other data sets to enable greater value and insight.

**Will any expenses be incurred during the study?**
You will receive an honorarium in order to cover time and travel expenses, this will be paid as the following instalments.

- You will be paid £10 on completion of Stage 1.
- If you take part in stages 2 and 3, and providing blood, urine and saliva in the stage 3 visits, then you will be paid £150 on completion of all 4 related visits.
- If you take part in stages 2 and 3 but are not providing blood, urine and saliva then you will be paid £70 on completion of all 4 related visits.

**Who is organising and funding this research?**
University of Reading is organising the study in collaboration with the University of Sussex. The research is funded by the Biotechnology and Biological Sciences Research Council (BBSRC), a government funded research council, in collaboration with 7 Food Industry Partners. Direct contributions to the research has been provided by Unilever and Pepsico, the following industrial partners contribute to the direction and design of the study at stakeholder meetings; Arla, Mars Wrigley Confectionery, Mondeléz International, Pladis and Premier Foods.

**Who has reviewed the study?**
The University of Reading Research Ethics Committee have reviewed the study and given a favourable ethical opinion for conduct.

**How do I arrange to take part?**
If you wish to take part in the study, please contact the person who sent you this information leaflet.

**If you have any concerns or complaints about the research, we will do our best to resolve them. Please contact: *(as appropriate)***

Dr Lisa Methven: l.methven@reading.ac.uk Tel: 0118 3788714
Xirui Zhou : xirui.zhou@reading.ac.uk. Tel: 0788 5972287

If you remain unhappy and wish to complain formally, you can do this through the Head of the Department of Food & Nutritional Sciences, Professor Richard Frazier (tel: 0118 378 8709; email: r.a.frazier@reading.ac.uk). In the event that something does go wrong and you are harmed during the study, the University of Reading has in place Professional Indemnity Insurances.

**Thank you for taking the time to read this Participant Information Sheet.**

# Gene Factsheet: Taste Receptor, Appetite and Reward Genes

Human genes contain the information needed to make functional molecules called proteins. It has been estimated that humans have between 20,000 to 25,000 genes, 99.9% of which are the same in all people. There is much interest in the genes that differ between people and the impact that these may have on our health and eating preferences. At the University of Reading, we are interested in how these variations affect people's response to foods. For this reason, in some of our studies we ask you to provide a blood or saliva sample that we use to determine if you have variations of a particular gene. In this particular study we will ask you for a buccal cell sample for genotyping.

Your buccal cell sample will be tested for variations in taste receptor, appetite and reward genes. These may include Type 2 Taste receptor genes, *TAS2R,* (responsible for bitter taste perception), *CD36* (a gene responsible for fat taste perception) and Gustin (*CA6*) a gene related to our ability to produce new taste receptor cells. They will also include the *FTO* gene (a gene associated with energy intake), *CNR1* gene (associated with appetite stimulation) and *DAT1* (which influences reward mechanisms). Only genes related to sensory perception, gut hormone release and food reward mechanisms will be investigated.
This factsheet is designed to explain what these receptors do in the body and what impact variations in this gene may have, if any, on your health.

## What are *TAS2R, CD36* and *CA6*; and what do they do?

There are numerous different protein receptors on the tongue that detect taste. *TAS2R* are a family of receptor genes responsible for detecting bitter taste compounds. *CD36* is a fatty acid translocase, it helps to move fatty acids across cell membranes, and has been linked to fat taste perception. *CA6* is the gustin gene which codes for the production of taste cells.

## What are *FTO, CNR1* and *DAT1*; and what do they do?

The *FTO* gene encodes the fat mass and obesity-associated protein (also known as alpha-ketoglutarate-dependent dioxygenase) which has been associated with food intake and satiety. The *CNR1* gene encodes the Cannabinoid Receptor type 1 (CB1) which facilitates the release of the gut hormone grehlin, causing appetite stimulation. *DAT1* is the dopamine transporter gene encoding for dopamine release and influencing reward and addiction mechanisms, including food reward.

## Does everyone have the same form of these genes?

The genetic code for these receptors can differ slightly from person to person. In each case there are different common forms of the gene which differ in amino acid sequence at specific nucleotide positions; termed Single Nucleotide Polymorphisms (SNPs). These SNPs cause variation in the functionality of the gene and, hence, can cause variation in taste sensitivity or appetite control.

**How does this affect me as an individual?**

Individual differences in the ability to taste certain bitter compounds have been shown to be related to variability in coding for the *TAS2R* genes. The intensity of perception of fat had been shown, in some studies to relate to the *CD36* genotype. Variation in *FTO* and *CNR1* have been related to differences in appetite and satiety following food intake and *DAT1* variations have been related to reward (pleasure) from eating control. Whether such differences have any effect on dietary choice and, therefore, potentially on health has yet to be fully researched and there are no firm conclusions to date. This is partly because there are many other factors, such as sex, age, learned behaviours or environmental conditions that play a large role in what and how we choose to consume.

