The effect of a higher-protein low energy diet on weight loss in obese women

yutongliu

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Health Sciences in Nutrition and Dietetics, The University of Auckland, 2020

Supervisor: Professor Sally D Poppitt

School of Biological Sciences
Human Nutrition Unit
Submitted: April 2020

Abstract

Background. Obesity has become a global epidemic with New Zealand having the third highest adult obesity rate among all OECD countries. The optimal dietary macronutrient composition to achieve weight loss has been long debated. Whilst higher protein diets have been shown to promote weight loss, many of these diets are also low carbohydrate (CHO). It is not established whether the higher protein or the lower CHO content may be the most important factor for successful weight loss. Manipulating the macronutrient content of a low energy diet (LED) provide the opportunity to investigate these effects. LED is one of the most effective dietary treatments for obesity, it provides only 4MJ of energy while still manages to deliver all the essential micronutrients. For that reason, LED is widely used in studies to induce short term weight loss.

Aim. The aim of this project was to investigate the effect of an 8-week LED which varied in protein and CHO content on (i) body weight loss; (ii) fat mass loss; (iii) lean mass loss; and (iv) satiety in a group of obese women.

Methodology. Obese but otherwise healthy female adults (BMI 30kg/m²-45kg/m²) completed an 8-week LED, where they were randomized to one of four diet interventions, in a 2×2 design. The presenting project took part within the study (sub-study) where only a subset of data (from the first 6 months) was collected and presented. Throughout the day, they were asked to consume 3 fixed meals (an oatmeal breakfast and 2 LED meal replacement products) and a variable meal where the intake was *ad libitum*. Two high (50en%) and two normal (35en%) protein diets, each with either low (28en%) or normal (40en%) CHO, were tested as follows: High protein, normal CHO diet (HPNC; % en from protein/CHO/fat: 50/40/10); high protein, low CHO diet (HPLC; % en from protein/CHO/fat: 50/28/22); normal protein, normal CHO diet (NPNC; % en from protein/CHO/fat: 35/40/25); and normal protein, low CHO diet (NPLC; % en from protein/CHO/fat: 35/28/38). Food records were used to assess energy and macronutrient intake at baseline, 4 weeks (mid LED), and 8 weeks (post LED). Body weight was assessed at baseline, 2 weeks (mid LED), and 8 weeks (post LED); body composition was not measured by dual x-ray (DXA) body scans at baseline and 8 weeks (post LED).

Results. 46 females completed all anthropometric measurements and dietary records at the end of 8 weeks. A good level of dietary compliance to the LED treatment was observed in all diet groups of >85%. The high protein diet groups significantly increased their protein intake from baseline and achieved a significantly greater protein intake than the normal protein diet groups. There was no significant difference detected between the 4 diet groups over the 8-week LED (weight loss: treatment*time

interaction, P=0.9997; fat loss: treatment*time interaction, P=0.9781). Body weight and body fat mass decreased significantly in all diet groups over 8 weeks (Body weight: P<0.0001 for NPLC, NPNC, HPLC groups, P=0.0017 for HPNC group; Body fat mass: P<0.0001 for NPLC and HPLC groups, P=0.0003 for NPNC group, P=0.0006 for HPNC group). Mean weight loss ranged from -3.5 to -2.6 kg and mean % fat loss was between -4.0 to -2.9%. Lean mass decreased significantly in NPLC, NPNC, and HPLC groups, but not in HPNC group, yet the changes were also not significantly different between diet groups (treatment*time interaction, P=0.9974). There is also no significant difference in energy intake (EI) between four diet groups mid-LED (week 4) and post-LED (week 8) (treatment*time interaction, P=0.6473).

Conclusions. In this present sub-study, a good level of dietary compliance for the LED treatment was achieved by most participants, and those who were compliant lost a significant amount of body weight. No diet group achieved a greater weight loss outcome or changes in body composition. A higher-protein LED was equally beneficial in promoting weight loss and fat loss while preserving lean mass when compared with a lower-protein LED with the same CHO content. A higher-protein LED also did not promote satiety and limit EI any better than a lower-protein LED with the same CHO content. When protein was kept constant, a lower-CHO higher-fat LED was not better at the loss of body weight and body fat loss along with the preservation of lean mass when compared with a higher-CHO lower-fat LED. However, a greater sample size might show a more significant effect, which might add precision for the designing of future weight loss diets.

Acknowledgements

Firstly, I would like to show my gratitude to my supervisor, Professor Sally Poppitt for giving me this opportunity to complete my Masters project at the Human Nutrition Unit. It would be impossible to complete the project without her professional support and guidance.

Secondary, I would like to thank Dr Clare Wall, Dr Andrea Braakhuis, Clare Wallis and Julia Sekula for supporting me throughout the two years of my Masters program. It has been a pleasure being a part of the Nutrition and Dietetics program.

Thirdly, I would like to thank the HNU research team, especially Dr Louise Lu and Jia Jit Lim, they have been extremely patient and offered their professional guidance with the completion of my Thesis. I would also like to thank the staff and many other students who have helped and supported me greatly throughout the years, it would be extremely difficult to complete my Thesis without their hard works.

Finally, a special thanks to all participants involved in the study. They showed great patience and passion while taking part, and it has been a pleasure working with them.

Table of content

Figure of tables

Table of Figures

Chapter 1: Introduction and literature review

1.1 Obesity in New Zealand

World Health Organisation described overweight and obesity as ‘abnormal or excessive accumulation of body fat that may impair health’ (WHO, 2018). According to the statistical report by WHO, the obesity rate has nearly tripled since 1975. By 2016, more than 1.9 billion adults were considered overweight. Within those, over 650 million were obese (World Health Organisation, 2020b). New Zealand is no exception to this epidemic. New Zealand has the third highest adult obesity rate among all OECD countries. According to The New Zealand Health Survey 2018/19, 1 in 3 adults (aged 15 years and over) was obese (30.9%), which increased from 28.6% in 2011/12. In New Zealand, obesity showed significant ethnic variation, with 66.5% Pacific, 48.2% Maori, 29.1% European/other, and 13.8% Asians being obese (Ministry of Health, 2019). It was hypothesized that the main driver for the obesity epidemic is a combination of policy and environment, leading to an over-supply of energy-dense foods, which ultimately results in energy imbalance in combination with inadequate physical activity levels (Boyd A Swinburn et al., 2011). Obesity increases the risk of many chronic illnesses, such as cardiovascular disease, thromboembolism, obesity hypoventilation syndrome, obstructive sleep apnoea, and metabolic syndrome (Neema Tiwari, 2016). The concept of metabolic syndrome was firstly introduced in 1876, where a link was found between abdominal obesity and the diagnosis of multiple conditions, such as hypertension atherosclerosis, dyslipidaemia, sleep apnoea-hypopnea, and hyperuricemia (Silva, Stanton, & Grande, 2013). One is

defined as having metabolic syndrome once he/she met three of the five criteria, including waist circumference, blood pressure, plasma triglycerides, HDL-cholesterol, and fasting plasma glucose (Alberti, K. G. M. M, Zimmet, & Shaw, 2006). Obesity and its comorbidities greatly reduce one's quality of life and act as a major burden on the healthcare system. In 2006, the estimated cost attributable to overweight and obesity was $623.9 million, which equates to 4.4% of New Zealand's total health care expenditure, includes both hospitalization cost and a loss of productivity cost (Lal, Moodie, Ashton, Siahpush, & Swinburn, 2012). As such, treating and preventing obesity at the early stage are paramount.

1.2 Measurements of obesity

1.2.1 BMI as a measurement of obesity

The standard measurement used to classify overweight and obesity is the body mass index (BMI), which is calculated using a person's weight (in kilograms) divided by the square of his or her height (in meters) (World Health Organisation, 2020b). A High BMI is associated with an increased risk for cerebrovascular and cardiovascular diseases, diabetes, and a higher colon cancer mortality rate (Waaler, 1984). According to WHO, a BMI of less than 18.5kg/m^2 is classified as underweight, a person with a BMI equal to or more than 25kg/m^2 is considered overweight, a person with a BMI equal to or more than 30kg/m^2 is considered obese. Within the obesity range, BMI over 30kg/m^2 and below 35kg/m^2 is defined as class I obesity, over 35kg/m^2 and below 40kg/m^2 is defined as class II obesity, above 40kg/m^2 is defined as class III obesity (World Health Organisation, 2020a). However, although BMI is commonly

used in epidemiological studies, it is only a crude measurement of overweight and obesity at a population level, therefore often flawed when applied to individuals. It was recognized that a person's stature, body proportions, and body composition might affect the interpretation of the body mass ratio (Nuttall, 2015). For example, BMI calculated based on only height and weight is less likely to distinguish the difference between absolute and relative fatness, and between individuals with different distributions of body fat (Baumgartner, Heymsfield, & Roche, 1995). A person's gender, age, and ethnicity were all shown to have an impact on BMI (Nuttall, 2015). Numerically same value of BMI may not represent the same level of body fatness, or the same degree of obesity-related health risks in male and female subjects, where for the same BMI, males tend to have a lower proportion of body fat mass than their female counterparts (Ogden, Yanovski, Carroll, & Flegal, 2007). Ageing is accompanied by a progressive increase in the ratio of body fat and lean mass, even when BMI is kept at constant, which is even more evident with a sedentary lifestyle (Gallagher et al., 2000). BMI is also a poor indicator of body fatness in people with a well-developed musculature, such as in athletes, military, and civil forces personnel, who are usually leaner for their BMI (Kahn & Bullard, 2016). Other factors such as weight history and physical background can all have impacts on the result, where a profound difference in lean mass preservation was found between individuals who lost weight with exercise and those without exercise (Pavlou, Steffee, Lerman, & Burrows, 1985). Furthermore, with the same numerical number of BMI, many Asian ethnic groups were shown to have a higher level of body fatness compared with New

Zealand Europeans, which potentially is associated with a greater risk of obesity-related diseases (McAuley, Kirsten A., Williams, Mann, Goulding, & Murphy, 2002). In view of this marked variation between ethnic groups, there have been debates regarding the adjustments of BMI cut-offs by different ethnic groups (Ulijaszek, 2003). Several studies have justified the needs for developing a more ethnic-specific set of cut-offs for BMI, waist circumference, and waist to hip ratio, both globally (Katzmarzyk et al., 2011), and within New Zealand (Chiu, Austin, Manuel, Shah, & Tu, 2011). New Zealand is an ethnically diverse country with 80% New Zealanders are Europeans, 14.7% are Maori, 6.6% are Asians, and 6.5% are Pacific Islanders. In comparison, Maori and Pacific Island adults are shown to have a higher lean-to-fat mass ratio than other ethnic groups of the same BMI (Swinburn, Craig, Daniel, Dent, & Strauss, 1996). In 2018, BMI for overweight was adjusted to 26kg/m^2 and 32kg/m^2 for obesity in the Maori population (Ministry of Health, 2018).

