Study Protocol

A physiological study on the effect of macronutrients delivery to the small bowel on satiety and gut hormone responses

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Start-up funds to Professor le Roux

Contents

1 Summary

Obesity is a chronic disease that has become a major nutritional health problem worldwide.

When we eat food we feel more full, but different foods with the same energy value can induce a variable amount of fullness. We previously showed that foods that have higher protein content increases fullness the most. We have also previously shown that many of the signals that induces fullness comes from the lower part of the small bowel when natural hormones such as Peptide YY (PYY) and glucagon like peptide 1 (GLP-1) is released from endocrine cells within the bowel. Our hypothesis is that the intake of mixed meals containing natural protein, fats and carbohydrates will result in more satiety gut hormones (PYY, GLP-1) when these foods can bypass the digestion processes in the upper gut, but rather be fully digested in the lower gut. To achieve this we can use microcapsules made of natural protein only which will protect the food from digestion in the upper gut. These protein capsules will release the food in the lower gut and generate maximum stimulation of the satiety gut hormones. The higher functions of the brain will respond to these strong neuroendocrine signals by ensuing fullness

The current study will provide important information on the physiological function when digestion of foods is moved from the upper to the lower small bowel as regards postprandial satiety, and food intake.

Methods: Ten healthy volunteers, aged 18 to 65 years will be recruited. Each subject will be studied on six occasions one week apart. Prior to each study day, the subjects will consume an identical meal between 19.00 and 20.00 on the night before and refrain from alcohol and strenuous exercise for the 24 hours before and after each study day.

Subject will arrive to the Clinical Research Centre at St Vincent University Hospital at 08.30 having fasted the night before from 20.00.
At 9:00 am a cannula will be inserted into the antecubital vein of the forearms. About 5 mL (1 teaspoon equivalent) will be taken to measure baseline hormone level. Another blood samples will be taken every 30 minutes thereafter. At 12:00, a final blood sample will be withdrawn and the cannula will be removed (total blood samples 7).

They will be asked to answer a questionnaire regarding your feeling of hunger, satiety and if there is any nausea on a linear scale. Then a drink containing 200 grams of macronutrient microcapsules suspension equating to 1087 kcal will be offered.

In a randomized way all subjects will receive a mixed meal containing 200 grams of macronutrient microcapsules suspension equating to 1087 kcal in their successive weekly visits:

- Option 1 High protein mixed meal in capsules that break down in the stomach,
- Option 2 High protein mixed meal in capsules that break down in the distal small bowel,
- Option 3 High fat mixed meal in capsules that break down in the stomach,

- Option 4 High fat mixed meal in capsules that break down in the distal small bowel,
- Option 5 High CHD mixed meal in capsules that break down in the stomach,
- Option 6 High CHD mixed meal in capsules that break down in the distal small bowel.

After 3 hours (i.e. at 12:00) participants will be offered standard *ad libitum* meal to measure their food consumption and another blood sample will be drawn
All subjects will be asked to rate their appetite on a Visual Analogue Scale (VAS) (see appendix B). Upon VAS completion the subjects will be allowed to go home.

Blood sample will be withdrawn from participants at the beginning and at the end of each visit. These samples will be used to quantify gut hormone levels

Conclusion:

The current study will provide important information on the physiological function of protein supplement on increasing postprandial satiety, reducing appetite and food intake. Understanding the underling neuroendocrine signalling pathway that translate the exposure of intestinal lumen to macronutrients into a healthier eating behaviour will facilitate the development of food products that will have considerable implications on controlling food consumption and managing overweight and obesity.

2 Overview

The worldwide prevalence of overweight and obesity is expected to reach 57.8% by 2030. A considerable amount of evidence supports the belief that bariatric surgery is the most effective weight-loss intervention.

The global, rapidly increasing prevalence of overweight and obesity has triggered research into food or food products that have therapeutic potential in the management of overweight and obesity. Nutrition, along with physical activity and behavioural changes, are cornerstones of obesity and T2D management. The nutrient composition of ingested food and meals varies substantially between individuals and within the same individual over time. Macronutrient composition of the diet has been shown to influence appetite and metabolic responses. Sustained satiety is a key component in the therapeutic approach to induce a negative energy balance and to promote weight loss.

Our hypothesis is that the altered exposition of the proximal small bowel to high protein microcapsules will increase satiety and would help developing natural protein formulas that would induce maximal anorexic effect.

2.1 The effect of protein intake on appetite and satiety

Bariatric surgery is the most effective method of maintaining long-term weight loss in patients with morbid obesity and for treating Type 2 Diabetes. Understanding the physiological changes after bariatric surgery has been challenging as many factors are implicated at different levels with extremely variable magnitude of effects. Following the operation patients generally prefer meals with higher protein content but lower fat and carbohydrate contents. Decrease in appetite has been described following oral application of high protein containing meals in obese patients. Dietary proteins are generally described as the most satiating nutrient. Diets high in protein have been shown to be a potential tool for weight loss. Recent studies have shown that proteins have more satiating effect than carbohydrates, which in turn are more satiating than fats.