**Implications for health insurance**

The genotyping we do is what is called 'predictive testing' and as such there is no need to disclose the results of these tests~~, at present or any time in the future,~~ to your insurance company.

**Why are researchers interested in these genes?**

We are interested to further determine if individuals of different genotype respond differently to foods, specifically to taste of foods and to satiety. In the future, rather than providing everyone with general dietary advice, it may be that a more personalised approach is taken, providing advice to suit an individual's genetic make-up

**Sources of further information**

It must be emphasised that genotyping is a relatively new area which is still at the research stage, with information in this area far from complete. If you would like to read more on this topic, you may find the following website of the Human Genetics Commission useful:

http://webarchive.nationalarchives.gov.uk/20120504100111/http://www.hgc.gov.uk/Client/Content.asp?ContentId=5

**If you have any questions or would like further information please contact the study investigators, or Dr Lisa Methven.**

# Appendix F: Mouthbehavior Screening Tool

Participants are shown the four groupings of foods and asked (1) Which is most like you, and (2) Which is not like you at all.


They are then asked the following validation questions:

1. Do you prefer products that you can chew? Yes / No
2. Do you prefer hard crunchy biscuits over soft chewy cookies? Yes / No
3. Do you prefer hard granola bars over soft chewy bars? Yes / No
4. Do you prefer hard candy (confectionary) over soft candy (confectionary) ? Yes / No
5. Do you like to suck on candy (confectionary) for a long time ? Yes / No
6. If you have breakfast cereals with milk do you let the cereal go soft before you eat it ? Yes / No
7. Do you like foods that are soft and spread through the mouth? Yes / No
8. Do you prefer ice cream with no pieces ? Yes / No
9. Do you like to eat ice cream as soon as it is out of the freezer ? Yes / No

## Appendix G: Examples of Line Scales Participants will Use for Rating in Stage 2

| | | | |
|---|---|---|---|
| How much did you like this sample? | Dislike Extremely | | Like Extremely |
| | | | |
| How sour was the sample? | Not Sour | | Very Sour |
| | | | |
| How salty was the sample? | Not Salty | | Very Salty |
| | | | |
| How fatty was the sample? | Not Fatty | | Very Fatty |
| | | | |
| How thick was the sample? | Very Thin | | Very Thick |
| | | | |
| How mouthcoating was the sample? | Not mouthcoating | | Very Mouthcoating |
| | | | |
| If you were to eat a full potion of the sample (*show picture*): | | | |
| How full do you think you would feel? | Not Full | | Very Full |
| | | | |
| How long would it be before you felt hungry? | A short time (< 30 minutes) | | A long time (> 2 hours) |

## Appendix H: Examples of Line Scales Participants will Use in Stage 3

| | | | |
|---|---|---|---|
| How hungry do you feel? | Not Hungry at all | | Extremely Hungry |
| | | | |
| How strong is your desire to eat? | Not at all | | Extremely |
| | | | |
| How full (satiated) do you feel? | Not Full at all | | Very Full |
| | | | |
| How much do you think you could (or would want to) eat right now? | Nothing at All | | A Very Large Amount |

## Appendix I: SCOFF Screening Questionnaire for Eating Disorders

1. Do you ever make yourself sick because you feel uncomfortably full?
2. Do you worry you have lost control over how much you eat?
3. Have you recently lost more than one stone in a three month period?
4. Do you believe yourself to be fat when others say you are too thin?
5. Would you say that food dominates your life?

Though not diagnostic, participants with a score of 2 or more positive answers will be screened out as at risk of eating disorder.

## Appendix J: Three-Factor Eating Questionnaire

**Volunteer No. _____** **Date:**

Directions: Please answer True of False by circling the appropriate characters (T or F)

**Part I**

| | | | | Factor Number |
|---|---|---|---|---|
| 1. | When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal. | T | F | 2 |
| 2. | I usually eat too much at social occasions, like parties and picnics. | T | F | 2 |
| 3. | I am usually so hungry that I eat more than three times a day. | T | F | 3 |
| 4. | When I have eaten my quota of calories, I am usually good about not eating any more. | T | F | 1 |
| 5. | Dieting is so hard for me because I just get too hungry. | T | F | 3 |
| 6. | I deliberately take small helpings as a means of controlling my weight. | T | F | 1 |
| 7. | Sometimes things just taste so good that I keep on eating even when I am no longer hungry. | T | F | 2 |
| 8. | Since I am often hungry, I sometimes wish while I am eating, an expert would tell that I have had enough or that I can have something more to eat. | T | F | 3 |
| 9. | When I feel anxious, I find myself eating. | T | F | 2 |
| 10. | Life is too short to worry about dieting. | T | F | 1 |
| 11. | Since my weight goes up and down, I have gone on reducing diets more than once. | T | F | 2 |
| 12. | I often feel so hungry that I just have to eat something. | T | F | 3 |
| 13. | When I am with someone who is overeating, I usually overeat too. | T | F | 2 |
| 14. | I have a pretty good idea of the number of calories in common food. | T | F | 1 |
| 15. | Sometimes when I start eating, I just can't seem to stop. | T | F | 2 |
| 16. | It is not difficult for me to leave something on my plate. | T | F | 2 |