In summary, although BMI is a useful tool in detecting obesity at a population level, it can be misleading when applied to individuals. As such, developing other alternative measurement of obesity becomes necessary.

1.2.2 other alternative measurement of obesity

1.2.2.1 Waist circumference

Compared with the total body fatness, other aspects of obesity, such as the regional distribution of excess body fat, is shown to be more closely associated with obesity-related health risks. The fat mass stored in the abdomen, typically referred to as ectopic body fat, is associated with multiple metabolic abnormalities, such as

adverse lipid profiles, decreased glucose tolerance, reduced insulin sensitivity (Huxley, Mendis, Zheleznyakov, Reddy, & Chan, 2010). And the risk is substantially higher compared to fat storage in other locations, such as subcutaneously or at lower body areas (Baumgartner et al., 1995). As a proxy measure of abdominal adiposity, waist circumference (WC) was widely employed in many studies (André Tchernof & Jean-Pierre Després, 2013). It has been established that the degree of abdominal obesity estimated by waist circumference, is associated with many obesity-related health risks. Several studies have shown that waist circumference is a better predictor of obesity-related health risks than BMI (Janssen, Katzmarzyk, & Ross, 2004).

However, waist circumference also has its own limitations. Ageing is often accompanied by the significant redistribution of body fat tissue. From middle age to late 80s, there is a relocation of subcutaneous fat tissue to the visceral depot, accompanied by a decline in lean body mass and an accumulation of fat in muscle, liver, and bone marrow (Cartwright, Tchkonia, & Kirkland, 2007). Again, like BMI, the application of WC varied between ethnic groups. With the same level of body fatness, many Asian races have a greater predisposition for central obesity, especially Asian Indians (Hughes, Aw, Kuperan, & Choo, 1997). An ethnic-specific cut-off for WC is also called for (Huxley et al., 2008).

Due to the inherent limitations of BMI in predicting body fatness and obesity-related health risks, it was suggested that combining BMI with other additional assessments such as waist circumference and body composition, can better predict obesity and the associated health risks than BMI alone (Ardern, Katzmarzyk, Janssen, & Ross, 2003;

Janssen, Katzmarzyk, & Ross, 2002). In an evidence report published by the National Heart, Lung, and Blood Institute of the National Institutes of Health, for adults with a BMI of 25 to 34.9 kg/m^2, gender-specific waist circumference cut-offs should be used in conjunction with BMI when assessing the health risks. Within each category of BMI, adults with high WC has higher obesity-related health risks than those with normal WC values, regardless of age or gender (Bethesda, 1998). Waist circumference was also positively associated with the risk of all-cause mortality throughout the whole course of adult BMIs (Seidell, 2010). The table below showed a new set of reference values and their predictions of disease risks (for type 2 diabetes, hypertension, and CVD) after combining BMI and waist measures (Goodwin, 2002).

Table 1 Classification of Overweight and Obesity by combining BMI, Waist Circumference, and Associated Disease Risk for type 2 diabetes, hypertension, and CVD (Goodwin, 2002)

	Body mass index	Obesity class	Disease risk (relative to normal weight and waist circumference)	
			Men < 102 cm Women < 88 cm	Men >102 cm Women >88 cm
Underweight	<18.5			
Normal	18.5–24.9			
Overweight	25.0–29.9		Increased	High
Obesity	30.0–34.9 35.0–39.9	I II	High Very high	Very high Very high
Extreme obesity	>40.0	III	Extremely high	Extremely high

1.2.2.2 Waist-height ratio

Waist-height ratio, calculated as waist measurement divided by height measurement, can also be used to assess body composition and central obesity, however less commonly than waist circumference. The use of waist-height ratio in detecting abdominal obesity and its associated health risks was firstly introduced in the mid-1990s. Compared with BMI and waist circumference, it roughly controls for

variation in adult stature, therefore remains a preferable method to use in tall or short individuals (Meseri, Ucku, & Unal, 2014; Roriz et al., 2014). Waist to height ratio was shown to be a superior screening tool for metabolic risk factors than BMI and waist circumference in adults (Ashwell, Gunn, & Gibson, 2012).

1.2.2.3 laboratory-based techniques

Another common way to assess obesity is through the measurement of body composition. In clinical studies, out of practical reason, bio-impedance (BIA) and skinfold callipers are two commonly used methods to estimate body fat mass and percentage. Skinfold thickness predicts the amount of total body fat based on the assumption that there is a constant relationship between subcutaneous fat and total body fat in a given age or sex group, and the total fat can be estimated by measuring the thickness of the subcutaneous adipose tissue using skinfold callipers. However, this method can result in errors even when conducted by trained and experienced operators, especially when measuring obese subjects. In addition, the use of skinfold thickness is potentially flawed in the elderly, mainly due to an increased level of internal fat compared with the younger age groups (Deurenberg & Yap, 1999). BIA is also commonly used in many studies to estimate body fatness. It assumes that the human body is a cylindrical-shaped ionic conductor, different tissues present with different levels of resistivity after applying a small alternating current through the body (Kotler, Burastero, Wang, & Pierson, 1996). However, the prediction equation is influenced by one's age and gender, leading to a difference in the distribution of body water. Body shape, body build, body posture during measurement, and whether or not

the subject is in the fasting state, or whether they took strenuous exercise prior to measurement can all have impacts on the results (Deurenberg & Yap, 1999).

Dual-energy x-ray absorptiometry (DXA) scanning was originally developed in the 1980s as a method to diagnose osteoporosis. Now it is widely employed in clinical studies to measure body composition. DEXA scanning estimates body composition based on the differential attenuation by bone, fat, and lean tissue of transmitted photons at two energy levels (Mazess, Barden, Bisek, & Hanson, 1990). In the end, it provides measurements of three compartments of the body: body fat mass, lean body mass, and total body bone mineral (A. Pietrobelli, C. Formica, Z. Wang, & S. B. Heymsfield, 1996). There is evidence showing that DEXA can provide a more precise estimation for body fat percentage than underwater weighing, which is currently recognized as the 'gold standard' method (Pritchard et al., 1993). In the present thesis, whole-body dual-energy x-ray absorptiometry (DXA) scanning was used to estimate body composition.

1.3 Current nutrition recommendation and advice for weight reduction

Several leading health authorities have recommended weight loss as a primary treatment strategy in reducing obesity and its associated comorbidities (Ministry of Health, 2017; Ulijaszek, 2003). Evidence has shown that a moderate weight loss of 5-10% has a positive effect on several metabolic and cardiovascular risk factors, such as hypertension, hyperglycaemia and dyslipidaemia (Van Gaal, Mertens, & Ballaux, 2005). It is well-known that the key to successful weight loss is energy-deficit, which

can be created either through dietary energy restriction or increased physical activity (Funk, Lee, Vidoni, & Reininger, 2019). According to the Clinical Guideline for Weight Management in New Zealand Adults released in 2017, overweight and obese individuals are encouraged to reduce the total energy intake by decreasing the overall consumption of energy-dense and nutrient-poor foods and drinks, as well as improving the quality of fat and carbohydrate in their diets (Ministry of Health, 2017).

Previous studies reporting no difference between various dietary strategies and diet compositions in weight loss have concluded that as long as an energy deficit is created, weight loss would occur (Aragon et al., 2017; Johnston, B. C. et al., 2014).

1.3.1 Low Energy Diet

Low Energy Diet (LED) is defined as hypo-caloric diets containing 3350 to 6280 kJ (800 to 1500 kcal) or 50 to 80 kJ (12 to 20 kcal/kg) of ideal body weight per day. Very low energy diet (VLED) further restricts the energy content and provides less than 3350 kJ (800 kcal) or less than 50 kJ (<12 kcal/kg) of ideal body weight per day (Atkinson, 1989). LED and VLED are two common approaches used in the treatment of obesity. The key concept is to limit the total energy intake by using formulated nutrition powders or other forms of nutrition products. As a result, LED/VLED has inspired the development of many meal replacement products in the form of shakes, bars and other products, which have gained popularity in UK for 35 years. Now, LED/VLED is widely used in academic studies to support weight loss (Leeds, 2014).

VLEDs are restricted to individuals with a BMI higher than 30 who have failed the conventional weight-loss approaches in the past. For these individuals, the VLED

products are commonly used to replace one or two meals in a day for a period of 12 to 16 weeks. Comparing with the traditional concept of fasting, VLED has its own advantage: it provides sufficient essential macronutrients, vitamins and minerals to prevent malnutrition, the high protein content also protects human body from the loss of lean body mass due to starvation (Atkinson et al., 1993). VLED is also low in carbohydrate of delivering ~50g of carbohydrate per day, which serves many functions such as maintaining blood glucose level and preventing the loss of electrolytes. More importantly, it induces a mild physiological ketosis, where ketones are produced by liver and then used to fuel the normal function of the brain (Saris, 2001).

Evidence supporting the use LEDs as an effective approach for weight reduction is extensive. In a study by Foster et al (1992), three LED/VLED treatments ranging from 1.8 to 3.3MJ/day in energy induced significant weight loss in 68 obese females, ranging from 7.2 to 8.9kg at 5 weeks, and from 19.5 to 22.6kg at 24 weeks. Rossner and Flaten et al (1997) conducted a similar study, where significant weight loss ranging from 12.9 to 14.7kg after applying three LED treatments ranging from 1.8 to 3.7MJ/day for 6 weeks, and 17.7kg to 20.2 kg for 26 weeks. A study by Christensen et al compared VLED and LED and their effectiveness in weight loss, both dietary strategies resulted significant weight loss at week 8 (11.4kg weight loss in VLED group and 10.7kg in LED group) and at week 16 (13.3kg weight loss in VLED group and 12.2kg in LED group) (Christensen, P., Bliddal, Riecke, Leeds, Astrup, & Christensen, 2011a). Theoretically, VLED would result in a greater amount of weight

loss than LED due to a bigger energy deficit. However, for the three studies discussed above, similar weight loss was observed with the uses of both dietary treatments.

There are several reasons favouring the use of LED. Firstly, there are more adverse effects reported by those consuming VLED, including but not limited to, constipation, cold intolerance, dry skin, dry mouth, headache, dizziness/orthostatic hypotension, fatigue, menstrual irregularities, and hair loss (Saris, 2001). Others such as gout, gallstones and cardiac disturbances are less frequently reported, and most of them occurred after the reintroduction of solid food (Everhart, 1993; Sours et al., 1981). As a result, the dietary compliance was often lower in those consuming VLED (Christensen, P. et al., 2011).

