Study Protocol

Higher-protein vs lower-carbohydrate energy-restricted diets: effects on eating behaviour, weight loss, and body composition.

'*Do higher protein or lower carb diets differentially promote weight loss?'*

Sally Poppitt, David Cameron-Smith, Jia Jiet Lim, Amy Liu, Louise Lu

University of Auckland, New Zealand.

In collaboration with

Dr Manish Khanolkar, Auckland District Health Board (ADHB)

A/Prof Lindsay Plank, Department of Surgery Body Composition Unit, University of Auckland, New Zealand.

This project is a part of the Riddet CoRE Programme, Platform 3.

Principle Investigators

Prof. Sally Poppitt, PhD

Professor of Human Nutrition
Director, Human Nutrition Unit (HNU),
School of Biological Sciences & Department of Medicine,
University of Auckland.
Telephone: 09 630 5160
Email: s.poppitt@auckland.ac.nz

Jia Jiet Lim, BSc (Hons)

PhD Candidate
Human Nutrition Unit,
University of Auckland.
Telephone: 09 630 1662
Email: jlim287@aucklanduni.ac.nz

Associate Investigators

Prof. David Cameron-Smith, PhD

Professor of Nutrition
Liggins Institute,
University of Auckland.
Email: d.cameron-smith@auckland.ac.nz

Amy Liu, MSc

Human Nutrition Unit,
University of Auckland.

Practice Supervisor Diabetes Dietitian and Community Adults,
Auckland Diabetes Centre,
Auckland District Health Board.
Telephone: 09 630 9980
Email: amy.liu@adhb.govt.nz

Dr Louise Lu, PhD

Post-doctoral research fellow
Human Nutrition Unit,
University of Auckland.
Telephone: 09 630 1662
Email: louise.lu@auckland.ac.nz

Collaborators

Dr Manish Khanolkar, MBChB

Diabetes Consultant
Auckland District Health Board.

Department of Medicine,
University of Auckland.
Telephone: 021 815 901
Email: manishk@adhb.govt.nz

A/Prof. Lindsay Plank, PhD

Head, Body Composition Unit
Department of Surgery
University of Auckland
Email: l.plank@auckland.ac.nz

Summary

Approximately a third of the world population is overweight or obese. Obesity and its metabolic consequences are arguably the most important public health issues of the 21st century. Irrespective of the underpinning environmental, genetic or dietary driver, an inability to match energy intake to energy expenditure is the primary cause of weight gain. In essence, weight loss can be achieved by reducing energy intake and/or increasing physical activity. Therefore, most dietary strategies that focus on energy restriction, e.g. various low-energy meal plans, will achieve some weight loss in the short-term. However such strategies, have varying degrees of success in the long-term, with weight re-gain very common. Few if any are successful in maintaining weight loss, which is likely associated with the difficulty in adhering to a diet over many weeks or months, arising in turn from the concomitant increase in the drive to eat that accompanies restricted intake or 'dieting'. How best to achieve successful long-term weight loss is a topic that attracts considerable attention, with much focus on the mechanisms that both drive and suppress appetite, and the role that various foods and nutrients play in this process.

Recently there has been growing interest in studies focusing on protein-induced weight loss. It has long been shown that the macronutrients differentially affect appetite when consumed on an iso-energetic (same amount of energy) basis. Explanations for the apparent success of protein-induced weight loss revolve around (i) the appetite control, (ii) the higher diet-induced thermogenesis (DIT) due to the high costs of protein digestion and absorption, as well as (iii) the changes in body composition in favour of lean mass if a higher protein diet is accompanied by increased physical activity. Short-term studies have shown that a higher-protein preload reduces hunger and increases feelings of satiety, in turn leading to a reduction in *ad lib* food intake, when compared with an iso-energetic non-protein preload. Additionally, long-term studies have shown that prescription of a higher-protein weight loss diet leads to a greater reduction in fat mass while sparing fat-free mass, which in turn prevents a drop in resting energy expenditure during and after the weight loss period.

In higher-protein weight loss diets, commonly protein is added into the diet in exchange for the carbohydrate (CHO) or fat portion in order to achieve an iso-energetic diet. Notably, low-CHO diets (e.g. the Atkins diet) have long been popular among the public for body weight management, with some evidence of efficacy in the scientific literature, however, it is not well understood whether it is the higher-protein or the lower-CHO component of the diet that leads to successful weight loss. This intervention trial intends to unravel this problem by carrying out a weight-loss intervention which compares the effects of higher-protein and lower-CHO diets on change in body weight, as well as change in appetite responses and eating behaviour, and change in body composition associated with those diets.

1. Background

1.1 The obesity 'epidemic'

Obesity is defined as "an excessively high amount of body fat (adipose tissue) in relation to lean body mass" (Ministry of Health New Zealand, 2017c). International cutoffs for overweight and obesity are body mass index (BMI) >25 and >30kg/m^2 respectively. The global prevalence of overweight and obesity has escalated over the past few decades, from 1980 (28.8% in males and 29.8% in females) to 2013 (36.9% in males and 38.0% in females) and it is expected to continue to rise (Ng et al., 2014). Obesity is a critical public health issue, with no evidence of improvement in any country although public health strategies are in place. Furthermore, New Zealand ranks third in the prevalence of obesity among OECD countries (OECD, 2017), with approximately a third of adults being overweight and a third of adults being obese, which has been tripled since 1977 (Ministry of Health New Zealand, 2017b).

Obesity becomes a heavy burden for health as each 5kg/m^2 increment in BMI increases the overall mortality rate by 30%, primarily from vascular disease, renal disease, liver disease, diabetes, or cancer (Prospective Studies Collaboration, 2009). As a result, weight management becomes crucial to reduce the risks of these non-communicable diseases. A modest weight loss between 5% and 10% of body weight is considered as a realistic and clinically relevant goal for the management of type-2 diabetes and the improvement of metabolic and cardiovascular health (Lau and Teoh, 2013, Wing et al., 2011).

1.2 Macronutrient composition of weight loss diets

There have been many weight loss diets that focus on the different macronutrients of protein, carbohydrate (CHO) and fat. There is currently no optimal macronutrient composition for weight management, with most diets focused on CHO and fat. In a recent study, Thom and Lean (2017) did not find a clinically significant difference in terms of body weight and metabolic health between diets of various macronutrient composition (eg. low-fat vs low-CHO diet). The current Clinical Guidelines for Weight Management in New Zealand Adults agrees with these findings by allowing the practice of various diets for weight management based on the individual's preference, including very-low energy diets (VLED) (≈1937kJ/day), low-CHO (≤40% total energy intake from CHO) diets, very-low-CHO diets (≤20% total energy intake from CHO, 20 to 60g per day), low-fat diets (≤10% total energy intake from fat), high-protein diet (≥35% total energy intake from protein) and high-CHO diets (≥65% total energy intake from carbohydrate), the Mediterranean diet, DASH diet, intermittent fasting diet, low glycaemic index/load diet and vegetarian diet (Ministry of Health New Zealand, 2017a).