| | | | | |
|---|---|---|---|---|
| 17. | At certain times of the day, I get hungry because I have gotten used to eating then. | T | F | 3 |
| 18. | While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it. | T | F | 1 |
| 19. | Being with someone who is eating often makes me hungry enough to eat also. | T | F | 3 |
| 20. | When I feel blue, I often overeat. | T | F | 2 |
| 21. | I enjoy eating too much to spoil it by counting calories or watching my weight. | T | F | 1 |
| 22. | When I see a real delicacy, I often get so hungry that I have to eat right away. | T | F | 3 |
| 23. | I often stop eating when I am not really full as a conscious means of limiting the amount that I eat. | T | F | 1 |
| 24. | I get so hungry that my stomach often seems like a bottomless pit. | T | F | 3 |
| 25. | My weight has hardly changed at all in the last ten years. | T | F | 2 |
| 26. | I am always hungry so it is hard for me to stop eating before I finish the food on my plate. | T | F | 3 |
| 27. | When I feel lonely, I console myself by eating. | T | F | 2 |
| 28. | I consciously hold back at meals in order not to gain weight. | T | F | 1 |
| 29. | I sometimes get very hungry late in the evening or at night. | T | F | 3 |
| 30. | I eat anything I want, any time I want. | T | F | 1 |
| 31. | Without even thinking about it, I take a long time to eat. | T | F | 2 |
| 32. | I count calories as a conscious means of controlling my weight. | T | F | 1 |
| 33. | I do not eat some foods because they make me fat. | T | F | 1 |
| 34. | I am always hungry enough to eat at any time. | T | F | 3 |
| 35. | I pay a great deal of attention to changes in my figure. | T | F | 1 |
| 36. | While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods. | T | F | 2 |

**Part II**

Directions: Please answer the following questions by circling the number above the response that is appropriate to you.

37. How often are you dieting in a conscious effort to control your weight?

| 1 | 2 | 3 | 4 | |
|---|---|---|---|---|
| rarely | sometimes | usually | always | +1 |

38. Would a weight fluctuation of 5lbs /2.3kg affect the way you live your life?

| 1 | 2 | 3 | 4 | |
|---|---|---|---|---|
| not at all | slightly | moderately | very much | +1 |

39. How often do you feel hungry?

| 1 | 2 | 3 | 4 | |
|---|---|---|---|---|
| only at mealtimes | sometimes between meals | often between meals | almost always | +3 |

40. Do your feelings of guilt about overeating help you to control your food intake?

| 1 | 2 | 3 | 4 | |
|---|---|---|---|---|
| never | rarely | often | always | +1 |

41. How difficult would it be for you to stop eating half way through dinner and not eat for the next four hours?

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | |
| easy | slightly difficult | moderately difficult | very difficult | +3 |

42. How conscious are you of what you are eating?

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | |
| not at all | slightly | moderately | extremely | +1 |

43. How frequently do you avoid 'stocking up' on tempting foods?

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | |
| almost never | seldom | usually | almost always | +1 |

44. How likely are you to shop for low calorie foods?

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | |
| unlikely | slightly unlikely | moderately likely | very likely | +1 |

45. Do you eat sensible in front of others and splurge alone?

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | |
| never | rarely | often | always | +2 |

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | |
| unlikely | slightly likely | moderately likely | very likely | +1 |

47. How frequently do you skip dessert because you are no longer hungry?

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | |
| almost never | seldom | at least once a week | almost every day | -3 |

48. How likely are you to consciously eat less than you want?

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | |
| unlikely | slightly likely | moderately likely | very likely | +1 |

49. Do you go on eating binges though you are not hungry?

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | |
| never | rarely | sometimes | at least once a week | +2 |

50. On a scale of 0-5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?

| | |
|---|---|
| 0 | |
| eat whatever you want whenever you want it | +1 |
| 1 | |
| usually eat whatever you want, whenever you want it | |
| 2 | |
| often eat whatever you want, whenever you want it | |
| 3 | |
| often limit food intake, but often 'give in' | |
| 4 | |
| usually limit food intake, rarely 'give in' | |
| 5 | |
| constantly limiting food intake, never 'giving in' | |