Secondly, for the same treatment duration, those following VLEDs lost significantly more lean tissue than LEDs. Christensen et al (2011) reported 17% of lost weight as lean mass with the use of VLED compared with 11% with the use of LED (Christensen, P. et al., 2011). Foster et al (1992) reported a similar difference where lost lean mass contributed to 14.6% of weight lost in those following VLED (1758kJ/day) compared with 11.8% in those following LED (3349kJ/day) for 19 weeks. Wadden et al (1994) compared the loss in lean mass between the uses of both VLED (420kcal/day) and LED (1200kcal/day), the lean mass constituted 18.25% and 27.89% of the weight lost in those following LED and VLED respectively (Wadden, Foster, & Letizia, 1994). Although there are concerns of lean mass loss with the use of VLED, there are studies showing there might be a better lean mass preservation

when combined with physical activity and adequate protein, yet direct evidence is lacking (Prentice et al., 1991).

Thirdly, there is growing evidence showing that VLED leads to more weight loss than LED initially, but the weight regain was much quicker in individuals consuming VLED in the long-term. A meta-analysis by Tsai et al (2006) extracted data from 6 studies with a mean follow-up period of 1.9 years. The results reported that, on average, those following VLED lost 1.3kg body weight than those following LED, yet the weight regain was much higher in VLED patients of 62% compared with 41% in LED patients (Tsai & Wadden, 2006).

Overall, both LED and VLED are shown to be effective in inducing clinically meaningful weight loss. However, for the reasons discussed above, LED is considered a safer and more sustainable approach for weight loss, which is why LED was chosen in the present sub-study.

1.3.2 Weight loss diets varying in macronutrient compositions

There is increasing debates regarding whether there is an optimal macronutrient composition for weight loss diets. Thom and Lean (2017) compared five commonly utilized diet approaches, including low-fat diet, low-carbohydrate (CHO) diet, Mediterranean type diets, meal replacements, and intermittent fasting. In the end, they found that each diet can lead to significant weight loss and improvements in metabolic health with no diet being superior to others, indicating that the key to any weight loss approach is moderate energy restriction combining with persisting dietary compliance (Thom & Lean, 2017). Similarly, the Clinical Guidelines for Weight

Management in New Zealand Adults published in 2017 also described a successful diet as ‘a diet that meets one’s nutritional needs while being maintained in the long term’ (Ministry of Health, 2017). Nevertheless, diets restricting fat and CHO while promoting protein were encouraged on many occasions.

1.3.2.1 Low-fat diet

There is increasing evidence supporting the role of dietary fat control as an important strategy in the regulation of body weight (Astrup, 1999). In comparison with other macronutrients, fat has a higher energy density. One gram of fat contains 37.6kJ of energy, which is more than twice as much as an equal gram of protein or CHO. Therefore, an increase in dietary fat will inevitably lead to an overall increase in energy density, and consequently, a passive increase in energy intake and body weight (Poppitt, S. D. & Prentice, 1996). In addition, evidence supported a preference for high-fat foods in human subjects (Mela, 1990) and an even stronger preference for fats in obese subjects (Drewnowski, Brunzell, Sande, Iverius, & Greenwood, 1985; Mela, 1990).

Fat is the least satiating among all macronutrients. In comparison with protein and CHO, fat has the smallest suppressive effect on the subsequent food intake (de Castro, 1987). On top of having a low satiating effect, fat is also highly palatable, which results in a tendency for overconsumption and weight gain in human subjects (Cotton, Burley, Weststrate, & Blundell, 2007; Green & Blundell, 1996). In addition, it was reported that the conversion from dietary fat to body fat is the most efficient when compared with protein and CHO. In the state of positive energy balance, addition of

dietary fat is associated with greater body fat deposition with no increase in fat oxidation (Leveille & Cloutier, 1987).

The role of low-fat intake as an important weight loss strategy has been well established over the years. It was reported that a 10% reduction in fat intake as percentage energy intake was associated with a 16g reduction in body weight per day among overweight individuals (Bray & Popkin, 1998). According to Eating and Activity Guidelines for New Zealand Adults, high consumption of dietary fat can contribute to excess energy intake and lead to weight gain. The Ministry of Health also recommends a fat-restricting diet to New Zealand adults for weight loss and weight gain prevention (Ministry of Health, 2003).

One of the main mechanisms for weight loss on a low-fat diet is through the control of overall energy intake. A study by Lissner et al (1987) reported a significantly higher *ad lib* energy intake in participants following a diet with 45-50% energy as fat than those following a diet with 15-20% energy as fat (Lissner, Levitsky, Strupp, Kalkwarf, & Roe, 1987). A meta-analysis of literature comprised of 12 prospective randomized control trials found that a mean reduction of 2.8-18% in self-reported fat intake was associated with a mean of 40-570 kcal/day reduction in total energy intake (Hill, Melanson, & Wyatt, 2000). This effect on energy intake and weight loss was especially obvious when food intake is kept at *ad libitum (ad lib)*. Astrup et al (2000) conducted a meta-analysis comprised of 16 trials and a total of 1728 participants investigating the effect of low-fat diets on *ad lib* energy intake and weight loss. The meta-analysis reported an extra 3.2kg weight loss and 1138kJ more reduction in total

energy in the low-fat group, after achieving a mean of 10.2% EI reduction in fat intake. A conclusion was drawn where low-fat diets in the absence of active energy restriction promote weight loss in overweight individuals, and the effect was particularly obvious in participants presented with a higher body weight at baseline (Astrup et al., 2000). Kendell et al (1991) found a lower *ad lib* energy intake and a greater weight loss (twice as much) in participants consuming a low-fat diet (20-25% energy) than those consuming a higher-fat diet (35-40% energy) (Kendall, Levitsky, Strupp, & Lissner, 1991). Lissner et al (1987) displayed a similar finding where a 2-week *ad lib* dietary treatment with 15-20% energy as fat resulted in an 11.3% deficit in energy intake and an average of -0.4kg extra weight loss than a medium fat diet (30-35% fat) (Lissner et al., 1987). Meanwhile, there is evidence showing the reduction in fat intake (%EI) plays a bigger role than energy restriction alone. In the study by Sheppard et al (1991), 303 women (aged between 45 to 69 years) were randomized into either following a low-fat diet with 20% daily energy as fat or into a control group. After a year of treatment, both groups achieved a mean of 25% reduction in energy intake, yet women following a low-fat diet had an extra of 2.7kg weight loss than the control group (Sheppard, Kristal, & Kushi, 1991). This finding was further supported by Jeffery et al and Shah et al. In their studies, fat intake as percentage of EI influences weight loss independent of total energy intake (Shah, McGovern, French, & Baxter, 1994) and the fat restriction is considered a better predictor of weight loss (Jeffery, Hellerstedt, French, & Baxter, 1995). Another meta-analysis by Yu-Poth et al (1999) showed that without active energy restriction,

for every 1% decrease in energy intake from fat, there is a 0.28kg decrease in body weight (Yu-Poth et al., 1999).

On the contrary, the use of low-fat diets in long-term weight control was questioned by many researchers. McManus et al (2001) indicated that a low-fat diet could only yield a greater weight loss outcome for up to 6 months, and the advantage was no longer obvious at 18 months. In their study, participants following a low-fat diet regained body weight due to having difficulty adhering to the diet (McManus, Antinoro, & Sacks, 2001). Powell et al randomly assigned 35 obese women to four isocaloric diets (1,200 kcal/day) with 10%, 20%, 30%, and 40% energy as fat respectively for 12 weeks. No significant difference in weight loss and body fat loss was found between treatment groups (Powell, Tucker, Fisher, & Wilcox, 1994), indicating that energy restriction is the primary predictor of weight loss, not dietary fat intake. An intervention study by Schlundt et al (1993) further supported this finding. They randomized 49 individuals into either consuming a low-fat *ad lib* diet or an-energy restricted low-fat diet, where both groups reduced fat intake from 90 to 30g per day. Over the course of 16-20 weeks, the low-energy group lost significantly more weight (males 11.8 kg; females 8.2 kg) than the low-fat group (males 8.0 kg; females 3.9 kg) (Schlundt et al., 1993).

1.3.2.2 Low-CHO diet

Although low-fat diet has been the mainstay for weight loss diet during the early 19th century, a book published in 1972 by John Yudkin, *Pure, White and Deadly*, challenged the traditional concept of a ‘weight-loss’ diet. In his book, John argued it

is the increase in sugar consumption that contributes to the rise in obesity prevalence, not fat. The excessive CHO intake, especially from sugar-sweetened beverages, had more effect on body weight than dietary fat. In the 1950s and 1960s, low-CHO diet replaced low-fat diet as the mainstay of weight loss, which inspired the creation of many branded weight loss programs, such as the Atkins diet, South Beach diet, and Zone diet. Low-CHO diet is defined as a diet with less than 40% total energy from carbohydrates, which was substantially lower than the 50-55% recommended by MOH for New Zealand adults. A very low CHO diet further restricts the intake to less than 20% total energy or 20-60g per day (Ministry of Health, 2017).

The use of low-CHO diet as a weight-loss strategy has been a controversial topic over the years. Yancy et al (2004) conducted a randomized control trial investigating the effect of a low CHO diet on weight loss and body composition. The study randomized 120 overweight individuals with hyperlipidaemia to either consuming a low CHO, ketogenic (<20g/day) diet or an energy-restricted (500-1000kcal/day deficit) low fat (<30% energy from fat) diet for 24 weeks. The result indicated that participants in the low CHO group lost significantly more body weight (mean change: -12.9% vs -6.7%), more body fat (-9.4kg or -5.8% vs -4.8kg or -2.8%) and fat free mass (-3.3kg vs -2.4kg) than those following the low diet (Yancy, Olsen, Guyton, Bakst, & Westman, 2004). Yet there was limited generalizability due to a short study duration of only 24 weeks. Another RCT by Brehm et al compared an *ad lib* very low carbohydrate diet (maximum intake of 20g/day) and an energy-restricted diet (30% energy from dietary fat) in 52 obese but otherwise healthy female subjects. Results showed that the very

low CHO diet group lost significantly more weight after 3 months (7.6 kg vs. 4.2 kg) and after 6 months (8.5 kg vs. 3.9 kg). The very low CHO diet group also lost significantly more body fat than low-fat diet group (Brehm, Seeley, Daniels, & D'Alessio, 2003). Hession et al (2009) conducted a systematic review investigating the effectiveness of a low CHO diet on weight loss when compared with a low-fat diet. The review included 13 randomized control trials and a total of 1222 participants, ranging from 6 to 36 months in duration. Results indicated that participants in the low CHO group lost a mean of 4.02kg more body weight than those who followed a low-fat diet at 6 months ($P<0.0001$). However, this difference shrunk to 1.05kg at 12 months ($P<0.05$). It is worth mentioning that this review showed a high heterogeneity at 6 months, where there is a large variation in the study design and the CHO allowance in low-CHO group of the studies included. A high drop-out rate was also reported (attrition rate 36%), with low-fat groups having a significantly higher attrition rate (Hession, Rolland, Kulkarni, Wise, & Broom, 2009). In alignment with the results from this study, another meta-analysis by Nordmann et al (2006) compared the efficacy of an a*d lib* low CHO diets and energy-restricted low-fat diets in participants with BMI over 25kg/m^2. The analysis included five randomized control trials and a total of 447 individuals, the results indicated participants following a low CHO diet lost a mean of 3.3kg more body weight than those following a low-fat diet at 6 months ($P=0.02$). However, similar to what was found by Hession et al (2009), this advantage diminished at 12 months, where no obvious difference was found between two diet groups. This review also reported high heterogeneity after 6 months,