Cholecystokinin (CCK) reduces food intake and meal size and induces satiety in a variety of mammalian species rats and humans [1]. Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are a key incretin secreted from enteroendocrine cells in the intestinal epithelium in response to an oral nutrient load. GLP-1 is synthesized and secreted by enteroendocrine L-cells which are expressed over a large portion of the GI tract, starting in the proximal small intestine and progressively increasing in density down to the distal part of the colon. PYY is present throughout the intestinal tract, with the highest concentrations in distal segments [2]. Two forms are released postprandially: PYY1–36 and PYY3–36 [3] [4], but the latter has the most potent anorectic effects when administered peripherally [5]. Several hormonal signals, including PYY, are generated during a meal, and it has been suggested that several of these signals may contribute to satiety. Recent studies have advocated a significant physiological role for PYY as a regulator of energy homeostasis and demonstrated that it mediates the satiating and weight reducing effects of dietary protein. PYY release from the intestinal tract may be inhibited in the obese, thus leaving obese subjects with a functional deficiency in PYY-induced satiety. Obese subjects have a PYY deficiency that would reduce satiety and could thus reinforce their obesity [6]. Food induced activation of gut peptides such as peptide YY (PYY) and Glucagon-Like Peptide- 1 (GLP-1) is known to reduce food intake and hunger feelings in humans [7]. The secretion of GLP-1,

CCK and peptide YY (PYY) seem to be increased in response to a high-protein diet whereas concentrations of ghrelin seem to be reduced [8] [9] [10]. The responses of the gut hormone peptide YY (PYY) to food were investigated in 20 normal-weight and 20 obese humans in response to six test meals of varying calorie content concluded that obese subjects have a PYY deficiency that would reduce satiety and could thus reinforce their obesity. In the postprandial PYY study, obese volunteers had a significantly lower fasting PYY than the normal-weight subjects. Plasma PYY peaked 90 min after the meal was ingested. A graded rise in peak plasma PYY was observed for both normal-weight and obese subjects in response to increasing calorific meals. Obese subjects, however, had a lower peak PYY response than normal-weight subjects for each calorie load. Thus, approximately double the meal calorie content was required to achieve equivalent PYY levels to those observed in normal-weight subjects. Moreover, this lower PYY level in the obese subjects was matched by a lower level of fullness after the 1000, 2000, and 3000-kcal meals as measured by VAS. The difference was significant at 30 min and was sustained until 180 min postprandially. The PYY response after 1000 kcal was not significantly different in the 500- and 900-ml protocols, evidence against a significant effect of volume on PYY response (Figure 1)

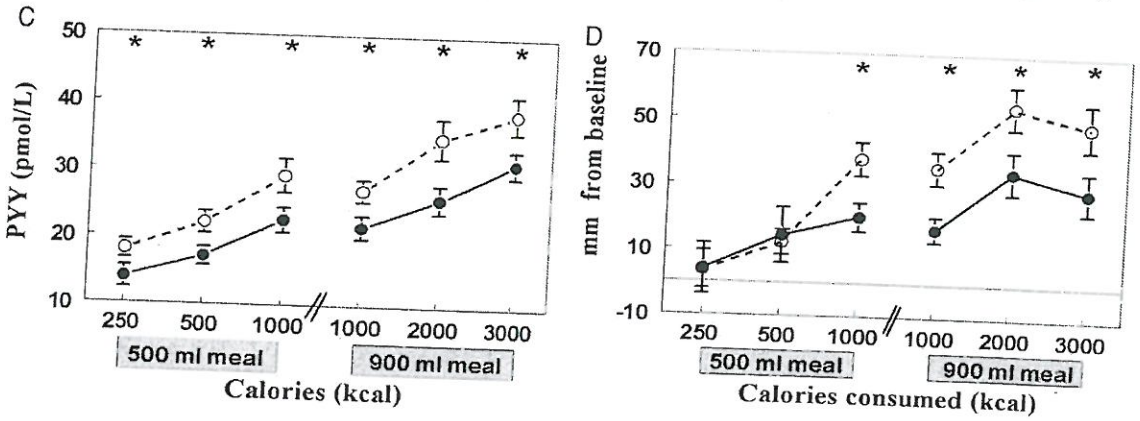

Figure 1: C, peak PYY levels in obese (●) and normal-weight (○) subjects at 90 min after the meal; D, fullness scores at 180 min after the meal, measured by VAS in the obese (●) and normal-weight (○) subjects. $P < 0.05$ (unpaired t test).