Although most energy-restricted weight loss diets or programmes can induce some weight loss, low-CHO/higher-protein diets have been shown to promote better weight maintenance for a period of at least 12 months (Clifton et al., 2014). Furthermore, the DioGenes study demonstrated that a high-protein diet when coupled with low-glycaemic index (GI) is associated with least weight regain during weight maintenance (Larsen et al., 2010). Although low-fat diets were originally popularised for weight loss, more recently low-CHO diets have been shown to be as efficacious or even superior to low-fat diets (Hession et al., 2009). Also more recently, higher-protein weight loss diets have gained substantial interest among the public as well as the researcher community (Westerterp-Plantenga et al., 2012). Interestingly, the higher protein composition of these diets is often accompanied by a lower-CHO content, such as in the Atkins diet. Therefore, in this study, we aim to investigate the causal role of higher-protein diets in weight loss, independent of the CHO component of the diet, when consumed as part of a low energy diet (LED) regime.

A commercial LED generally provides at least 3.3MJ/day energy, and consists of meal replacements. Despite its low energy content, an LED regime is nutrient-rich, delivering a complete range of micronutrients and minerals for good health. Therefore, LEDs can be consumed as the sole source of nutrients, but can also be consumed alongside other self-prepared meals. LEDs are commonly consumed over periods of 6-8 weeks, but can be continued over many months in morbidly obese individuals aiming for significant body weight loss. The average amount of weight loss when adhering to a total diet replacement regime using a commercial 4MJ/day LED is 1 to 2 kg per week, with greatest weight loss in the first 2 weeks. Physical activity is restricted during the LED phase, with participants following a sedentary lifestyle. The efficacy of an LED regime to induce weight loss over period of 8 weeks have been shown to be similar to a VLED regime (Christensen et al., 2011). An example of the commercially available LED is the Cambridge Diet (Cambridge Weight Plan™ Ltd, UK), which will be used in this study. Body weight loss for an LED over an 8 week period is expected to be at least 8% of baseline body weight (Christensen et al., 2018), with absolute weight loss predicted by baseline body weight when LED intake is fixed. Modification of the LED regime, where the meal replacements are supplemented with a restricted home diet, is expected to result in more variable daily energy intake and hence more variable weight loss. Flexibility of energy intake is required in this intervention in order to identify differences induced by the higher protein and/or higher CHO component of the diet.

1.3 Blood biomarkers of appetite and the response to weight change

The success of a weight loss diet depends on the long-term adherence to a diet, which makes the concomitant increase in hunger during periods of weight loss one of the biggest obstacles for successful weight management. Therefore, maintaining a prolonged feeling of satiety has been proposed to improve adherence to a weight loss programme (Hetherington et al., 2013). However, change in appetite sensations (e.g. increased hunger and desire to eat) is a strong physiological response during energy deficit to defend the body against weight loss, as an evolutionary survival instinct (Sumithran and Proietto, 2013). Consequently, the goal to suppress feelings of hunger during energy deficit is a challenge.

Trying to understand the physiology of appetite and its interaction with the diet may provide some insight on successful weight management. For the past two decades, investigations into the signals that underpin appetite response has been growing, such as changes in the circulating gastrointestinal peptides including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). They are known as the anorectic hormones, and have been shown to significantly reduce appetite sensations following direct intravenous injection into human participants (Lieverse et al., 1995, Verdich et al., 2001, le Roux et al., 2008). Dieting results in a disadvantageous shift in anorectic GI peptides and this alteration may even persist up to a year (Sumithran et al., 2011). This observation suggests that there is a conflict between the physiological adaptation to prevent energy deficit and the need to control food intake for better metabolic health. Consequently, this inherent conflict may eventually undermine the long-term effectiveness of a weight-loss diet, where strong feelings of hunger and associated appetite responses prevent an overweight individual from maintaining an energy restricted weight loss regime.

In contrast, the opposite shift in anorectic GI peptides is associated with the improvement in body weight and metabolic health as evidenced by bariatric surgery studies. A previous study has shown that postprandial peaks of anorectic GI peptides were significantly higher for obese individuals after Roux-en-Y Gastric Bypass (RYGB) surgery weight-loss compared to energy-balanced lean individuals (le Roux et al., 2006). This observation is in contrast to dietary-induced weight loss. The increase in response of GI peptides-secreting cells as a result of RYGB has led to the proposal that sustained elevated levels of these anorectic GI peptides, for example induced by a higher-protein diet, may help with weight management without the need for surgery. There is no doubt that in short-term studies, a higher-protein meal has resulted in favourable changes in the biomarkers of appetite under energy-balanced condition (Brennan et al., 2012, Chungchunlam et al., 2015, Hutchison et al., 2015). Mimicking the

change in GI peptides post-RYGB to a smaller degree could be promising with a higher-protein diet. Despite limited evidence on the long-term effect of higher-protein diet on biomarkers of appetite, long-term voluntary reduction in energy intake has been demonstrated when adhering to higher-protein diet (Weigle et al., 2005). In this current study, we are interested to see if higher-protein diet can at least ameliorate or prevent the unfavourable shift in GI peptides, commonly seen during energy deficit and the subsequent restoration of energy balance, to help with the success of protein-induced weight loss.

1.4 Exploring plasma amino acid profile as a unique signature for appetite sensation, obesity and type-2 diabetes

The relationship between postprandial plasma amino acids (AAs) and appetite was first hypothesised by Mellinkoff et al. (1956) to suggest that the abundance of AAs in plasma may result in the waning of appetite, with the fall in plasma AA concentrations having the opposite effect. Several studies have been conducted recently to test this hypothesis (Luscombe-Marsh et al., 2016, Chungchunlam et al., 2015, Korompokis et al., 2016, Veldhorst et al., 2009) to demonstrate that it is a particular profile of AAs, rather than the total concentration, that may be involved in the regulation of appetite control. This is in line with previous reports that have shown that the difference in postprandial total circulating concentration of AAs may not directly translate into satiety or satiation (Poppitt et al., 2013). Therefore, plasma AA profiles could potentially provide information on the possible role of protein quality and protein source in the regulation of appetite.

It is noteworthy that individuals with type 2 diabetes (T2D) have elevated fasting AA concentrations (Nakamura et al., 2014). Mook-Kanamori et al. (2016) added to the existing evidence that there are notable differences in postprandial plasma AA response between healthy and diabetic individuals. One hypothesis is that the increase in branched-chain amino acids (BCAA) in blood may activate the mTOR pathway which contributes to insulin resistance in a high fat diet, despite acknowledging the fact that supplementation of BCAA in the diet slows weight gain in a high fat diet (Newgard et al., 2009).

Since weight loss is one of the best strategies to improve metabolic health, improvement in plasma AA profile is commonly found to accompany weight loss (Tochikubo et al., 2016, Geidenstam et al., 2017). Although a higher-protein weight loss diet is reasonably successful in improving metabolic health, the longer term changes in plasma AA profile and how the profile responses to food intake are not known. In short, characterising plasma AA profile is a relatively new research area where more studies need to be conducted to identify a signature which defines an increased risk of impaired metabolic health.

1.5 Dietary protein requirement and body composition

A typical Australia and New Zealand diet contains 10% to 20% energy from protein, and the recommended dietary intake (RDI) is 0.84g/day protein per kg body weight for adult men and 0.75g/day protein per kg body weight for adult women (National Health and Medical Reseach Council et al., 2006). The RDI is set based on the estimated amount required for growth and maintenance of fat-free mass under energy-balanced circumstances.