possibly from the one trial allowing 10% energy from fat in the low-fat group, as opposed to the 30% in the other three trials (Nordmann et al., 2006). Involuntary reduction in energy intake in the low CHO diet group was reported by both Nordmann et al (2005) and Yancy et al (2004). They proposed three possible mechanisms, including higher satiety resulted from higher protein intake, a limited food selection, and the ketogenic effect from the low CHO intake (Nordmann et al., 2006; Yancy et al., 2004). Foster et al (2003) conducted a multi-centre, controlled trial involving 63 obese subjects, where participants were randomized into either consuming a low-CHO, high protein, high fat diet or an energy-restricted, high-CHO, low fat diet. Results indicated that subjects consuming a low-CHO diet lost significantly more weight than those following a low fat diet at 3 months (6.8% vs. 2.7% body weight) and 6 months (7.0 vs. 3.2 body weight), but not at 12 months (4.4 vs. 2.5 body weight) (Foster, Gary D. et al., 2003). It is important to note that in this study, a greater reduction in energy intake was found in the low-CHO group, although voluntary, which was possibly due to a limited food selection and an increased adherence to the diet plan. Several other studies with a particular focus of obese subjects with metabolic syndromes also reported similar results (Linda Stern et al., 2004; Samaha et al., 2003), where weight loss resulted from a low-CHO diet was greater than a low fat diet, at least in the short term (within 6 months).

In contrast, there are studies showing the opposite. A systematic review in 2003 included 94 interventional studies and a total of 3268 participants, aiming to investigate the efficacy of low-CHO diets. After a comprehensive analysis of results

from all studies, they found insufficient evidence to support the low-CHO diets of being superior in losing weight compared with higher carbohydrate diets. Instead, a better weight loss outcome was observed in studies with a greater degree of energy-restriction and a longer study duration, and in participants with higher baseline body weight. However, the majority of studies included in the review used completer-only approach, and the diets were not iso-caloric, indicating the percentage energy from CHO was not controlled. There were also other confounding components skewing the analysis, such as exercise, behaviour therapy, and other supportive measures (Bravata et al., 2003). Likewise, the intervention study conducted by Liu et al (2008) enrolled 50 overweight and obese females comparing a low CHO *ad lib* diet (CHO intake of 20g/day and increases 10g every week, 36.1% energy from CHO at the end of study) with an energy-restricted diet (CHO intake 156-205g/day, 35% reduction in energy, 51.1% energy from CHO at the end of study) for 12 weeks. The results indicated no significant difference in the amount of weight loss (LC: -5.27kg vs ER: -5.09kg) or the amount of percentage fat loss assessed by DEXA (LC: -1.19%, ER: -1.56%) between two diet groups (Liu et al., 2013). However, a high drop-out rate was reported by several studies. Nordmann et al reported a range of 31% to 48% drop out rate in low CHO diets, and between 37% and 50% drop out rate in low fat diets after 1 year of follow up (Nordmann et al., 2006).

1.3.2.3 High-protein diets

The role of protein in body-weight regulation comprised of several aspects. Firstly, protein promotes and sustains satiety. The acute protein-induced satiety and its

association with body weight were discussed by many studies (Lejeune, Westerterp, Adam, Luscombe, & Westerterp-Plantenga, 2006; Marisa Porrini, Roberta Crovetti, Giulio Testolin, Santino Silva, 1995; Westerterp, Wilson, & Rolland, 1999). Smeets et al (2008) conducted a randomized controlled trial involving 30 healthy subjects with a mean BMI of 23.8kg/m^2, aiming to compare the satiating effects of an appropriate protein lunch (10% energy as protein) and a high protein lunch (25% energy as protein). The result showed a significantly higher level of satiety in those consuming a higher protein single meal than those following a single meal with 25% energy as protein ($P<0.02$), at both 30 minutes and 120 minutes (Smeets, Soenen, Luscombe Marsh, Ueland, & Plantenga, 2008). In another study, a protein preload of 1 MJ in energy produced a significantly greater short-term satiating effects than an isocaloric CHO preload and an isocaloric fat preload, which ultimately resulted a lower energy being consumed at the next meal (Poppitt, Sally D., McCormack, & Buffenstein, 1998). This protein-induced satiety effect can even persist throughout the day (Westerterp et al., 1999).

Secondly, protein promotes and sustains energy expenditure. One important aspect of successful weight loss is the maintenances of basal energy expenditure, especially during the state of negative energy balance. The association between diet-induced thermogenesis and protein intake was widely investigated (Crovetti, Porrini, Santangelo, & Testolin, 1998; Karst, Steiniger, Noack, & Steglich, 1984; Lejeune et al., 2006). Tentolouris et al (2008) conducted a crossover study involving 30 participants, both lean and obese. They were randomized to either consuming a

high-protein breakfast (102g protein, 18g carbohydrate, 0g fat) or a high-fat breakfast (12g protein, 20g carbohydrate, 39g fat). The study reported an almost three-fold higher diet-induced energy expenditure in the high protein group, and the effect was evident in both lean (356.8kJ/h vs 125.2kJ/h) and obese subjects (348.8kJ/h vs 145.6kJ/h) (Tentolouris et al., 2008). Similarly, Smeets et al (2008) found a greater increase in the post-prandial energy expenditure after consuming a high protein meal compared with a lower protein meal (Smeets et al., 2008). Moreover, evidence showed that high protein intake is able to prevent decreases in sleeping metabolic rate/expenditure for participants following hypo-caloric diets. Whitehead et al (1996) found significantly slighter decrease in 24-hour energy expenditure and sleeping metabolic rate in participants consuming a high protein diet (36% energy as protein) compared with those following a lower protein diet (15% energy as protein) (Whitehead, McNeill, & Smith, 1996). Studies by Lejeune et al (2006) and by Mikkelsen et al (2000) consistently observed a significantly greater elevation in sleeping metabolic rate among participants consuming high protein diets. (Brita Stenius-Aarniala, Tuija Poussa, Johanna Kvarnström, Eeva-Liisa Grönlund, Mikko Ylikahri, Pertti Mustajoki, 2000; Lejeune et al., 2006). The elevated energy expenditure induced by high protein intake also promotes satiety, mainly through the increases oxygen consumption and body temperature, which ultimately results in a feeling of oxygen deprivation (Westerterp et al., 1999). Westerterp-Plantenga et al (1999) observed a positive relationship between 24-hour diet-induced energy expenditure and satiety in participants consuming a high protein diet (Westerterp et al.,

1999). A finding that was consistent with Lejeunen et al (2006) and Crovetti et al (1998) (Crovetti et al., 1998; Lejeune et al., 2006).

Thirdly, protein promotes weight loss in a manner that spares fat free mass, which is of great significance during both weight loss and weight maintenance. Loss in body weight is usually accompanied by a reduction in both fat mass and lean body mass (Ian Janssen, Anne Fortier, Robert Hudson, & Robert Ross, 2002). As the single best predictor of energy expenditure, reduction in fat-free mass inevitably results in a lower resting energy expenditure, an undesirable outcome during weight loss (Nelson, Weinsier, Long, & Schutz, 1992). There is increasing evidence showing that a relatively high-protein diet improves body composition by increasing the FFM/FM ratio, which prevents the reduction in energy expenditure induced by weight loss (Westerterp-Plantenga, M. S., Nieuwenhuizen, Tome, Soenen, & Westerterp, 2009).

There is increasing evidence supporting the effectiveness of high-protein diets in promoting weight loss and improving body compositions. In a 12-month RCT comparing an energy-restricted high protein diet (32% en protein, 41% en CHO, 25% en fat) with an iso-caloric high-CHO diet (20% en protein, 58% en CHO, 21% en fat) in 71 young females with a BMI of more than 27.5 kg/m^2. At 6 months, HP group lost significantly more weight (HP vs HC: -8.9kg vs -4.6kg) and body fat (HP vs HC: -8.0kg vs -3.4kg) than HC group. Yet this difference was no longer significant at 12 months. However, this study used completers-only method in analysis, which inevitably leads to bias (Griffin et al., 2013). Completers only analysis ignores those with missing data, and it only analyses those who strictly adhered to the protocol and

completed the study. Although this approach is often used to provide an estimate of the true efficacy of an intervention, it inevitably exaggerated the treatment effect (Ranganathan, Pramesh, & Aggarwal, 2016). Another RCT with a similar design also examined the difference in weight loss among 13 obese males induced by either a hypo-energetic (80% of resting metabolic rate) high protein diet (45% en protein, 25% en CHO, 30% en fat) or a high CHO diet (12% en protein, 58% en CHO, 30% en fat). After 4 weeks, HP diet group lost significantly more body weight than HC diet group (-8.3 vs. -6.0kg), and HC group showed a significantly greater decrease in resting metabolic rate ($P<0.05$) (Hwalla Baba et al., 1999). In a 12-month RCT by McAuley et al (2005), 93 overweight women were randomly assigned to either a high-fat diet group, a high-protein diet group (30% en protein), or a high-CHO diet group (>55% en CHO). By the end of 12 months, HP diet group achieved the greatest and the most sustained weight loss (HP: -6.6kg; HC: -4.4kg; HF: -5.4kg) and the greatest reduction in body fat mass among all groups (HP: -3.8kg; HC: -3.5kg; HF: -3.4kg) (McAuley, K. A. et al., 2006).