An investigational study on normal and obese human subjects found that high-protein diet caused the greatest reduction in hunger in both age-matched normal-weight and obese male volunteers. The high-protein meal resulted in the greatest increment in both total plasma PYY and integrated PYY levels in both groups, although post-meal levels were lower in obese subjects (Figure 2)

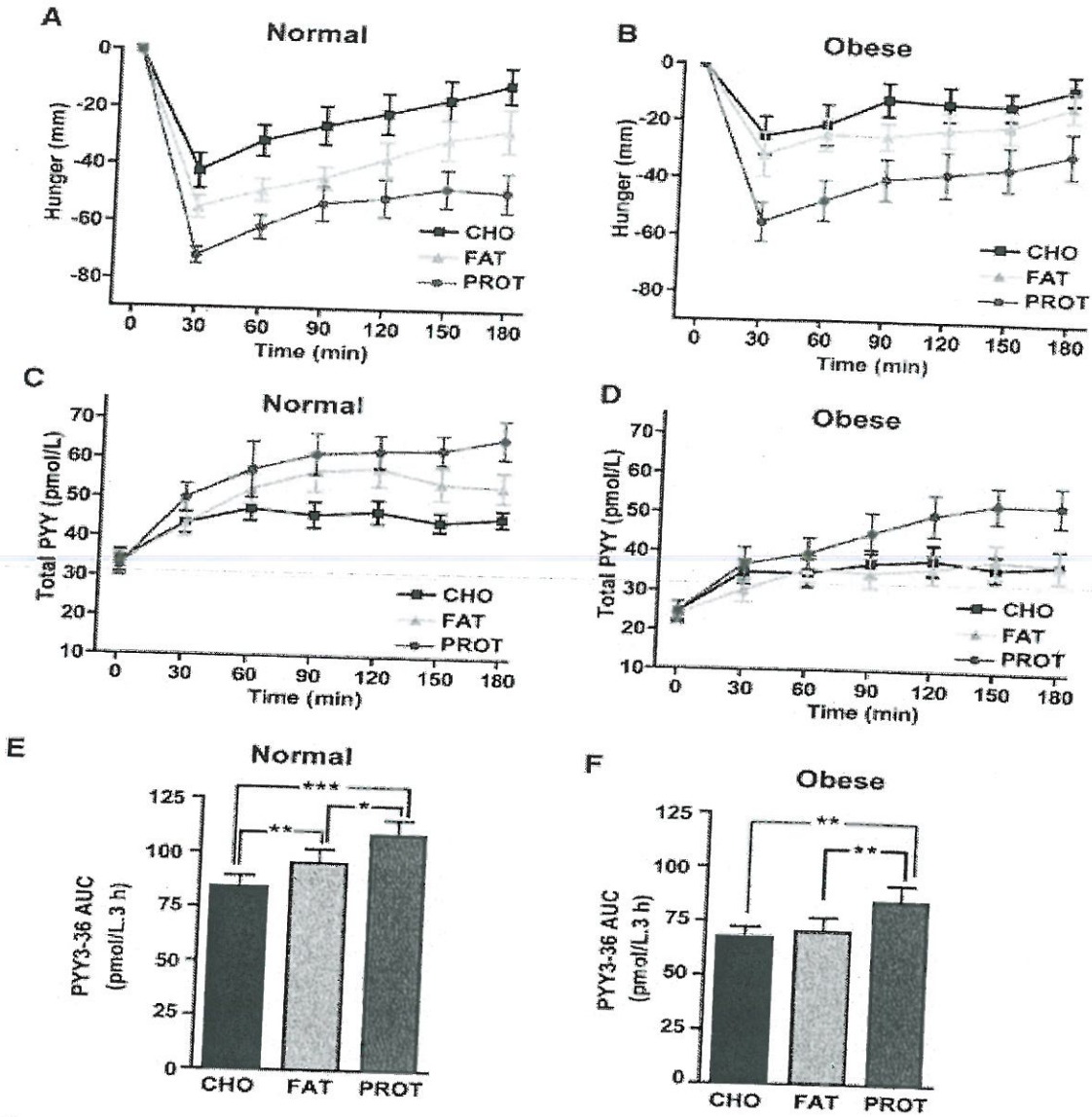

Figure 2: Hunger scores and PYY levels in normal weight and obese human subjects following high protein, high-fat, and high-carbohydrate isocaloric meals A and B) Time course of subjective visual analogue scale ratings (mm change from baseline) for hunger in normal-weight subjects (A) and obese subjects (B) after ingestion at time 0 of isocaloric meals. C and D) Time course of plasma PYY levels in normal- weight subjects (C) and obese subjects (D) after ingestion at time 0 of isocaloric meals. E and F) Area under curve (AUC) for PYY3-36 release in normal-weight subjects (E) and obese subjects (F) after ingestion at time 0 of isocaloric meals. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. PROT = high-protein; CHO = high-carbohydrate; FAT = high-fat. All values represent group mean 6 SEM, n = 10 for normal weight subjects, and n = 9 for obese subjects.