However, there is no standard guideline on the amount of protein required to exert its satiety-inducing properties and beneficial effects on weight management. Studies comparing lower-protein versus higher-protein, or normal-protein versus higher-protein have always been a relative comparison. The success of studies comparing two doses of dietary protein intake in weight management depends on two factors: (i) the difference of protein intake (g/kg/day) between groups, (ii) the change from protein intake (g/kg/day) during intervention from baseline (Bosse and Dixon, 2012). Improvement in anthropometric measurement is more likely when the protein intake of an intervention is approximately 50% higher than the RDI, or when the protein intake is approximately 40% above an individual's baseline intake (Bosse and Dixon, 2012). Hence, higher protein intake in this weight loss study is targeted at 1.2g/kg/day, which is approximately 50% higher than the RDI.

Since females and males have different body composition and energy requirement (Wu and O'Sullivan, 2011), it is sensible to consider that a dietary intervention may affect weight loss and body composition differently between genders (Wirth and Steinmetz, 1998). A recent large-cohort PREVIEW study showed that males lost significantly more weight than females using the same intervention diet, additionally males lose more fat than females, potentially led to greater improvement in metabolic risk (Christensen et al., 2018). Nevertheless, females were more likely than males to participate in a structured weight-loss program (Bhogal and Langford, 2014, Crane et al., 2017). On this basis, this study will recruit female participants only to decrease the variability of response in a smaller cohort. Therefore, the power of this study can be improved to identify a significant effect of higher-protein vs. lower-CHO diets on weight loss.

We are interested in two compartments of body composition, fat mass and fat-free mass. Fat-free mass contributes to energy expenditure at rest whereas fat mass does not. Hence, weight loss should aim at reducing fat mass whilst preserving fat-free mass to prevent a drop in resting energy expenditure alongside the drop in energy intake. Under energy restricted conditions, it is recommended to maintain absolute protein intake at RDI (0.8g/kg/day) for weight loss and weight maintenance, whereas higher-protein intake (1.2g/kg/day) is

recommended for preservation of fat-free mass, and hence resting energy expenditure, during weight loss and weight maintenance (Soenen et al., 2013). As a result, the percentage of energy from protein turns out to be less important as compared to absolute protein intake (Westerterp-Plantenga, 2007). Therefore, the macronutrient composition for a standard diet could not be applied to a weight loss or weight maintenance diet. This view is also in agreement with protein leverage hypothesis, which proposed that there is an absolute protein intake requirement for our physiology. According to protein leverage hypothesis, there is a drive to eat until absolute protein requirement is met. Notably one has to increase the consumption of food hence total energy intake to achieve this requirement with a diet of low protein to CHO or fat ratio (=high energy intake); whereas eating is inhibited when this requirement is met even under energy deficit with a diet of high protein to CHO or fat ratio (=low energy intake) (Simpson and Raubenheimer, 2005). There is little clinical evidence as yet in support of the protein leverage hypothesis which has not been clearly demonstrated, but the hypothesis remains of some interest.

2. Aims, objectives and hypothesis

2.1 Aims

The study aims to evaluate whether a higher-protein diet can induce successful weight loss independent of a concurrent low-CHO content, under conditions of negative energy balance achieved through the use of a Low Energy Diet (LED) over 8 weeks, by assessing several determinants of successful weight loss:

i. Change in body weight
ii. Change in subjective appetite sensations
iii. Change in body composition

2.2 Objectives

The objectives of this 4-treatment study are to compare the effects of intervention with 2 x High-Protein vs 2 x Normal-Protein LEDs, each differing in CHO content, as follows:

High-Protein Normal-CHO diet (HPNC), High-Protein Low-CHO diet (HPLC), vs.

Normal-Protein Normal-CHO diet (NPNC), Normal-Protein Low-CHO diet (NPLC),

on:

i. Body weight loss following 8 weeks of intervention
ii. Biomarkers of appetite (plasma concentration of glucose, insulin, amino acids, CCK, GLP-1 and PYY), subjective appetite sensations (hunger, fullness, satisfaction and thoughts of food), assessed using a preload challenge at baseline and 8 weeks after intervention
iii. Retention of fat-free mass and reduction of fat mass following 8 weeks of intervention, compared to baseline.

2.3 Hypothesis

A higher-protein LED diet may induce greater body weight loss than a normal-protein LED diet, irrespective of CHO content, during a period of rapid weight loss. In addition, a higher-protein LED may suppress hunger and promote fat loss while retaining greater fat-free mass.

3. Methods

3.1 Trial Design

This trial is conducted as a 4-arm parallel intervention study, comparing 4 different weight loss LEDs based on a 2 x 2 factorial design. The study will comprise 2 higher-protein diet treatments and 2 lower-CHO diet treatments.

Table 1. Four treatments in the study.

Components	High-Protein (HP)	Normal-Protein (NP)
Normal-CHO (NC)	HPNC	NPNC
Low-CHO (LC)	HPLC	NPLC

Each eligible participant will be randomised to one of the 4 intervention arms and will need to adhere to the assigned diets for 8 weeks. The target daily energy intake during the 8-week weight-loss period is approximately 40% of their daily energy requirement at the time of screening, calculated using the Harris-Benedict's Equation and participants will be assigned to the nearest 3.9 or 4.6MJ diet plan. An LED will be used in part to achieve this dietary target.

HP diet (50en%) is aimed to provide 1.2g/kg/day protein; whereas NP diet (35en%) is aimed to provide 0.8g/kg/day protein. Conversely, the LC arm is aimed to provide 28en% CHO; whereas the NC arm is aimed to provide 40en% CHO. The balance of energy for each diet treatment will comprise dietary lipid. The macronutrient composition of each diet during the weight-loss period is shown in Table 2; whereas the absolute intake of macronutrients (grams) for each intervention is shown in Appendix 1. Throughout the intervention period, participants are asked to maintain their usual physical activity level.

Table 2. Macronutrient composition of the intervention diets.

High-Protein (HP)		Normal-Protein (NP)	
Normal-CHO (NC)	Low- CHO (LC)	Normal- CHO (NC)	Low- CHO (LC)
50en% Protein 40en% CHO 10en% Fat	50en% Protein 28en% CHO 22en% Fat	35en% Protein 40en% CHO 25en% Fat	35en% Protein 28en% CHO 38en% Fat

en%, percentage of energy

Investigators will explain the macronutrient composition of the diet to participants either individually or as a group of participants who are assigned to the same intervention arm.

3.2 Low Energy Diet (LED)

Commercial LED meal replacements (Cambridge Weight Plan™ Ltd) will be provided to participants at no cost to provide the base energy and macronutrient content of their daily diet. The LEDs offer a range of products, including shakes, soups, bars, porridge, pasta and savoury meals, which in turn are comprised of different macronutrient composition. Therefore, participants in different intervention arms will receive products with macronutrient composition that will align with the intervention arm (Table 2). Participants must consume the LED meal replacements throughout the 8-week weight loss period.

Participants will be recommended to have a total of 4 eating occasions every day, which will consist of 1 fixed oat breakfast, 2 fixed commercial LED meal replacements (participants must adhere to the recommended intake, ≈1.7MJ/day) and 1 variable home meal (participants may eat less if not hungry or eat a little more if needed). An example for the recommended fixed and variable meals is shown in Table 3. The recommended total daily energy intake is either 3.9MJ or 4.6MJ, depending on the participants' energy requirement.

Table 3. Recommended eating occasions and meal format in a day.