Similar weight loss was also evident when food intake was kept at *ad lib.* Skov et al (1999) compared a high-protein *ad lib* diet (25% en protein, 45% en CHO, 30% en fat) and a high-CHO *ad lib* diet (12% en protein, 58% en CHO, 30% en fat) in 65 overweight but healthy subjects. At 6 months, there was an extra 3.7kg reduction in body weight ($P<0.0001$) and an extra 3.3kg reduction in body fat ($P<0.0001$) among participants following the high protein diet, which is possibly due to a significantly lower energy intake in this group (Skov, Toubro, Rønn, Holm, & Astrup, 1999). In a

similar study by Dumesnil et al, a 6-day, low-GI, low-fat, high protein *ad lib* diet resulted in a significantly lower energy intake (by 25%) and greater weight loss (by 2.3kg) in comparison with a high-CHO, low-fat diet (Dumesnil et al., 2001). Due et al (2004) compared two *ad lib*, low-fat (30% en) diets either high in protein (25% en) or medium in protein (12% en) in 50 overweight and obese subjects. At 6 months, those consuming the high protein diet achieved a significantly greater weight loss than those consuming the medium protein diet (9.4 vs 5.9 kg). At 12 months, the difference in weight loss between groups was no longer significant, yet HP group had a greater reduction (by 10%) in intra-abdominal adipose tissue than MP group.

Whilst evidence supporting the role of high protein intake in promoting weight loss is extensive, some studies find no such relationship. Wycherley et al (2012) randomly assigned 123 overweight and obese males into either following a high-protein diet group (35% en protein, 40% fat CHO, 25% en fat) or an isocaloric high-CHO diet (17% en protein, 58% en CHO, 25% en fat) for 52 weeks. Results showed that an energy-restricted, low-fat, high-protein diet was able to achieve a greater improvement in body composition (better preservation of FFM), but not a greater weight loss than an isocaloric high-CHO diet. Both groups lost approximately 11% body weight at the end of 52 weeks (Wycherley, Brinkworth, Clifton, & Noakes, 2012). Similar to this study, Delbridge et al (2009) found no difference in weight loss outcomes resulted from two low-fat (<30% en fat) diets with either 30% en as protein or 15% en as protein for 12 months (Delbridge, Prendergast, Pritchard, & Proietto,

2009), indicating that as long as the low-fat component is present, the weight loss would be equally successfully when accompanied by either high protein or high CHO.

1.4 Rationale of the study

This thesis is a sub-study within a larger study: High-Protein vs Low-Carbohydrate Diets for Weight Loss, which is a randomised cross-over trial that compares the effects of high protein and lower-carbohydrate energy-restricted diets on eating behaviour, weight loss, and body composition in obese females. The main aim of the study is to find out the optimal macronutrient composition for weight loss in obese female participants.

Previous studies have investigated various macronutrient compositions and their effects on weight loss. Layman et al (2003) compared two iso-energetic low-fat (<30% energy as fat) diets with a CHO/protein ratio of 1.4 (high protein) and 3.5 (low protein), respectively (Layman et al., 2003). Another study by Due et al (2004) compared two *ad libitum* low-fat (<30% energy as fat) diets, a high-protein diet with 25% energy as protein and an average-protein diet with 12% energy as protein (Due, Toubro, Skov, & Astrup, 2004). The beneficial effect of high protein diet on weight loss was seen in both studies. However, these studies are unable to test whether protein per se is the sole cause of weight change, as the CHO content of the diet was often not controlled or considered. When the energy content is kept constant, a high protein diet is often achieved by decreasing the energy percentage (En%) from carbohydrate while increasing the energy percentage (En%) from protein and fat

(Clifton, Condo, & Keogh, 2013). For that reason, in the previous studies with a focus on high protein diets, the high protein component was often coupled with a low carbohydrate component. The evidence supporting the effects of high protein intake on weight loss, independent of the carbohydrate content of the diets, is limited.

Previously, there were studies attempting to separate the effects of low-CHO and high-protein in weight loss diets. Soenen et al (2012) conducted a study with the similar 2×2 factorial design, where two high (60% en) and two normal (30% en) protein diets, each with either low (5% en) or normal (35% en) CHO content, were tested as follows: High protein, low carbohydrate diet (% en from protein/CHO/fat: 60/5/35); high protein, normal CHO (% en from protein/CHO/fat: 60/35/5); normal protein, low CHO diet (% en from protein/CHO/fat: 30/5/65); and normal protein, normal CHO diet (% en from protein/CHO/fat: 30/35/35). In the end, the study concluded that the high-protein component of weight loss diets is mainly responsible for successful body-weight loss and weight-maintenance rather than the low-CHO component (Soenen et al., 2012). For a similar reason, the present study was designed to answer the question: which factor is more important in promoting weight loss, the high protein, or the low carbohydrate?

In addition, previous studies with a focus of macronutrient composition were often not sensitive enough to detect any significant difference in weight loss outcomes, possibly because the difference in protein intake was too small, or by including a control group with sufficiently large protein intake. For example, a study by Sacks et al created a difference in % energy intake as protein of only 10% between the

high-protein diet group and the average-protein diet group. The results showed no significant difference in weight loss between two protein diet groups (Sacks et al., 2009).

Although protein has been the focus of many weight loss studies, the interpretation of high protein diets seems to vary between studies. According to Westerterp et al, the absolute high protein intake is more important than the % En as protein when designing a weight loss diet, since it is necessary to prevent loss in FFM and reduction in REE induced by energy restriction (Westerterp-Plantenga, Margriet S., 2007). Soenen et al., recommended a protein intake of 1.2g/kg/d for weight loss diets to preserve FFM and to prevent a drop in REE (Stijn Soenen, Eveline A P Martens, Ananda Hochstenbach-Waelen, Sofie G T Lemmens, & Margriet S Westerterp-Plantenga, 2013). In addition, Bosse et al., proposed a protein spread theory, where a sufficient % difference in g/kg/day protein intake between groups is necessary to detect any body composition and anthropometric differences. According to the theory, greater anthropometric benefits are more likely to be generated when there is a 58.4% difference in the g/kg/d protein intake between groups (Bosse & Dixon, 2012). Considering the above, for the present study, the protein target was set at 1.2g/kg/d for participants following the high protein diets, which is 50% higher than the target of 0.75g/kg/d for the normal protein diets.

Low energy diet (LED) is defined as a diet providing between 800 and 1200 kcal/day, either as Total Diet Replacement (TDR) or as Partial Diet Replacement (PDR), by which formula products are consumed alongside modified conventional meals (Brown

& Leeds, 2019). Meal replacement (MR) is frequently used for weight loss purposes in commercial diets. It reduces the total daily energy intake by replacing at least one meal per day. MR includes formulated products such as shakes, bars, and soups, and it is considered a safe approach with support from health care professionals. A meta-analysis by Astbury et al showed that an MR diet could induce weight loss equally or even better than a diet-only approach (Astbury et al., 2019).

Very low energy diet (VLED) refers to a dietary regimen providing fewer than 800 kcal/day. It is commonly designed as TDR, indicating the diet is replaced with 3-4 formulated products per day (Brown & Leeds, 2019). These meal replacement products are commonly formulated to contain an adequate amount of vitamins, minerals, and electrolytes, so they can be consumed as the sole source of nutrition while still being low in energy. In most clinical trials, the TDR phase lasts for 8-16 months, where individuals can achieve a 10 to 16 kg or 10% to 15% reduction in body weight, with the greatest weight loss seen at the beginning (Brown & Leeds, 2019).

There are also many studies supporting the effectiveness of PDR in weight loss. A meta-analysis by Heymsfield et al showed a significantly better weight loss outcome among participants following a PDR when compared with participants consuming a conventional reduced calorie diet with same amount of energy (Heymsfield, van Mierlo, C A J, van der Knaap, H C M, Heo, & Frier, 2003). Furthermore, Leader et al supported the use of 2 PMR over 1 PMR for better weight loss in overweight/obese subjects with diabetes (Leader, Ryan, Molyneaux, & Yue, 2013). It is also more cost-friendly for self-use. A study in 2004 approved the use of PMR as an effective

and affordable weight loss approach, especially within the low-income community (Huerta et al., 2004).

In the present study, participants in all treatment groups were following a LED comprising 2 MR products and 2 restricted home meals with a fixed macronutrient composition. This *ad lib* design allowed flexibility in energy intake between treatment groups, in order to detect differences in weight loss and body composition changes induced by the dietary treatments with various macronutrient compositions, with an emphasis on higher protein and/or higher carbohydrate component of the diet.

It is well established that there is a gender difference in the distribution of body fat. Females have a higher tendency of storing body fat around the gluteo-femoral region, whereas males are more likely to store body fat around the abdominal region (Nielsen, Guo, Johnson, Hensrud, & Jensen, 2004). With the same degree of central adiposity, women turn to store more subcutaneous fat while men have more storage of visceral fat (Lemieux, Prud'homme, Bouchard, Tremblay, & Després, 1993). There is also a gender difference found in the metabolic patterns. As the main component of energy expenditure, resting metabolic rate turned to be higher in men due to a greater amount of FFM (Owen, 1988). Consequently, with the same degree of energy restriction (such as during periods of LED), the weight loss outcomes varied between men and women. A systematic review found a greater reduction in both body weight and body fat in male subjects for the same dietary intervention (Williams, Wood, Collins, & Callister, 2015). Evidence also showed that female subjects turned to lose more FM than LM whereas male subjects experienced an equal reduction in both FM and LM

(Mauriege et al., 1999). A more recent study, named the PREVIEW study, applied an 8-week LED in 2500 overweight women and men with pre-diabetes. After adjusting for the differences in weight loss (%), male participants lost significantly more body weight and fat mass than female participants ($p<0.001$) (Christensen, Pia et al., 2018). Considering the gender variability in the body composition and weight loss outcomes, only females were recruited for the present study.

Chapter 2: Aim and hypothesis

2.1 Aim

The aim of this project is to evaluate whether an 8-week LED with higher-protein results in greater weight loss than an 8-week LED with lower-protein independent of carbohydrate content in obese females. Several indicators for successful weight loss were assessed, including change in body weight, body fat, lean body mass, and self-reported energy intake.

2.2 Hypotheses

- Primary hypothesis:

An 8-week higher-protein LED treatment may result in significantly greater body weight loss among obese women when compared to a normal-protein LED treatment independent of carbohydrate content.

- Secondary hypothesis:

1, An 8-week higher-protein LED treatment may result in a significantly greater reduction in body fat and a significantly better preservation of lean mass among obese women when compared to a normal-protein LED treatment independent of carbohydrate content.

2, An 8-week higher-protein LED treatment may result in greater satiety among obese women when compared to a normal-protein LED treatment, which will in turn be reflected in the *ad libitum* energy intake and so the total energy intake, independent of the carbohydrate content.

Other hypotheses

1, An 8-week lower-carbohydrate higher-fat LED treatment may result in significantly greater body weight loss among obese women when compared to a higher-carbohydrate lower-fat LED treatment, independent of protein content.

2, An 8-week lower-carbohydrate higher-fat LED treatment may result in significantly greater loss of body fat and better preservation of lean mass when compared to a higher-carbohydrate lower-fat LED treatment, independent of protein content.