Dietary proteins are generally described as the most satiating nutrient, an effect that may be partly mediated by the stimulation of anorexigenic GI peptides secretion, including that of GLP-1. It was demonstrated that the satiating effect after a high-protein preload was significantly larger than preloads containing an iso-energetic amount of carbohydrates or fats [11] [12]. The recent expert opinion on recommended dietary allowance (RDA) for protein in older adult's ranges from 1.0 – 1.2 g/kg/d [13].

Most proteins cannot be delivered orally due to problems related to degradation in the acidic environment of the gastrointestinal tract. Most previous studies used catheters for the delivery of macronutrient. Parenteral administration of proteins has now become more common practice for daily protein administration. For oral proteins supplements it is necessary to maintain protein bioactivity during microspheres or microcapsules formation as much as possible to induce maximal stimulatory effect on gut hormones. Encapsulation is a physico-chemical or mechanical process whereby bioactive components are completely enveloped by a matrix for physical protection against potentially hazardous processes and adverse environmental conditions. The capsules are also classified as a food substance as they have a pure protein matrix that contains no synthetic elements and allows controlled disintegration due to intestinal digestion.

The goal of the present study is to investigate the physiological effect of natural macronutrients when delivered exogenously to the proximal and distal small bowel. This will mimic some aspects of the physiological changes after Roux-en-Y gastric bypass. The oral administration of macronutrients in a protective capsule will enable us to deliver them to the proximal or the distal part of the small intestine depending on the chemical and physical properties of the capsules. Our study will provide important information on the physiological role of protein, fat, and carbohydrate content of the ingested food on specific gut hormone secretion and their effects on satiety

3 Potential side effects and risks

Blood sampling can cause mild pain and/or bruising. A doctor or a trained phlebotomist will insert this with experience in obtaining blood samples to minimise the discomfort caused. We do not expect any severe side effects

4 Study hypothesis

The delivery of high protein supplement to the proximal and distal gut will increase the release of anorexigenic gut hormones that have powerful satiety effect and will reduced food intake.

5 Study Objectives and Design

5.1 Primary Aim:

To identify the physiological satiety gut hormone response and the satiety response of subjects when macronutrients are digested in the distal rather than proximal small bowel.

5.1.1 Objective 1:

To quantify the physiological gut hormones response of differential macronutrient delivery to the proximal and distal small bowel.

5.1.2 Objective 2:

To quantify the physiological satiety response of differential macronutrient delivery to the proximal and distal small bowel.

5.1.3 Objective 3:

To quantify the amount of food consumed after a preload of differential macronutrient delivery to the proximal and distal small bowel.

5.2 Study design

This is a randomized six-way crossover study. The study will include 10 healthy volunteers with each subject being studied on six separate occasions at weekly intervals (Figure 3).