Eating occasions	**Meal format**	**Comment**
Breakfast	Fixed – oat breakfast	To prepare and consume the oat breakfast completely
Lunch	Fixed – meal replacement	To consume meal replacements completely.
Afternoon Tea	Fixed – meal replacement	
Dinner	Variable – home prepared meal	To consume additional food items to meet the absolute protein (g/kg/day) and overall macronutrient composition (en%) targets.

Participants will buy the ingredients to prepare oat breakfasts and home prepared meals. They will be given oral and written instructions on how to prepare this breakfast. Participants must consume the oat breakfast completely. In addition, the participants will consume 2 fixed LED meal replacements every day as lunch and afternoon tea. Following that, participants will be advised to prepare and consume a variable home meal, comprising high/normal protein in combination with normal/low CHO each day, in order to achieve the differential macronutrient composition of the 4 intervention diets. A diet plan booklet containing various food items and portion sizes, specific for each intervention arm, will be given to participants to help the preparation of the variable home meal. The food items used to achieve protein,

CHO or fat targeted intake will be identical across different arms where possible (Table 4). For example, poultry and fish are recommended to attain the protein target; however a greater portion size is recommended to achieve a HP diet whereas a smaller portion size is recommended to achieve a NP diet.

The variable meal will allow intake to vary (*ad lib*) in response to feelings of hunger and fullness. This will ensure that total intake for the day (fixed and variable meals), and hence body weight, can vary throughout the 8-week LED intervention. This is essential to promote a differential energy intake between the treatment arms. Despite *ad lib* intake, participants will be advised at the dietary counselling sessions to adhere to dietary recommendations (Table 4) to meet the absolute protein (g/kg/day) and overall macronutrient (en%) targets, as well as to maintain a substantial energy-deficit in order to promote weight loss. Additionally, the guidelines/principles governing the dietary recommendations are:

HPNC: Consume high protein items, plus some carbohydrate but no fat.

HPLC: Consume high protein items, plus some 'good' (unsaturated) fat but no carbohydrate.

NPNC: Consume moderate protein items, plus some carbohydrate and 'good' (unsaturated) fat.

NPLC: Consume moderate protein items, plus greater consumption of 'good' (unsaturated) fat but not carbohydrate.

Table 4. Examples of dietary recommendations in a day.

Intervention arm	**HPNC 50/40/10en%**	**HPLC 50/28/22en%**	**NPNC 35/40/25en%**	**NPLC 35/28/38en%**
Recommended food	Cambridge LED[1] Poultry, fish Fat-free dairy Couscous, rice WPI	Cambridge LED[1] Poultry, fish Olive oil, avocado WPI	Cambridge LED[1] Poultry, fish Low-fat dairy Couscous, rice Olive oil, avocado	Cambridge LED[1] Poultry, fish Olive oil, avocado

en%: protein/CHO/fat

[1]Participants must consume Cambridge LED meal replacements as part of the fixed meal intake.

WPI, Whey Protein Isolate.

In addition to reaching the target macronutrient composition, all participants will also be recommended to drink 9 glasses of water per day and avoid all energy-containing beverages (e.g. alcohol, sugar-sweetened beverages SSBs). Additional supplements on all arms to

avoid constipation due to the low fibre intake of the LED may be prescribed by the research team if required.

3.3 Randomisation

Randomisation will be undertaken using an online randomiser tool (https://www.randomizer.org/) to randomise eligible participants into one of the four intervention arms.

3.4 Participants

Inclusion criteria

i. Females aged between 18 and 65 years.
ii. Obese (BMI 30 kg/m^2 - 45kg/m^2) with a maximum body weight of 130kg
iii. Otherwise healthy

Exclusion criteria

i. Recent body weight loss/gain >5% within previous 3 months
ii. Currently taking part in an active diet program
iii. Current medications or other conditions known to affect body weight and appetite
iv. Previous bariatric surgery
v. Diagnosed with impaired liver or kidney function
vi. Significant current disease such as type 2 diabetes, cardiovascular disease, or cancer; or digestive disease including inflammatory bowel syndrome/disease (IBS/D), ulcerative colitis (UC), Crohn's disease
vii. Systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100mmHg
viii. Depression or any other anxiety disorder known to affect appetite
ix. Unable to consume food items included in the study, or hypersensitivities or allergies to these foods (based on Food Preference Questionnaire)
x. Smokers or ex-smokers who have given up smoking for less than 6 months
xi. Pregnant or breastfeeding women
xii. Unwilling/unable to comply with the study protocol

3.5 Power calculation

The power calculation formula for detecting the difference between two means is used:

$$k = \frac{n_2}{n_1} = 1$$
$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$
$$n_1 = \frac{(4^2 + 4^2/1)(1.96 + 0.84)^2}{2.82^2}$$
$$n_1 = 32$$
$$n_2 = K * n_1 = 32$$

n_1, n_2 = Sample size for group 1 and group 2

K = Ratio of sample size for group 2 to group 1

ó = Standard deviation using previous data

$Z_{1-\alpha/2}$: This depends on level of significance, for 5% this is 1.96

$Z_{1-\beta}$: This depends on power, for 80% this is 0.84

Δ = Clinically significant difference between two groups

It is performed based on the data from a similar study conducted in The Netherlands (Soenen et al., 2012). The HP diet in this study resulted in a mean body weight reduction of 14kg over 12 weeks, with a standard deviation of 4kg. By applying the data into the formula, it showed that a sample size of 32 participants per diet arm is required to detect a difference of 2.8kg (20% of 14kg) in body weight between two diet groups (HP vs LC) with 80% power and a 5% level of significance. Assuming a dropout rate of 10%, we target recruitment of 35 participants per diet arm. Since there are 4 intervention diets (HPNC, HPLC, NPNC, and NPLC) in this study, a total of 140 participants will be recruited.

3.6 Participant recruitment and pre-screening

Recruitment of participants will be conducted in the Auckland region using convenient sampling method. Participants will be recruited through various media, by posting the ethics-approved advertisement on newspapers, press release, email, social gatherings, public notice boards, blog sites and medical centres. Participants who completed previous studies at HNU will be invited back for this study if they wish to participate.

Individuals who are interested in this study may contact the investigators for a remote pre-screening (either online questionnaire or telephone interview) and a Participant Information

Sheet (PIS), which will be emailed or posted to them. Data on age, ethnicity, reported body weight and height, brief medical record, current medications will be collected during the pre-screening to ensure that inclusion/exclusion criteria are met before attending Human Nutrition Unit (HNU) for a screening visit.

3.7 Clinic visits

The summary of the screening and clinical investigation days (CIDs) are shown in Table 5. Participants are required to attend all visits.

Table 5. Agenda during each visit at HNU.

Agenda	Screening	Baseline	8-week weight loss	
		CID 1 Week 0	CID 2 Week 4	CID 3 Week 8
Informed consent	✓			
Anthropometric measurement[1]	✓	✓	✓	✓
Blood pressure	✓	✓	✓	✓
Fasting blood sample	✓	✓	✓	✓
24-hour urine		✓	✓	✓
4-day food record		✓	✓	✓
DXA		✓		✓
Preload challenge		✓		✓
3-factor eating questionnaire		✓		✓
Baecke Questionnaire		✓		✓
Dietary consultation		✓	✓	✓

[1] Anthropometric measurements include body weight, body height and waist/hip circumference.