Chapter 3: Methodology

3.1 Study design

This project took part within the study: High-Protein vs Low-Carbohydrate Diets for Weight Loss, which is a randomised cross-over trial that compares the effects of high

protein and lower-carbohydrate energy-restricted diets on eating behaviour, weight loss, and body composition in obese females. The full High-Protein vs Low-Carbohydrate Diets for Weight Loss study aims to investigate 140 females across multiple ethnicities, including Maori and Pacific population. The whole study was 9-month in duration, consisting 5 cohorts and a total of 140 participants. Whereas the author's sub-study was carried out during the first 6 months of the main study, and the data was collected from the participants of the first 3 cohorts and 46 participants. The study was conducted as a 4-arm parallel intervention study with a 2 x 2 factorial design, including 2 higher-protein diet treatments and 2 lower-carbohydrate diet treatments. Eligible participants were randomly allocated to 4 intervention arms (HPLC, HPNC, NPNC, NPLC) where they were required to adhere to the pre-planned diets with respective energy and macronutrients ratios for 8 weeks. The four intervention arms were:

1. High protein, low carbohydrate diet (HPLC),
2. High protein, normal carbohydrate diet (HPNC),
3. Normal protein, normal carbohydrate diet (NPNC),
4. Normal protein, low carbohydrate diet (NPLC).

Participants within the same intervention arm were further allocated to two energy treatment based on their energy requirement. The daily energy requirement is estimated as follows: calculated the BMR using the Harris-Benedict equation for women (BEE/BMR (kcal/d) = 655.0955+9.5634(weight in kg)+1.8496(height in cm)-4.6745(age)) (Frankenfield, Muth, & Rowe, 1998), then multiplied the BMR

with the activity factor of 1.375 (lightly active, which is recommended for the participants), the target energy intake was set as 40% of their calculated daily requirement.

The participants were allocated to the 3.9MJ energy group when the energy requirement was 4.24MJ or below, and they were allocated to the 4.6MJ energy group when the energy requirement was 4.25MJ or above.

Table 2, Target total macronutrient intake for the four treatments.

	HPNC *50en% Protein* *40en% CHO* *10en% Fat*	**HPLC** *50en% Protein* *28en% CHO* *22en% Fat*	**NPNC** *35en% Protein* *40en% CHO* *25en% Fat*	**NPLC** *35en% Protein* *28en% CHO* *38en% Fat*
3.9MJ meal plan				
Energy (kJ)	4043	3957	3995	3946
Protein (g)	120	120	81	81
CHO (g)	95	65	95	65
Fat (g)	12	23	28	40
4.6MJ meal plan				
Energy (MJ)	4707	4646	4647	4661
Protein (g)	138	138	96	96
CHO (g)	110	77	110	77
Fat (g)	15	28	32	47

3.2 Ethics approval and consent of participants

Ethnic approval was gained on 18/12/2018 for the study: High-Protein vs Low-Carbohydrate Diets for Weight Loss from the Northern A Health and Disability Ethics Committee, reference number 18/CEN/238, trial registration number ACTRN12619000209190. All participants read a Participants Information sheet and signed a consent form (attached) before participating the study.

3.3 Subgroup participants

The Weight loss study started on 18th March 2019 and ended on 7th December 2019; the recruitment process took place from February 2019 to September 2019.

This sub-study (for the presenting thesis) took place from April 2019 to September 2019. Participants were recruited from the Auckland region across different ethnic groups. Recruitment process occurred via a variety of methods, including online advertisement on social media such as Facebook, and Instragram, as well as on websites such as my neighborly, and events. Advertising posters were distributed in various locations, including local medical centres, universities, gyms and community boards. Participants were also approached through the recommendations of their friends and family members. Potentially eligible individuals from previous studies were also contacted to ask if they were interested in participating.

The main criteria indicating eligibility to apply included:

- Female
- Available for an 8-week weight loss study
- Body weight not more than 130kg
- Between BMI of 30-45 kg/m^2
- Between 18-65 years of age
- Otherwise healthy
- Able to attend Human Nutrition Unit (HNU) located in Mt Eden, Auckland

Individuals who fit the criteria and showed interests to participate were contacted by researchers either through telephone interviews or emails. The emails containing a

link to a Qualtrics online survey form were sent to participants to assess their eligibility. For participants who preferred the telephone survey, the surveys were filled out by researchers through verbal communication. The surveys provided the assessment of eligibility, including gender, age, self-reported weight, self-reported height, self-reported weight history, self-reported diet history. The eligibility criteria also included no major disease complications (such as cancers, cardiovascular disease, thyroid problems, digestive disease, diabetes, liver and renal disease), previous bariatric surgery, history of depression/anxiety, and other factors that potentially affect body weight (such as certain medication or diet programs).

The full exclusion/inclusion criteria are shown in Table 3.

Individuals who completed the survey and found eligible were contacted again via telephone or email to explain the study briefly, including the aim of the study, the expected time-commitment and the tests involved if participate. The individuals found eligible and showed interests in the study were then scheduled a date for a screening visit at HNU and asked to bring the supplements/medication used in the last 6 months. A copy of Participant Information Sheet (PIS) was also emailed to participants to read before attending the screening visit.

Eligible participants were then recruited into cohorts of approximately n=28 subjects (depending on their availability to participate). Participants in each cohort were further randomised into four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).

Table 3, Inclusion and exclusion criteria for the study

Inclusion criteria	Exclusion criteria
Female Age 18-65 years Obese (BMI 30 kg/m^2 - 45kg/m^2) with a maximum body weight of 130kg Otherwise healthy	Recent body weight loss/gain >5% within previous 3 months Currently taking part in an active diet program Current medications or other conditions known to affect body weight and appetite Previous bariatric surgery Diagnosed with impaired liver or kidney function 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 Systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100mmHg Depression or any other anxiety disorder known to affect appetite Unable to consume food items included in the study, or hypersensitivities or allergies to these foods (based on Food Preference Questionnaire) Smokers or ex-smokers who have given up smoking for less than 6 months Pregnant or breastfeeding women Unwilling/unable to comply with the study protocol

3.4 Intervention

3.4.1 Randomisation

Participants were randomised using an online randomising tool (https://www.randomizer.org/) to randomise eligible participants into 1 of the 4 dietary treatment groups, as follows:

- High protein, low carbohydrate diet (50%en protein, 28%en carbohydrate, 22%en fat)
- High protein, normal carbohydrate diet (50%en protein, 40%en carbohydrate, 10%en fat)

- Normal protein, normal carbohydrate diet (35%en protein, 40%en carbohydrate, 10%en fat)
- Normal protein, low carbohydrate diet (35%en protein, 28%en carbohydrate, 38%en fat)

The study proceeding of events is shown below.

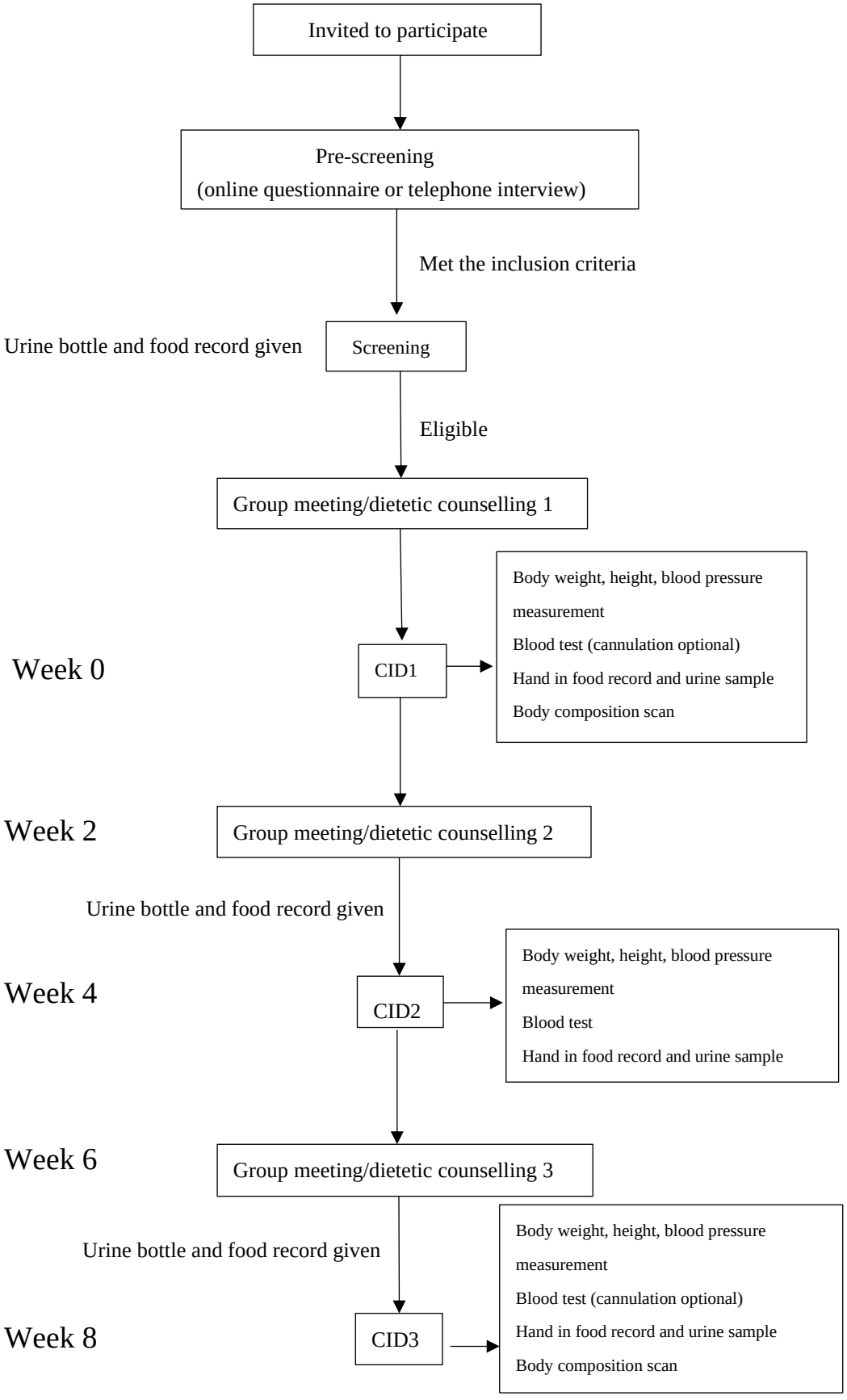

Figure 1, Flow diagram of participants in the present sub-study.

3.4.2 Dietary counselling

Dietary counselling was provided to participants in the form of group meeting at week 0, week 2, week 4, and week 6. Group meetings were treatment-specific, where participants within the same treatment group were given oral and written instructions to promote the compliance to their allocated diet plan.

3.4.3 Food record

Participants were asked to keep a food diary at week 0, week 4, and week 8. The food intake data was analysed using Foodworks (Xyris 8.0 Professional, Australia).