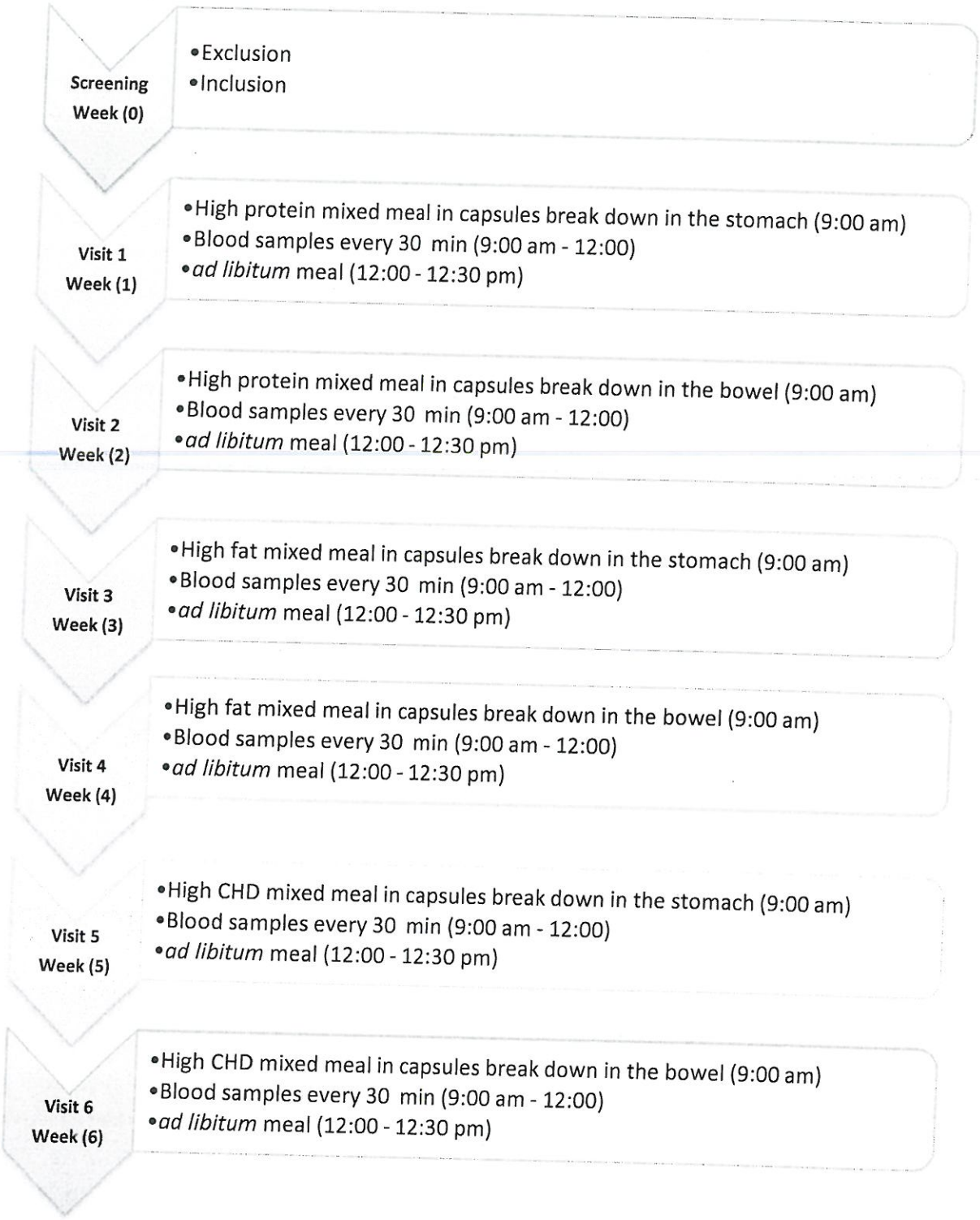

Figure 3: Schematic of Study Design

5.3 Primary endpoint

Changes in appetite as measured by visual analogue scale

5.4 Secondary endpoints

Changes in gut hormone concentrations as indicated by serum levels of these hormones

5.5 Study subjects

10 healthy volunteers of both sexes age 18 to 65 years will be recruited. Power analysis with amount energy ingested *ad lib* as endpoint indicate need for n=10 at power 80% and alpha of 0.05.

5.5.1 Design (10 subjects)

5.5.2 Macronutrient microcapsules

- The microcapsules will be stored in line with manufacturer instructions.
- As the study drug will be blinded to the research investigator
- The Microcapsules will be introduced by the research nurse and labelled with the appropriate code drink A, B, C, D, E or F.
- It will be given at 9:00 am on an empty stomach prior to the meal.

5.5.3 Study procedures

At the screening visit a doctor or nurse will then perform the following tests and procedures to determine if the subject is eligible for the study:

- Medical history
- Physical examination
- Body measurements including height, weight and waist circumference
- Record of your medications or illnesses
- Urine pregnancy test (if applicable)

Visit 1-6 (1 week apart):

Subjects will be randomised to consume a one the 6 microcapsules in each week. Internet-based randomisation software will be used to allocate microcapsules to each subject at each visit

Eligible subjects will be invited to six consecutive visits in the clinical research centre at St Vincent University Hospital. On each occasion we would request you to have identical meals the night before between 19:00 and 20:00 hours and fast thereafter. Please refrain from alcohol and strenuous exercise for 24 hours before each study visit.

1. Food intake for the 24 h before each study day should be similar.
2. Participants will be asked to consume an identical meal between 19.00 and 20.00 on the night before each study (fish or pasta meal).
3. They will also be asked to be fasted and drink only water from 20.00 the night before the study.

In each visit, participants will be asked to be in the clinical research centre at 8:30. At 9:00 am a cannula will be inserted into the antecubital vein of the forearms. About 5 mL (1 teaspoon equivalent) will be taken to measure baseline hormone level. Another blood samples will be taken every 30 minutes thereafter. At 12:00, a final blood sample will be withdrawn and the cannula will be removed (total blood samples 7). They will be asked to answer a questionnaire regarding your feeling of hunger, satiety and if there is any nausea on a linear scale. Then a drink containing 200 grams of macronutrient microcapsules suspension equating to 1087 kcal will be offered. At 12:00 an *ad libitum* meal will be offered. After the meal they will also be provided with a similar questionnaire to fill in, in order to establish your fullness and satiety level. The total visit time is around five hours and there is a total of approximately 35 ml of blood (less than 2 table spoons) at each visit 1 to 6.

6 Methodology

6.1 Recruitment

Healthy free-living individuals will be recruited through community, university campus and hospital based efforts.