Screening visit

During the screening visit, participants are required to arrive at HNU after an overnight fast of at least 10 hours, having consumed nothing but water since the prior evening meal.

Participants will receive a Participant Information Sheet (PIS) and the study will be explained by a researcher. Participants will be given the opportunity to ask questions and written informed consent will be obtained. Participants will then be screened for eligibility. Demographics (age and ethnicity) and anthropometry (height, body weight, waist and hip circumference, BMI and blood pressure) will be recorded. A venous blood sample will be collected to check for fasting plasma glucose (FPG) concentration on site to screen for diabetes. When FPG is ≥ 7.0mmol/L, another venous blood sample will be collected to check for HbA1c. When HbA1c ≥ 50mmol/L, participant's GP will be contacted to confirm whether the participant has diabetes.

After confirming the participant's eligibility, participants will be given a urine bottle, alongside verbal and written instruction on how to collect urine samples. Additionally, participants will be taught to complete a 4-day food record (3 weekdays and 1 weekend) by investigators. Participants are asked to collect the urine sample on one of the days of the food record. The urine samples and 4-day food records will be completed during the week before attending CID1 and CID 3.

Clinical Investigation Days (CIDs)

For all CIDs, participants are required to arrive at HNU after an overnight fast of approximately 10 hours, having consumed nothing but water since the prior evening meal.

At CID1 (Week 0), the visit will take approximately 5 hours. Participants are required to arrive at HNU at 8am for a preload challenge (see Section 3.7). After the preload challenge, participants will be given a lunch meal. Participants are also required to complete a 3-factor eating questionnaire (3FEQ) and a Baecke Questionnaire. It will be followed by a dietary consultation session with an investigator. Finally, participants will be invited to the Auckland City Hospital to perform a body composition scan using DXA (see Section 3.9). The 8-week weight loss intervention period will be commenced after CID1, whereby participants are required to adhere to their assigned intervention diets (HPNC/HPLC/NPNC/NPLC). The intervention comes to the end upon the completion of CID3.

At CID 2 (Week 4), the visit will take approximately 30 minutes. Participants are required to arrive in the morning at any time between 8:00am and 11:00am for their anthropometric measurements to be taken, following by blood pressure. Then a Research Nurse will collect 7mL of fasting blood. Finally, there will be a dietary consultation session with an investigator.

At CID 3 (Week 8), the visit itinerary will be the same as CID 1. The study is completed upon the end of CID 3 visit.

3.8 Preload challenge

The protocol for preload challenge is adapted from the standard international guidelines as recommended by Blundell et al. (2010). The purpose of this preload challenge is to investigate the change in appetite associated with each of the 4 weight-loss intervention diets.

Schedule for the preload challenge visit

The preload challenge will take place at the Appetite Research Centre in HNU. Participants will arrive at HNU at 8:00am after an overnight fast of approximately 10 hours, having consumed nothing but water since the prior evening meal. Upon arrival, the participant will be given a glass of water (250mL) to drink and their anthropometric measurements will be taken, followed by blood pressure.

For all participants, baseline Visual Analogue Scale (VAS) ratings of appetite will be measured at 8:50am (t = 0 min), before the breakfast. At the same time, a 10mL fasting blood sample will also be collected (t = 0 min). At 9:00am participants will be given a preload breakfast and allowed 15 minutes to consume all components of the meal. The meal will be given within an individual dining booth. Participants will rate the palatability of the preload breakfast using standard VAS methods. At specific time points over a period of 3.5 hours (t = 0 , 15, 30, 60, 90, 120, 150, 180 and 210 mins), participants will rate appetite-related sensations (hunger, fullness, satisfaction, thoughts of food, thirsty and nausea) using standard VAS methods. At 11:05am, participants will be given a glass of water (250mL) to drink for rehydration. Participants should finish the glass of water within 10 minutes.

A subset of participants who consent for repeat blood sampling will undergo forearm venous cannulation by the Research Nurse. Apart from fasting blood sample being collected (t = 0 min; 10mL blood), blood samples will also be collected intermittently after preload breakfast (t = 15, 30, 60, 90, 120, 150, 180, 210 mins; 7mL blood at each time point). Collectively, a total of 66mL of blood will be collected from these participants on each of the 2 preload study days. The participants will complete the preload challenge study at 12:30pm, and the cannula will be removed.

The summary of the visit schedule for the preload challenge is as follows:

08:15am – 250mL water to drink
08:30am – cannulation
08:50am – VAS, baseline blood sample (t0)
09:00am – preload breakfast
09:15am – VAS, blood sample (t15)
09:30am – VAS, blood sample (t30)
10:00am – VAS, blood sample (t60)
10:30am – VAS, blood sample (t90)
11:00am – VAS, blood sample (t120)
11:05am – 250mL water to drink
11:30am – VAS, blood sample (t150)
12:00pm – VAS, blood sample (t180)
12:30pm – VAS, blood sample (t210)

Preload breakfast

The Cambridge LED meal replacement along with peanut butter toast and egg will be used as the preload breakfast. A glass of 250mL water will also be given to drink for hydration and aid swallowing. The identical preload will be used at Week 0 and Week 8, irrespective of the diet intervention arm. For example the preload may be a 227mL meal replacement shake, which contains approximately 1.8MJ, 29g protein, 39.2g CHO and 16.8g fat. The purpose of using identical preload for all participants at all preload challenge occasions is so that participants will be exposed to the same acute stimuli (i.e. the preload breakfast), and any difference in the appetite response can be expected to be linked to the differences in long-term stimuli (i.e. high-protein vs low-CHO diets).

Visual analogue scales (VAS)

Appetite-related VAS will be used following the international methodology of Blundell et al. (2010). The following questions will be used: “How hungry do you feel? / How full do you feel? / How satisfied do you feel? / How much do you think you can eat now / How nauseous do you feel?” anchored on the left by “I am not hungry at all / I am not full at all / I am completely empty / nothing at all / not nauseous at all” and “I am as hungry as I have ever been / I am totally full / I cannot eat another bite / a large amount / very nauseous” on the right. Participants will mark their responses by placing a vertical line across the 100-mm scale according to their subjective feelings (Fig 1).

Hunger

- How hungry do you feel?
- ANCHOR: 'I am not hungry at all' vs. 'I am as hungry as I have ever been'

Fullness

- How full do you feel?
- ANCHOR: 'I am not full at all' vs. 'I am totally full'

Satisfaction

- How satisfied do you feel?
- ANCHOR: 'I am completely empty' vs. 'I cannot eat another bite'

Thoughts of food

- How much do you think you can eat now
- ANCHOR: 'nothing at all' vs. 'a large amount'

Thirst

- How thirsty do you feel?
- ANCHOR: 'I am not thirsty at all' vs. 'I am as thirsty as I have ever been'

Nausea

- How nauseated do you feel?
- ANCHOR: 'I do not feel nauseated at all' vs. 'I am as nauseated as I have ever been'

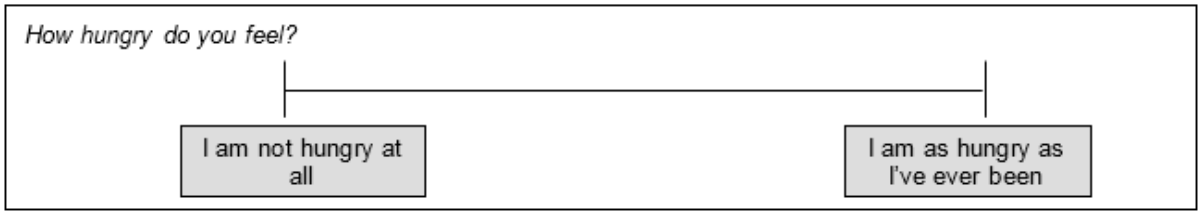

Figure 1. Example of VAS.