3.4.4 Diet intervention

During the 8 weeks of intervention, all participants were recommended to have a total of 4 eating occasions across the day, which included 1 fixed oatmeal breakfast, 2 fixed commercial LED meal replacements, and 1 variable home-cooked meal. Participants were asked to consume all the fixed meals (oatmeal and LED meal replacement) but were asked to consume the variable meal till they were comfortably full. This design created an *ad libitum (ad lib)* feeding condition, as in the food intake varied in response to satiety resulted from different dietary treatments. The purpose was to generate a difference in daily energy intake between treatment groups, which ultimately resulted in a variation in weight loss outcomes during the 8-week LED treatment. The recommended eating occasions across a day is outlined in Table 3.

Table 4, Recommended eating occasions across a day.

Eating occasions	Meal format	Comment
Breakfast	Fixed – oatmeal porridge	Same for all treatment groups, participants were asked to consume all
Lunch	Fixed – Cambridge LED meal replacements	Vary between treatment groups, participants were asked to consume all
Afternoon Tea	Fixed – Cambridge LED meal replacements	
Dinner	Variable – home cooked meals	Vary between treatment groups, Participants were asked to prepare the meals according to the cookbook, recommended to eat till comfortably full – *ad lib*

3.4.4.1 Oatmeal breakfast

Participants were instructed to prepare a standard serving of oatmeal porridge for breakfast every morning, which were asked to consume completely. A standard serving of oatmeal porridge consists of Australian quick oats (25g), Alpine skim milk powder (10g), Macro psyllium husk (5g), Nutrawhey Natural protein powder from Nutratech (15g), artificial sweetener (optional), and Hansells vanilla flavoured essence (10ml, optional). An instructive sheet was given to each participant, (attached) including a shopping list, cooking instructions, the amount of each ingredients, and the estimated cost for a standard serving. Participants were expected to purchase all the ingredients except for the Nutrawhey protein powder (provided by the research unit). Certain condiments were allowed, including coffee powder, salt, sweetener, flavour essence, and sugar-free chocolate powders. No fruits, vegetables, additional milk/milk powder, cream, or sugar were allowed.

The ingredients and nutrition profile for the oatmeal breakfast are outlined in Table 5. Overall, a standard serving of the oatmeal porridge provides 839kJ energy, 18.7g protein, 3.2g fat, and 20.7g of carbohydrate.

3.4.4.2 Cambridge LED meal replacements

All participants were provided with 8-week supply of meal replacement products (LED, Cambridge diet, UK), two products per day. Each treatment group was given a combination of LED meal replacement products according to their treatment groups. The allocation of meal replacement products per treatment group is outlined below, along with their nutrition profiles.

The products came in the form of shakes, soups, porridge, and bars. Participants were asked to follow the instructions displayed on the back of the packet:

- Porridge: Pour contents into a deep microwaveable bowl. Add 200ml hot water and stir thoroughly. Microwave on full power for 30 seconds. Stir and microwave again for another 30 seconds. Stir and allow to cool as it will be very hot. Consume within 15 minutes. Once prepared, do not store or re-heat.
- Shakes: Pour 227ml of cold water into a container and add sachet contents. Using a blender or whisk, mix until smooth or shake vigorously in a Cambridge weight plan drink shaker for 45-60 seconds. Consume within 15 minutes.
- Soups: Pour 227ml of hot (not boiling) water into a container and add sachet contents. Using a blender or whisk, mix until smooth. Consume within 15 minutes.
- Bars: Open the packet and consume.

Participants were asked to prepare the shakes, soups, porridges with only water, and no other food items were allowed to be consumed with the meal replacement products, such as skim milk.

Table 5a, Macronutrient composition of LED meal replacements per treatment group

	HPNC		**HPLC**		**NPNC**		**NPLC**	
Energy (kJ)	1560		1560		1560		1560	
Protein	37.3g	39.9% En	37.3g	39.9% En	30.3g	32.4% En	33.3g	35.6% En
CHO	43.3g	46.3% En	43.3g	46.3% En	50.3g	53.8% En	42.3g	42.3% En
Fat	6.8g	16.0% En	6.8g	16.0% En	6.8g	16.0% En	9.8g	23.0% En
Note: participants in 3.9MJ and 4.6MJ of each treatment group received the same combination of meal replacement products.								

Table 5b, Allocation of LED meal replacement products per treatment group

	HPNC	HPLC	NPNC	NPLC
Product name	Fruits of forest shake Strawberry shake Banana shake Chocolate mint shake Chocolate shake Lactose free chocolate shake Lactose free vanilla shake Oriental chilli soup Chicken & mushroom soup Tomato & basil soup	Fruits of forest shake Strawberry shake Banana shake Chocolate mint shake Chocolate shake Lactose free chocolate shake Lactose free vanilla shake Oriental chilli soup Chicken & mushroom soup Tomato & basil soup	Vanilla flavoured shake Vanilla rice pudding Maple & pecan porridge Apple & cinnamon porridge Original flavoured porridge Cherry & strawberry smoothie Vegetable soup Chicken & mushroom soup	Lactose free vanilla shake Lactose free chocolate shake Cappuccino flavoured shake Lemon yoghurt bar Chocolate flavoured bar Orange flavoured bar Toffee flavoured bar Tomato & basil soup

3.4.4.3 Variable meals

Apart from the fixed oatmeal porridge and 2 LED meal replacement products, participants were also instructed to self-prepare and consume a variable home-cooked meal every day. These meals varied between treatment groups and were designed specifically to meet the differential macronutrient target of each intervention arm, either being high or normal in protein while being normal or low in carbohydrate. Before the study started, participants were provided a cookbook outlining the variable meals that were specifically designed for the treatment they were allocated to. Each booklet contains the ingredients of the variable meals, the amount of each ingredient, the cooking instructions, pictures of example meals, reference price for a standard serving of the variable meals, the tips for food safety and hygiene. The booklets were designed specifically according to the energy treatment (3.9MJ vs 4.6 MJ) and further categorized into the four intervention arms (HPLC vs HPNC vs NPNC vs NPLC). There were at least 8 dishes in each booklet, and all dishes were designed and tested by two researchers and a cook in the research unit.

The ingredients used in the booklet were selected as following:

- The majority of the protein came from tuna fish (in both spring water and olive oil), Tarakihi fish, chicken breast, milk powder, protein powder, and eggs (with yolk and white only).
- The majority of the carbohydrate came from fresh vegetable and fruit, including fresh potatoes, tomatoes, apples, bananas, canned corn kernels, and pumpkins.

- The choice of fat includes walnuts, olive oil (from both the canned tuna and cooking addition), sesame oil, regular fat mayonnaise, regular fat cheese, and egg yolk.
- The recipes also include fresh vegetables, such as coleslaw salad, capsicum, kale, spinach, tomato, green beans, frozen vegetable mixes, lettuce, cucumber, celery, and onion.
- Condiments were also included, such as garlic, Dijon mustard, salt, pepper, soy sauce, lime juice, lemon pepper, Natvia, fresh chili, and dried parsley.

The food items used to meet the macronutrient target were kept as identical as possible across treatment groups, with only the portion sizes being variable. For example, a greater portion size for lean poultry was recommended for HP diet groups. In addition, a combination of milk powder and whey protein powder was also used to supplement the protein intake in HP diet groups.

An example of a recipe from NPLC group, 3.9MJ was attached below.

Chicken and vegetable stir fry

Ingredients

One Serve
10g olive Oil, regular
150 g mixed vegetables, frozen, Watties international stir fry (chinese)
125 g chicken breast, no skin, raw
10 g soy Sauce, Pearl River dark
5 g garlic, peeled and chopped
5 g Greggs chinese 5 spice
5 g sesame oil
20 g walnuts, roughly chopped

Cost for one serving: 3.7$

Method:

Slice the chicken breast and place in a dish with a lid

Mix the garlic, soy sauce, sesame oil and spice
Pour over the chicken, cover and marinate in the fridge 1 hour
Add the vegetables and walnut and stir
Heat a pan and olive oil on high 1 minute
Add the chicken mix and cook stirring continuously for 3 minutes
Sprinkle the chopped walnut on top
Serve

Figure 2, Example of a cookbook of the 3.9MJ energy treatment, NPLC diet group.

Table 6, Target macronutrient intake for the variable meals

	HPNC		**HPLC**		**NPNC**		**NPLC**	
3.9MJ meal plan								
Energy (kJ)	1600		1600		1600		1600	
Protein	62g	64.7% En	62g	64.7% En	32g	33.4% En	32g	33.4% En
CHO	31g	32.4% En	1g	1.0% En	24g	29.6% En	2g	2.1% En
Fat	1g	2.3% En	13g	29.8% En	16g	35.0% En	27g	61.9% En
4.6MJ meal plan								
Energy (kJ)	2200		2200		2200		2200	
Protein	82g	62.2% En	82g	62.2% En	47g	35.7% En	47g	35.7% En
CHO	46g	34.9% En	13g	9.9% En	39g	29.6% En	14g	10.6% En
Fat	2g	3.3% En	17g	28.4% En	21g	35% En	33g	55.1% En

3.4.4.4 Allowed items

Certain herbs, spices and condiments were allowed to consume. Participants were encouraged to drink 9 glasses of water and to avoid all energy-containing beverages.

Table 7, Allowed food items and beverages

	Allowed			**Not allowed**
Herbs and spices	All spices Basil Celery flakes Chilli Chives Cinnamon clove Coriander Cumin	Curry powder Dill Garlic Ginger Mint Mustard seed Nutmeg Oregano	Paprika Parsley Pepper Rosemary Sage Thyme Turmeric Tarragon	
Condiments and Sweeteners	Lemon/lime juice Fish sauce Vinegar Worcestershire/soy sauce Tomato paste	Artificial sweeteners (e.g. Equal, Splenda, Stevia)		Sugar, honey
Drinks	Aim for 2L of water per day in addition to the LED Tea and coffee (black in small amounts)	Water 6-8 glass/day Diet cordial Diet fizzy (<2 cans/day)		alcohol, fruit juice

3.5 Data collection

Data was collected during the clinical investigation days (CID) as follows:

- Pre-screening / recruitment: Age, ethnicity

- Week 0 – CID 1 (baseline): Weight, height, BMI, waist and hip circumferences, 4-day food record, and body composition

- Week 4 - CID2 (after 4 weeks of LED): Weight, BMI, waist and hip circumferences, 4-day food record, and body composition

- Week 8 - CID3 (after 8 weeks of LED): Weight, BMI, waist and hip circumferences, 4-day food record, and body composition.

3.6 Study procedures

3.6.1 General information

Ethnicity and age data were obtained through the online pre-screening survey, where participants could self-select their ethnicity and age.