6.1.1 Recruitment procedure

Recruitment will be focused on recruiting healthy free-living individuals who have normal blood sugar levels. These subjects will include those recruited by the community and hospital based protocol.

6.1.2 Proposed Recruitment methods

Different recruitment platforms will be used in order to reach our target sample size; the following sources will be used:

- Posters outlining the study initiative will be placed in public areas including hospital waiting rooms and universities, contact information for the research investigator will be available.

- Study information will be circulated internally by email at University College Dublin as well as at its affiliated hospital, St. Vincent's University Hospital.

- Social media websites / advertisements / news stories / trial websites etc. will only contain essential documentation approved by the ethics committee (patient information

sheet, trial contact details etc.). We may set up social media pages such as Twitter / Facebook which will contain basic trial contact details and referral to the website.

Potential participants identified through those methods will be able to make contact with the research investigator and they will receive written information detailing the study and be invited to attend a screening visit. If required, an open advertisement will be placed in national newspaper.

6.2 Inclusion/Exclusion criteria

The inclusion and exclusions criteria for the study are outlined below and in order to participate in the study patients must comply with these criteria.

6.2.1 Inclusion Criteria

- Age: 18-50 years
- Normal fasting glucose
- Stable body weight for at least last three months
- BMI < 30 Kg/m^2
- Capacity to consent to participate
- Independently mobile

6.2.2 Exclusion Criteria

Patients who meet any of the following criteria will be excluded:

- Pre-diabetes
- Diabetes
- Obesity
- Smoking
- Substance abuse
- Pregnancy
- Use of medications (except for oral contraceptives)
- Chronic medical or psychiatric illness
- Any significant abnormalities detected on physical examination, electrocardiography, or screening blood tests (measurement of complete blood count, electrolytes, fasting glucose, and liver function).

6.3 Visit and procedures

Table 1: Schedule of Study Visits and Events									
Trial Periods	Screening	V1	V2	V3	V4	V5	V6	Premature discontinuation	Follow up/ Premature discontinuation
Timing of visit (weeks)	**-1**	**1**	**2**	**3**	**4**	**5**	**6**		
Informed Consent	X								
Eligibility Assessment	X								
Record medications and illnesses	X							X	X
Anthropometric Measurements	X						X	X	
Blood Samples		X	X	X	X	X	X		
Randomisation		X	X	X	X	X	X		
Visual Analogue Scales		X	X	X	X	X	X		
Adverse events		X	X	X	X	X	X	X	X

6.3.1 Screening Visit

- Informed Consent
- Eligibility Assessment
- Anthropometric Measurements (Weight, height, BMI and waist circumference).

6.3.2 Subsequent study visits

6.3.2.1 Study Visit 1 - 6 (Week 1 - 6)

- Record current and past medications and illnesses
- Randomisation to protein microcapsules or placebo
- Visual Analogue Scales

6.4 Description of procedures and measurements

6.4.1 Participant Information and Consent

In advance of the study, volunteers will have received a copy of the participant information. Any questions and concerns that remain can be discussed with the investigator at the screening visit. If the volunteer meets the inclusion criteria and is satisfied to proceed with recruitment to the study, they will be asked to sign the consent form prior to the initiation of any further elements of the study visit. As part of this process, every participant will be advised of their right to refuse to participate or to withdraw at any time without impediment to their medical care in accordance with Good Clinical Practice guidelines. Withdrawal from the study can be done at any time by contacting the hospital or research team. If a participant chooses to participate, and subsequently withdraws their consent, all blood samples, records or footage in storage will be destroyed and the information stored will be deleted so it cannot be used again.

6.4.2 Medical history

A medical history will be performed at the screening visit to record illnesses, disorders and medications. This information needs to be updated on all follow-up visits.

6.4.3 Vital Signs

Pulse should be recorded at all visits after resting for five minutes in a sitting position. Systolic and diastolic blood pressure will be measured preferably in sitting position at all visits.

6.4.4 Anthropometric measurements

To minimise variability all anthropometric measurements will be taken by the same investigator throughout the study, as is practically feasible. Measurements will be taken between 8 and 10am while the volunteer is in a fasted state. Volunteers will be asked to void before measurements are performed. All measurements should be conducted in the presence of a chaperone

6.4.4.1 Weight measurement

Study participants will have their weight meaured on a digital eqilibrated scale. Volunteers will be weighted in their clothing with their shoes off and in barefeet or socks. Measurements will be recorded to the nearest 0.1Kg.

6.4.4.2 Height measurement

Study participants will have their height measured using a stadiometer. This measuremet will be taken in bare feet or socks. Patients will be asked to stand upright with their heels, buttocks and occiput touching the backboard of the stadiometer. The measuring arm are of the stadiometer will be brought down to the top of the subjects head. Height is recorded to the nearest 0.1cm.