Following the preload breakfast participants will also rate pleasantness, visual appeal, smell, taste, aftertaste and overall palatability using VAS.

Pleasantness

- How pleasant was the meal?
- ANCHOR: 'not at all pleasant' vs. 'as pleasant as I have ever tasted'

Appearance

- How did the meal look?
- ANCHOR: 'good' vs. 'bad'

Smell

- How did the meal smell?
- ANCHOR: 'good' vs. 'bad'

Taste

- How did the meal taste?
- ANCHOR: 'good' vs. 'bad'

Aftertaste

- How much aftertaste was there?
- ANCHOR: 'much' vs. 'none'

Palatability

- How appealing was the meal?
- ANCHOR: 'good' vs. 'bad'

3.9 Group meetings

In addition to the clinic visits described above, there will also be group meetings at Week 0, Week 2, Week 4 and Week 6 to provide behavioural counselling and also provide participants with general knowledge to overcome any difficulties they face during the weight loss intervention. This is also aimed to promote adherence to the intervention. The group meetings will be led by a trained health professional, supported by a postgraduate nutrition & dietetics research student. Participants will attend different meetings according to the intervention arms wherever possible. Each group meeting will take approximately one hour.

3.9 Compliance

Compliance to the diet arms will be monitored using a 24-hour urinary nitrogen assessment and 4-day food records. Investigators will contact participants through phone calls to inquire about their dietary progress and promote compliance throughout the 8-week intervention. Participants may also contact the investigators if they require any dietary or other advice between group visits.

3.10 Dual Energy X-Ray Absorptiometry (DXA)

DXA body composition measurement will take place at Department of Surgery Body Composition Unit at Auckland City Hospital. DXA is based on the 3 component model of body composition, and uses 2 x-ray energies to measure body fat mass, lean mass, and bone mineral density. A rapid scan iDXA (GE-Lunar, Madison, WI) designed to allow scanning of larger individuals with high body weight and BMI will be used. The participant is required to lie recumbent on the open scanner bed for approximately 10 minutes. Body composition comprising total body fat, fat-free soft tissue and bone mineral content as well as regional fat deposition will be determined from DXA whole-body and segmental scans. DXA scans are a low-risk research tool, commonly used in nutrition intervention trials. A single scan provides exposure similar to that of a 1 hour flight between Auckland and Wellington.

3.11 Outcome variables

Primary outcome

i. Change in body weight.

Secondary outcome

i. Plasma concentration of appetite-related biomarkers.
ii. VAS ratings for appetite sensations following a preload breakfast challenge.
iii. Change in body composition (fat mass and fat-free mass).

Other

i. Other metabolic parameters and urinary nitrogen.
ii. 4-day food records.

3.12 Blood and urine parameters

Fasting blood samples

i. Hb_{A1c}
ii. Glucose (FPG)
iii. Cholesterol (total-C, LDL-C, HDL-C) and triglyceride
iv. Liver function (AST, ALT, ALP, GGT)
v. CCK, GLP-1, PYY, GIP, insulin, C-peptide, glucagon and amylin
vi. Amino acids

Postprandial blood samples (a subset of participants during preload-test meal challenge only)

i. Glucose
ii. CCK, GLP-1, PYY, GIP, insulin, C-peptide, glucagon and amylin
iii. Amino acids

Urine parameters (for the assessment of protein intake compliance)

i. Urine nitrogen

3.12 Proposed research timeline

Recruitment process (see Section 3.6) will begin as soon as this study received human ethics approval. Recruitment will be ongoing until May unless target sample size has achieved. The 8-week weight-loss intervention will be running in cohorts, spanning through February and July. All cohorts and data collection are expected to complete by the end of July. Laboratory analysis of blood samples is expected to begin in June and completed in October. Data analysis and manuscript write-up is expected to begin in September and completed in December.

Table 6. A summary of proposed research timeline.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Recruitment	✓	✓	✓	✓	✓							
Data collection		✓	✓	✓	✓	✓	✓					
Lab analysis						✓	✓	✓	✓	✓		
Data analysis & write-up									✓	✓	✓	✓

4. Ethics approval

Human ethics approval to conduct this study will be obtained from the Auckland Health and Disabilities Ethics Committee (HDEC), Auckland, New Zealand.

5. Trial registration

The trial will be registered with the Australia New Zealand Clinical Trials Registry (ANZCTR).

6. Risks and benefits

Controversy exists among the public regarding the advantages and disadvantages of a high protein diet. Generally, intakes that exceed the capability of liver and kidney to ‘detoxify’ the AA-derived nitrogenous waste should be avoided (Wu, 2016), therefore individuals diagnosed with impaired liver or kidney function are excluded from this study.

According to the Australian and New Zealand Nutrient Reference Values (NRV), there is no strict upper level of intake for protein in terms of grams per day due to insufficient evidence, but an upper level of 25% energy from protein is generally recommended for sedentary individuals (National Health and Medical Reseach Council et al., 2006). Importantly, the NRV is recommended on the basis of an energy-balanced diet.

Since this is a negative energy balance study, the total daily energy intake (MJ) is lower and hence a low absolute protein intake (grams). The protein intake in the intervention group is high as a percentage of total energy but when expressed as absolute protein intake (grams), the intervention diets do not exceed the recommended upper level as illustrated in Table 4.

Table 4. An example illustrating the amount of protein when expressed in percentage of total energy and in absolute term.

Total energy intake (MJ)	% energy from protein	Absolute amount of protein (g)
11.9[1]	25%[3]	178
4.8[2]	50%	144

[1]Assumption of the daily energy requirement of a sedentary 130kg woman.

[2]Total energy intake and percentage energy from protein representing weight loss period when energy intake is restricted to 40% of daily energy requirement.

[3]NRV upper level of intake at energy balance.

Since the capability for liver and kidney to handle the nitrogen load is dependent on absolute intake (Bilsborough and Mann, 2006), therefore our intervention diet is safe for all healthy individuals. Furthermore, a recent large international study in high risk adults showed that after a 2-month LED for weight loss, an *ad lib* high protein diet (25% energy from protein during weight maintenance, [1]as per example above) did not worsen the kidney function of overweight, pre-diabetic but otherwise healthy older adults (Moller et al., 2018). All intervention diets to be used in this current trial (HPNC, HPLC, NPNC and NPLC) have previously been shown to promote weight loss (Soenen et al., 2012) with no adverse events reported.

Both obesity and rapid weight loss have been associated with increased risk of symptomatic gallstones. Hence, LEDs have been associated with this increased risk. Participants will be monitored for adverse events throughout this 8-week intervention, and any events reviewed by the study consulting physician.

Venous cannulation may result in mild discomfort for the participant. The participant will be monitored by a research nurse throughout the day and no adverse events are expected. Participants will be continuously monitored at all CIDs and following the visits by telephone interview, over the study period, by research staff.