51. To what extent does this statement describe your eating behaviour? 'I start dieting in the morning but because of any number of things that happen during the day, by

evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'

| 1 | 2 | 3 | 4 | |
|---|---|---|---|---|
| not like me | little like me | pretty good description of me | describes me perfectly | +2 |

## Appendix K: Inform General Practitioner about subjects' results

**Hugh Sinclair Unit of Human Nutrition**
Department of Food and Nutritional Sciences
University of Reading
PO Box 226
Reading RG6 6AP
*Phone* +44 (0)118 378 7771

Date

Dear
Your patient....., date of birth....., has volunteered to take part in our human nutrition intervention study in the University of Reading entitled:
**Sensory Perception and Satiety following breakfast, snack and lunch**

Following their consent it is our standard practice to screen participants in order to exclude any health factors which may affect the outcome of our study or indicate an issue which may require further investigation. Our inclusion criteria include healthy people between 18-70 with normal haemoglobin and blood glucose levels who have no chronic illness and are not taking any prescribed medication which may affect our study outcomes. Analysis is performed in a laboratory which is not accredited but is under external quality controls.
On this occasion **some of** your patient's results **fell outside** the standard reference range for this study. Please find the results enclosed.
Therefore your patient **will not** be able to participate in the study since his/her blood test results levels **do not meet** our inclusion criteria.
Yours sincerely,
Dr Lisa Methven, *Study Investigator, Email: l.methven @reading.ac.uk*

### Screening Test Results Report

**Participant Name**_____**Date: ___ ___/ ___ ___/ 201_**

**Date of Birth**:…… **Sex:** Male/Female (circle)

**Screening Observations:**

**Blood Pressure (Average of three measurements)**.....(Right arm/Left arm)
Pulse……
**BMI** ……
**Glucose**……..mmol/L
**Haemoglobin (Hb)**……...g/dL
The above ……. (BMI/BP/glucose/ /Hb – insert appropriate) result(s) will exclude your patient from the study.

Copies of blood results attached YES NO Not Applicable (delete as necessary)

Researcher :……..Signature:……Date:……

**Appendix L (Note : this questionnaire is used because personality traits may influence eating behaviour, and to ensure that we do not have a bias of personality types between volunteers that consenting to taking of blood samples and those that opt-out of blood sampling.**

**The Big 5 Personality Test**This is a personality test, it will help you understand why you act the way that you do and how your personality is structured. Please follow the instructions below, scoring and results are on the next page.

In the table below, for each statement 1-50 mark how much you agree with on the scale 1-5, where 1=disagree, 2=slightly disagree, 3=neutral, 4=slightly agree and 5=agree, in the box to the left of it.

**Test**

| Rating | I.... | Rating | I..... |
|---|---|---|---|
| | 1. Am the life of the party. | | 26. Have little to say. |
| | 2. Feel little concern for others. | | 27. Have a soft heart. |
| | 3. Am always prepared. | | 28. Often forget to put things back in their proper place. |
| | 4. Get stressed out easily. | | 29. Get upset easily. |
| | 5. Have a rich vocabulary. | | 30. Do not have a good imagination. |
| | 6. Don't talk a lot. | | 31. Talk to a lot of different people at parties. |
| | 7. Am interested in people. | | 32. Am not really interested in others. |
| | 8. Leave my belongings around. | | 33. Like order. |
| | 9. Am relaxed most of the time. | | 34. Change my mood a lot. |
| | 10. Have difficulty understanding abstract ideas. | | 35. Am quick to understand things. |
| | 11. Feel comfortable around people. | | 36. Don't like to draw attention to myself. |
| | 12. Insult people. | | 37. Take time out for others. |
| | 13. Pay attention to details. | | 38. Shirk my duties. |
| | 14. Worry about things. | | 39. Have frequent mood swings. |
| | 15. Have a vivid imagination. | | 40. Use difficult words. |
| | 16. Keep in the background. | | 41. Don't mind being the center of attention. |
| | 17. Sympathize with others' feelings. | | 42. Feel others' emotions. |
| | 18. Make a mess of things. | | 43. Follow a schedule. |
| | 19. Seldom feel blue. | | 44. Get irritated easily. |
| | 20. Am not interested in abstract ideas. | | 45. Spend time reflecting on things. |
| | 21. Start conversations. | | 46. Am quiet around strangers. |
| | 22. Am not interested in other people's problems. | | 47. Make people feel at ease. |
| | 23. Get chores done right away. | | 48. Am exacting in my work. |
| | 24. Am easily disturbed. | | 49. Often feel blue. |
| | 25. Have excellent ideas. | | 50. Am full of ideas. |