6.4.4.3 Waist circumference

A non-stretch tape measure will be used to measure waist circumference. The measurement will be taken with the subject in a standing position with the abdomen relaxed, the arms at the sides and the feet together and breathing normally. The measurer faces the subject and places a measuring tape around the subject in a horizontal plane, at the mid-point between the lowest rib and the supra iliac crest. The measurement should be taken at the end of a normal expiration, ensuring the tape is taut around the subject's waist, without compressing the skin. Waist circumference measurements will be taken in duplicate and values will be recorded to the nearest 0.5cm. The mean value of both measurements will be recorded as the waist circumference.

6.4.4.4 Visual analogue Scales

Visual analogue scales will be used to assess subjective feelings of malaise, nausea and symptoms of flushing (Appendix B)

6.5 Data collection and storage

All screening data will be entered on a Screening Excel Database. Only individuals who match the recruitment criteria should be entered on this database. The following information should be inserted into the Screening Database.

Subjects Initials	Volunteer Code Number	DOB (dd/mm /yy)	Gender (M/F)	Ht (m)	Wt (Kg)	BMI (kg/m2)	Pulse beat/min	Blood Pressure (mm Hg)	Waist Circ. (cm)

GLP-1	PYY	Glucose	AST	ALT			

Subjects Initials – A four-digit code, the first two letters of their first and surname (e.g. HERO for Helen Roche) assigned at the screening stage. This should be also be used on the Screening CRF. Volunteer code number – A Volunteer Number should only be assigned to volunteers who are suitable for the study. Only suitable volunteers should be included in the Screening Database. Volunteer codes numbers assigned following successful screening will be used throughout the study.

6.6 Sample transport

Samples will be transported from St. Vincent's University Hospital to the Conway Institute, University College Dublin where sample analysis will take place. Samples will be stored at this location in accordance with the above instructions

6.6.1 Blood samples processing

Serum will be separated immediately by centrifugation at 4°C and then stored at - 20°C freezer until analysis.GLP-1 and PYY assays will be performed on each sample.

7 Adverse event program

7.1 Subject premature Withdrawl

If a study volunteer fails to complete the study protocol it will be documented in the individual case report form. The reason for failure to complete the study will be included in the CRF. Study Subjects may be withdrawn for the following reasons

- Adverse Event(s) (AEs)
- Abnormal laboratory value(s)
- Protocol violation
- Subject withdrew consent
- Lost to follow-up
- Death

All patients who withdraw early from the study will have their GP informed in writing that their participation in the study is complete. In the event of adverse event, GP's will be informed and arrangements will be made for subjects who have a requirement for ongoing medical follow-up as a consequence of an adverse event.

7.1.1 Adverse Events

An adverse event (AE) is defined as "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment". The principal investigator (PI) will be informed of any adverse events affecting participants within 24 hours of the research investigator becoming aware of them. The suitability of the participant to continue

with the study will be determined by the PI. All adverse events will be recorded in the CRF and the adverse events log contained within the site file. A data safety monitoring committee will be establish consisting of a senior physicians. All events will be reported within 7 working days and decisions to progress or stop the study will be made in conjunction with the Ethics committee.

7.1.2 Serious Adverse Events

A serious adverse event (SAE) or reaction is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

The term "life-threatening" in the definition of "serious" refers to an event in which the patient 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 were more severe. The principal investigator (PI) will be informed of any adverse events affecting participants within 24 hours of the research investigator becoming aware of them. The suitability of the participant to continue with the study will be determined by the PI. All adverse events will be recorded in the CRF and the adverse events log contained within the site file. A data safety monitoring committee will be establish consisting of a senior physicians. All events will be reported within 7 working days and decisions to progress or stop the study will be made in conjunction with the Ethics committee.

8 Insurance and Indemnity

Clinical staff will be covered under the institutional Clinical Indemnity Scheme (CIS).

9 Reimbursement

All participants will be reimbursed for travel and parking expenses incurred while participating. Volunteers will not be paid to participate in this study.

10 Data management

Data will be collected and stored in both paper and electronic format. Paper data will be filed and stored in a locked office at the Clinical Research Centre, St Vincents University Hospital. Electronic data will be password encrypted. All data will be handled in line with data protection legislation.