DXA uses a low dose of ionizing radiation, similar to the natural radiation exposure of a 1 hour airplane flight. The exposure to participants represents a very low risk. Pregnancy in female participants is an exclusion criteria, as is metal implants such as cardiac pacemakers.

7. Data collection/privacy/confidentiality

Data will be de-identified and recorded in hard copy on case report forms (CRF) and also stored in electronic format using Microsoft Excel. All hard copy CRFs will be stored in secure locked cabinets and the electronic data stored on a secure server with an automatic backup facility at the University of Auckland Human Nutrition Unit.

8. Adverse event reporting

Adverse events (AEs) are classified as serious or non-serious. The investigator is responsible for reporting and recording adverse events. An adverse event is defined as an event that is undesirable occurring in a participant, whether related or unrelated to the study procedure.

Serious adverse events (SAEs) include:

i. Death.
ii. Life threatening event.
iii. Serious injury i.e. events which require hospitalisation or medical attention.

Non serious events include:

i. All events not defined as serious.

Any reported AEs and SAEs will be recorded at the clinic visit.

9. Data retention

All data will be retained for a period of 10 years, or as stipulated by the NZ National Human Ethics Committee (HDEC).

10. Clinical trial sites

The study visits will be conducted at the University of Auckland Human Nutrition Unit (www.humannutritionunit.auckland.ac.nz) and the body composition scan (DXA) will be conducted at Department of Surgery Body Composition Unit at Auckland City Hospital.

Appendix 1 Absolute macronutrient intake (grams) for each intervention.

(a) 3.9 MJ meal plan

Intervention arm	**HPNC 50/40/10en%**	**HPLC 50/28/22en%**	**NPNC 35/40/25en%**	**NPLC 35/28/38en%**
Protein	118g	118g	81g	81g
CHO	95g	65g	95g	65g
Fat	11g	23g	26g	40g

(b) 4.6MJ meal plan

Intervention arm	**HPNC 50/40/10en%**	**HPLC 50/28/22en%**	**NPNC 35/40/25en%**	**NPLC 35/28/38en%**
Protein	138g	138g	96g	96g
CHO	110g	77g	110g	77g
Fat	12g	27g	31g	46g

References:

BHOGAL, M. S. & LANGFORD, R. 2014. Gender differences in weight loss; evidence from a NHS weight management service. *Public Health, vol 128 issue 9, pp 811-813*.

BILSBOROUGH, S. & MANN, N. 2006. A review of issues of dietary protein intake in humans. *Int J Sport Nutr Exerc Metab,* 16**,** 129-52.

BLUNDELL, J., DE GRAAF, C., HULSHOF, T., JEBB, S., LIVINGSTONE, B., et al. 2010. Appetite control: methodological aspects of the evaluation of foods. *Obes Rev,* 11**,** 251-70.

BOSSE, J. D. & DIXON, B. M. 2012. Dietary protein in weight management: a review proposing protein spread and change theories. *Nutr Metab (Lond),* 9**,** 81.

BRENNAN, I. M., LUSCOMBE-MARSH, N. D., SEIMON, R. V., OTTO, B., HOROWITZ, M., et al. 2012. Effects of fat, protein, and carbohydrate and protein load on appetite, plasma cholecystokinin, peptide YY, and ghrelin, and energy intake in lean and obese men. *Am J Physiol Gastrointest Liver Physiol,* 303**,** G129-40.

CHRISTENSEN, P., BLIDDAL, H., RIECKE, B. F., LEEDS, A. R., ASTRUP, A., et al. 2011. Comparison of a low-energy diet and a very low-energy diet in sedentary obese individuals: a pragmatic randomized controlled trial. *Clin Obes,* 1**,** 31-40.

CHRISTENSEN, P., MEINERT LARSEN, T., WESTERTERP-PLANTENGA, M., MACDONALD, I., MARTINEZ, J. A., et al. 2018. Men and women respond differently to rapid weight loss: Metabolic outcomes of a multi-centre intervention study after a low-energy diet in 2500 overweight, individuals with pre-diabetes (PREVIEW). *Diabetes Obes Metab*.

CHUNGCHUNLAM, S. M., HENARE, S. J., GANESH, S. & MOUGHAN, P. J. 2015. Dietary whey protein influences plasma satiety-related hormones and plasma amino acids in normal-weight adult women. *Eur J Clin Nutr,* 69**,** 179-86.

CLIFTON, P. M., CONDO, D. & KEOGH, J. B. 2014. Long term weight maintenance after advice to consume low carbohydrate, higher protein diets--a systematic review and meta analysis. *Nutr Metab Cardiovasc Dis,* 24**,** 224-35.

CRANE, M. M., JEFFERY, R. W. & SHERWOOD, N. E. 2017. Exploring Gender Differences in a Randomized Trial of Weight Loss Maintenance. *American Journal of Men's Health,* 11**,** 369-375.

GEIDENSTAM, N., MAGNUSSON, M., DANIELSSON, A. P. H., GERSZTEN, R. E., WANG, T. J., et al. 2017. Amino Acid Signatures to Evaluate the Beneficial Effects of Weight Loss. *International Journal of Endocrinology,* 2017**,** 12.

HESSION, M., ROLLAND, C., KULKARNI, U., WISE, A. & BROOM, J. 2009. Systematic review of randomized controlled trials of low-carbohydrate vs. low-fat/low-calorie diets in the management of obesity and its comorbidities. *Obes Rev,* 10**,** 36-50.

HETHERINGTON, M. M., CUNNINGHAM, K., DYE, L., GIBSON, E. L., GREGERSEN, N. T., et al. 2013. Potential benefits of satiety to the consumer: scientific considerations. *Nutr Res Rev,* 26**,** 22-38.

HUTCHISON, A. T., PISCITELLI, D., HOROWITZ, M., JONES, K. L., CLIFTON, P. M., et al. 2015. Acute load-dependent effects of oral whey protein on

gastric emptying, gut hormone release, glycemia, appetite, and energy intake in healthy men. *Am J Clin Nutr,* 102**,** 1574-84.

KOROMPOKIS, K., OSTMAN, E. & DOUGKAS, A. 2016. The impact of liquid preloads varying in macronutrient content on postprandial kinetics of amino acids relative to appetite in healthy adults. *Appetite,* 107**,** 511-520.

LARSEN, T. M., DALSKOV, S.-M., VAN BAAK, M., JEBB, S. A., PAPADAKI, A., et al. 2010. Diets with High or Low Protein Content and Glycemic Index for Weight-Loss Maintenance. *New England Journal of Medicine,* 363**,** 2102-2113.

LAU, D. C. & TEOH, H. 2013. Benefits of modest weight loss on the management of type 2 diabetes mellitus. *Can J Diabetes,* 37**,** 128-34.

LE ROUX, C. W., AYLWIN, S. J., BATTERHAM, R. L., BORG, C. M., COYLE, F., et al. 2006. Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Ann Surg,* 243**,** 108-14.

LE ROUX, C. W., BORG, C. M., MURPHY, K. G., VINCENT, R. P., GHATEI, M. A., et al. 2008. Supraphysiological doses of intravenous PYY3-36 cause nausea, but no additional reduction in food intake. *Annals of Clinical Biochemistry,* 45**,** 93-95.

LIEVERSE, R. J., JANSEN, J. B., MASCLEE, A. A. & LAMERS, C. B. 1995. Satiety effects of a physiological dose of cholecystokinin in humans. *Gut,* 36**,** 176-9.

LUSCOMBE-MARSH, N. D., HUTCHISON, A. T., SOENEN, S., STEINERT, R. E., CLIFTON, P. M., et al. 2016. Plasma Free Amino Acid Responses to Intraduodenal Whey Protein, and Relationships with Insulin, Glucagon-Like Peptide-1 and Energy Intake in Lean Healthy Men. *Nutrients,* 8.

MELLINKOFF, S. M., FRANKLAND, M., BOYLE, D. & GREIPEL, M. 1956. Relationship Between Serum Amino Acid Concentration and Fluctuations in Appetite. *Journal of Applied Physiology,* 8**,** 535-538.

MINISTRY OF HEALTH NEW ZEALAND 2017a. Clinical Guidelines for Weight Management in New Zealand Adults. Wellington: Ministry of Health New Zealand.

MINISTRY OF HEALTH NEW ZEALAND. 2017b. *New Zealand Health Survey: Annual Data Explorer* [Online]. Available: https://minhealthnz.shinyapps.io/nz-health-survey-2016-17-annual-data-explorer/_w_f9f2e435/#!/explore-topics [Accessed 18 May 2018].

MINISTRY OF HEALTH NEW ZEALAND. 2017c. *Obesity* [Online]. Available: https://www.health.govt.nz/our-work/diseases-and-conditions/obesity [Accessed 18 May 2018].

MOLLER, G., RIKARDT ANDERSEN, J., RITZ, C., M, P. S., NAVAS-CARRETERO, S., et al. 2018. Higher Protein Intake Is Not Associated with Decreased Kidney Function in Pre-Diabetic Older Adults Following a One-Year Intervention-A Preview Sub-Study. *Nutrients,* 10.

MOOK-KANAMORI, D. O., DE MUTSERT, R., RENSEN, P. C., PREHN, C., ADAMSKI, J., et al. 2016. Type 2 diabetes is associated with postprandial amino acid measures. *Arch Biochem Biophys,* 589**,** 138-44.

NAKAMURA, H., JINZU, H., NAGAO, K., NOGUCHI, Y., SHIMBA, N., et al. 2014. Plasma amino acid profiles are associated with insulin, C-peptide and adiponectin levels in type 2 diabetic patients. *Nutrition & Diabetes,* 4**,** e133.

NATIONAL HEALTH AND MEDICAL RESEACH COUNCIL, AUSTRALIAN GOVERNMENT DEPARTMENT OF HEALTH AND AGEING & NEW ZEALAND MINISTRY OF HEALTH 2006. Nutrient Reference Values for Australia and New Zealand. Canberra.

NEWGARD, C. B., AN, J., BAIN, J. R., MUEHLBAUER, M. J., STEVENS, R. D., et al. 2009. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab,* 9**,** 311-26.

NG, M., FLEMING, T., ROBINSON, M., THOMSON, B., GRAETZ, N., et al. 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet,* 384**,** 766-781.

OECD. 2017. Obesity Update 2017. Available: https://www.oecd.org/els/health-systems/Obesity-Update-2017.pdf.

POPPITT, S. D., STRIK, C. M., MCARDLE, B. H., MCGILL, A. T. & HALL, R. S. 2013. Evidence of enhanced serum amino acid profile but not appetite suppression by dietary glycomacropeptide (GMP): a comparison of dairy whey proteins. *J Am Coll Nutr,* 32**,** 177-86.

PROSPECTIVE STUDIES COLLABORATION 2009. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *The Lancet,* 373**,** 1083-1096.

SIMPSON, S. J. & RAUBENHEIMER, D. 2005. Obesity: the protein leverage hypothesis. *Obes Rev,* 6**,** 133-42.

SOENEN, S., BONOMI, A. G., LEMMENS, S. G., SCHOLTE, J., THIJSSEN, M. A., et al. 2012. Relatively high-protein or 'low-carb' energy-restricted diets for body weight loss and body weight maintenance? *Physiol Behav,* 107**,** 374-80.

SOENEN, S., MARTENS, E. A., HOCHSTENBACH-WAELEN, A., LEMMENS, S. G. & WESTERTERP-PLANTENGA, M. S. 2013. Normal protein intake is required for body weight loss and weight maintenance, and elevated protein intake for additional preservation of resting energy expenditure and fat free mass. *J Nutr,* 143**,** 591-6.

SUMITHRAN, P., PRENDERGAST, L. A., DELBRIDGE, E., PURCELL, K., SHULKES, A., et al. 2011. Long-Term Persistence of Hormonal Adaptations to Weight Loss. *New England Journal of Medicine,* 365**,** 1597-1604.

SUMITHRAN, P. & PROIETTO, J. 2013. The defence of body weight: a physiological basis for weight regain after weight loss. *Clinical Science,* 124**,** 231-241.

THOM, G. & LEAN, M. 2017. Is There an Optimal Diet for Weight Management and Metabolic Health? *Gastroenterology,* 152**,** 1739-1751.

TOCHIKUBO, O., NAKAMURA, H., JINZU, H., NAGAO, K., YOSHIDA, H., et al. 2016. Weight loss is associated with plasma free amino acid alterations in subjects with metabolic syndrome. *Nutrition &Amp; Diabetes,* 6**,** e197.

VELDHORST, M. A., NIEUWENHUIZEN, A. G., HOCHSTENBACH-WAELEN, A., WESTERTERP, K. R., ENGELEN, M. P., et al. 2009. Effects of high and normal soyprotein breakfasts on satiety and subsequent energy intake, including amino acid and 'satiety' hormone responses. *Eur J Nutr,* 48**,** 92-100.

VERDICH, C., FLINT, A., GUTZWILLER, J. P., NASLUND, E., BEGLINGER, C., et al. 2001. A meta-analysis of the effect of glucagon-like peptide-1 (7-36)

amide on ad libitum energy intake in humans. *J Clin Endocrinol Metab,* 86**,** 4382-9.

WEIGLE, D. S., BREEN, P. A., MATTHYS, C. C., CALLAHAN, H. S., MEEUWS, K. E., et al. 2005. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *Am J Clin Nutr,* 82**,** 41-8.

WESTERTERP-PLANTENGA, M. S. 2007. How are normal, high- or low-protein diets defined? *Br J Nutr,* 97**,** 217-8.

WESTERTERP-PLANTENGA, M. S., LEMMENS, S. G. & WESTERTERP, K. R. 2012. Dietary protein – its role in satiety, energetics, weight loss and health. *British Journal of Nutrition,* 108**,** S105-S112.

WING, R. R., LANG, W., WADDEN, T. A., SAFFORD, M., KNOWLER, W. C., et al. 2011. Benefits of Modest Weight Loss in Improving Cardiovascular Risk Factors in Overweight and Obese Individuals With Type 2 Diabetes. *Diabetes Care,* 34**,** 1481-1486.

WIRTH, A. & STEINMETZ, B. 1998. Gender Differences in Changes in Subcutaneous and Intra-abdominal Fat during Weight Reduction: An Ultrasound Study. *Obesity Research,* 6**,** 393-399.

WU, B. N. & O'SULLIVAN, A. J. 2011. Sex Differences in Energy Metabolism Need to Be Considered with Lifestyle Modifications in Humans. *Journal of Nutrition and Metabolism,* 2011.

WU, G. 2016. Dietary protein intake and human health. *Food Funct,* 7**,** 1251-65.