11 Statistical analysis

Data will be analysed using GraphPad Prism (version 6.0) for Windows, GraphPad software (San Diego, CA, USA) and SPSS® (version 20.0) software (SPSS, Chicago, IL, USA). Central tendencies for patient and control group demographics, anthropometric data, caloric intake, VAS scores, QoL scores and macronutrient intake will be calculated and expressed using arithmetic mean ± standard error of the mean or median (range), as appropriate. Differences in continuous variables between surgical and control groups will be calculated using the Student's *t* test or Mann-Whitney U test for parametric or non-parametric data, respectively. Correlative analyses will be performed using the Spearman Rho rank correlation to assess the relationship between *ad libitum* caloric intake and macronutrient intake with peri-operative weight loss and anthropometric data. Univariate analysis of factors associated with perioperative and post-surgical weight loss will be performed using Student's *t* test or one-way ANOVA for categorical variables or simple logistic regression for continuous variables. For the multivariate analysis, independent variables which were significant on univariate analysis, including standard demographic, oncologic and operative parameters as well as food intake and food preference, will be entered into a multiple logistic regression model with a forward stepwise selection procedure to assess their impact on perioperative and post-surgical weight loss. All statistical analyses will be two-tailed with the threshold of significance set at $P < 0.05$. Caloric intake and visual-analogue scores within groups will be compared by the Wilcoxon signed rank, matched-pairs test. Areas under the curve and plasma hormone levels will be compared by Wilcoxon rank-sum analysis.

12 Duration of Project

The estimated duration of the study will be 12 months. Screening and recruitment will begin in May 2017. Completion of the study is planned for May 2018.

13 Administrative considerations

13.1 Changes to the conduct of the study/Amendments to study protocol

Any and all amendments to the protocol must be agreed upon by the named investigators of the study. All protocol amendments must submitted to the institutional ethics committee for approval. Informed consent documents must be updated with amendments appropriately and submitted with application for protocol revision. Changes to the protocol may not be implemented prior to ethical approval unless it is intented to eliminate and immediate hazard to the study subject. In this case study sponser and ethics committee must be informed as soon as possible of the deviation.

14 Ethical and Regulatory Considerations

These physiological studies will be conducted in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments laid down by the World Medical Assemblies, and the ICH guidelines for good clinical practice (GCP), all applicable laws, rules and regulations.

15 Premature termination of the study

The principal investigator reserves the right to terminate the study at any time. Adequate consideration must be given to the protection of all study volunteers on termination of the investigation. Subjects will be informed in writing immediately and appropriate follow-up is assured.

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Appendices

Appendix A- Recruitment poster

FOOD & HEALTH

Are you interested in participating in a research study?

The Metabolic Medicine Laboratory, University College Dublin are interested in recruiting individuals for a study looking at protein supplement effect on satiety and food intake.

If you are **18 years or over**

You may be eligible.

You will be reimbursed for your travel!

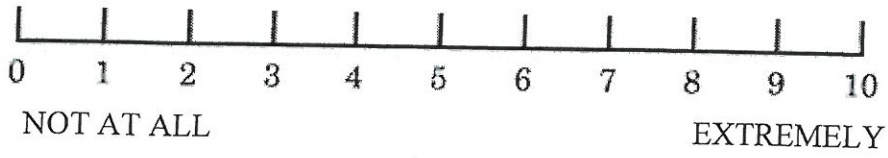
Further Information:

If you are interested and would like to know more please contact a member of the research team at 0834514783 or loai.shakerdi@ucd.ie

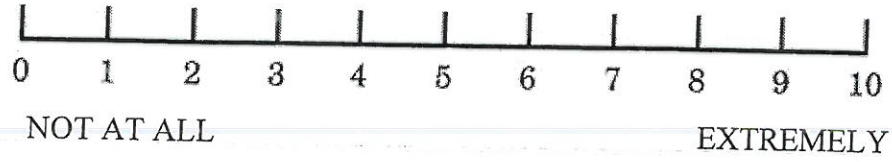
EMAIL: loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:**0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783	**EMAIL:** loai.shakerdi@ucd.ie **MOBILE:** 0834514783

Appendix B: VISUAL ANALOGUE SCALE

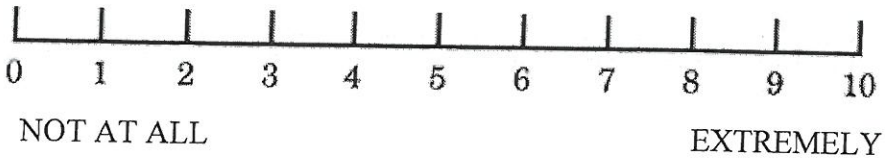
How anxious do you feel?



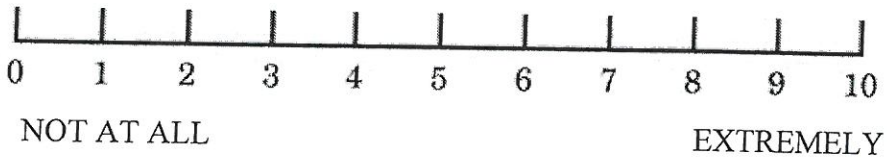
How stressed do you feel?



How sick/Nauseated do you feel?



How dizzy do you feel?



How flushed do you feel?