3.6.2 Anthropometry and body composition measurements

3.6.2.1 Weight

Body weight was measured using digital scales (Seca Sensortronic Scales, Auckland, NZ) within HNU, which was calibrated at least once a year. Participants were asked to remove shoes and any heavy clothes such as coat and sweater, and to transfer their wallet/purse, keys or any other items from their pockets onto the tray or table provided. Participants were then asked to step up onto the scales with their feet placed at the middle of the scale while looking straight ahead. All the measurements were performed twice and recorded to the nearest 0.1 decimal, the final result was recorded by calculating the mean of the two readings. If the two readings were >0.5kg apart, the third measurement was performed. In this case, the final recording was the mean of these three readings.

3.6.2.2 Height

Body height was measured using a wall-attached stadiometer (Seca, Auckland, NZ) within HNU during the screening visit. Participants were asked to remove their shoes and any headgear if applicable before measuring, then participants were instructed to stand straight, put their back against the instrument, put their feet together, keep their

knees straight, and keep their heels against the back of the instrument. They were not allowed to slump or stand on their toes. The height was measured to the nearest two decimals and all measurements were performed twice. The final result was recorded by calculating the mean of the two readings. If the two readings were >0.5cm apart, the third measurement was performed. In this case, the final recording was the mean of these three readings.

3.6.2.3 BMI

Body mass index was calculated using the below equation:

BMI (kg/m^2) =Weight (in kg)/Height^2 (in m)

3.6.2.4 Circumference measurements

3.6.2.4.1 Waist measurement

Waist circumference was measured using a retractable measuring tape within HNU. Participants were asked to wear only a thin top, if there were multiple layers of clothing, they were asked to lift the top layers and expose only one layer. Tight belt was not allowed since it may interference with the readings. Participants were instructed to stand with their feet 20cm apart and to have their hand reaching their lowest palpable ribs along the side of their body. The measuring tape was placed just below the lowest palpable rib without being too tight to wrinkle participants' skin or to buckle their clothes. For participants with overhanging midsection, the measurement was performed around the belly. Participants were instructed to breath normally (not deeply for them to hold in their abdomen) and to relax their arms on

their side of their bodies. Two measurements were performed with their mean value calculated and recorded. If two measurements were >2cm apart, a third measurement was performed. In this case, the mean of these three readings were calculated and recorded.

3.6.2.4.2 Hip measurement

Hip circumference was measured using a retractable measuring tape within HNU. Participants were asked to remove any heavily buckled belts and stand with their feet as close together as they can manage and balance. Participants were instructed to breath normally and have their arms relaxed on the either side of their body. The measuring tape was wrapped around the widest part of the buttock. Two measurements were performed with their mean value calculated and recorded. If two measurements were >2cm apart, a third measurement was performed. In this case, the mean of these three readings were calculated and recorded.

3.6.2.5 Blood pressure

Blood pressure was measured using a Dinamap Carescape Monitor within HNU. Participants were asked to sit with both feet flat on the ground and with their back against the chair, they were allowed at least two minutes of rest after climbing a fleet of stairs within HNU. Participants had their right arm placed on a pillow, a size-appropriate cuff was used, which was wrapped smoothly and snugly around the right upper arm of the participants at least 2.5cm above the antecubital space with the arrow on the cuff in line with the brachial artery. Two measurements were performed with at least 2 minutes apart (for the release of blood that was trapped in the arm

veins). The mean of both systolic and diastolic pressure was calculated and recorded. If the reading were >10mmHg apart for either systolic or diastolic blood pressure, a third measurement was performed with at least 2 minutes apart. In this case, the mean of these three readings were calculated and recorded.

3.6.2.6 Blood collection

Blood samples were collected by trained medical professionals within HNU. Fasting blood glucose level was measured using the Reflotron within HNU at screening visit.

3.6.3 Objective measurements

3.6.3.1 Food record

Four-day food diaries were completed by participants before CID1 (baseline), before CID2 (week 4), and before CID3 (week 8). Participants were asked to record all the food and beverages consumed for 4 consecutive days, including 3 weekdays and 1 weekend day (Wednesday, Thursday, Friday, Saturday or Sunday, Monday, Tuesday, Wednesday). Detailed description was also required, including the amount of food and beverage consumed, the brand name (if applicable), cooking methods, home-made recipes (if applicable), meal time, meal type, the weight of the raw ingredients, the weight of the cooked dish, and the weight of the leftovers.

Oral instructions were given to participants by the author of the thesis (a dietetic student), a phd student, and interns who worked on the study. An electronic scale was provided to each participant for accurate measurement. Written instruction was also given them to guide their recording process, as displayed in Appendix 5.

Participants were also reminded not to change their eating habits when they recorded their food intake at baseline, and to record the intake as they eat. If they consumed home-cooked meals, they were asked to record the recipe and serving size in detail if possible.

The empty food diaries and written instructions were given to participants at the screening visit, at the group meeting in week 2, and at the group meeting in week 6. And the completed food diaries were handed in by participants at CID1 (week 0), at CID2 (week 4), and at CID3 (week 8). If they were not able to provide the completed food diary at three CID visits, they were asked to bring it along at a later date where possible.

On the day of the CID visit, the completeness of the food diary was checked by the author or the phd student. If there were not enough details recorded or in doubt, participants were asked to provide further information via email, phone call, or preferably face-to-face right after they handed in.

3.6.3.2 Food intake data entry

Food diaries were manually entered into Foodworks (Xyris 8.0 Professional, Australia) by the author and other interns who worked on the study. All the entries were doubly checked by two separate workers of the study and all the changes made were recorded.

Foodworks® is an Australian/New Zealand food analysis software which analyses the TE, protein, fat, CHO and fibre content of the foods entered. Food items were entered and selected from the New Zealand and Australia food databases, where the user types in a food item and selects the best fit one from multiple options displayed. If the desired food item was not found in the system, the next best alternative option would be selected, especially when it comes to the different cooking methods. For example, if the desired food item was 'deep-fried potatoes', the closest option would be 'baked potatoes with fat'. If participants provided a nutritional label of a product that was not in the database, a new food item was created and saved in the database, where the macronutrient content was manually entered and calculated. Similarly, If the participants included a recipe for home-cooked meals, a new recipe was created using the software where each ingredient was entered separately.

Complicated food items were entered separately e.g. a hamburger was divided into the bread portion, the meat portion, the vegetable portion, and the sauce portion, the weight of each portion was entered individually.

3.6.3.3 Food intake data analysis

Foodworks (Xyris 8.0 Professional, Australia) analyses the macronutrient content, including energy, protein, carbohydrate and fat. The 4-day average energy, protein, carbohydrate, and fat intake was generated and exported into an excel spreadsheet after entering each participant's food record into the software.

However, even detailed verbal and written instructions were provided to participants along with regular follow-ups, some participants still were not able to provide

complete and interpretable food records at the end of this sub-study, and subsequently were excluded from the data analysis.

3.6.3.4 24-hour urine sample

In addition to the 4-day food record, participants were also asked to collect a 24-hour urine sample, which was used to assess urinary nitrogen and monitor the participants' compliance to their respective dietary treatments. Eligible participants were given a 1.5L urine bottle, a funnel, and an instruction/time recording sheet. They were required to collect their urine output for 24 hours on one of the four days of recording their food intake. The first urine was collected after breakfast on the day of urine collection, the last urine was collected before breakfast on the following morning, all the urines during the whole day and night in between were collected into the urine bottle. The exact time of the first and the last urination were asked to be recorded. However, it is important to point out that the urinary nitrogen analysis was not used to assess dietary compliance in the present thesis due to time constraint.

3.7 Study Visits

3.7.1 Study visit locations

All visits occurred at the Human Nutrition Unit (HNU), located in Mt Eden, Auckland, New Zealand. The DEXA scans were taken place at the Auckland City hospital.

3.7.2 Study visit outline

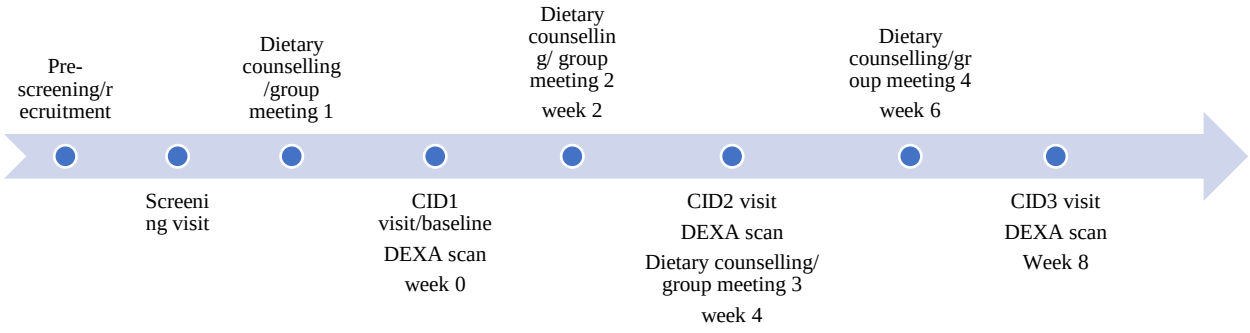

Figure 3, The study visit outline of the sub-study.

In summary, participants were expected to have 8 visits at HNU across the study period, as follows:

- Visit 1: screening (HNU), pick up empty food diary and urine collection kit
- Visit 2: dietary counselling/group meeting 1 (HNU)
- Visit 3: Week 0 of LED, CID 1/baseline visit (HNU), hand in completed food diary and urine sample, fasting blood glucose test, complete DEXA scanning, collect week 0-4 LED products. Beginning of the weight loss period.
- Visit 4: Week 2, dietary counselling/group meeting 2 (HNU), pick up empty food diary and urine collection kit
- Visit 5: Week 4 of LED, CID 2 visit (HNU), hand in completed food diary and urine sample, fasting blood glucose test, complete DEXA scanning, collect week 4-8 LED products
- Visit 6: Week 4, dietary counselling/group meeting 3 (HNU), pick up empty food diary and urine collection kit
- Visit 7: Week 6, dietary counselling/group meeting 4 (HNU), pick up empty food diary and urine collection kit

- Visit 8: Week 8 of LED, CID 3 visit (HNU), hand in completed food diary and urine sample, fasting blood glucose test, completed DEXA scanning. End of the weight loss period.

Body weight was measured at every CID visit. Body weight, fasting glucose test, circumference measurement, blood pressure measurement, and food intake measurement were performed on all CID days. Body composition assessment was undertaken at CID1 and CID3.

Table 8, Study visits and required data collection

Visit number	Data collected
Visit 1 Screening visit Informed consent gained	Ethnicity, age Height, weight, waist circumference, hip circumference, BMI, blood pressure, fasting blood glucose level Personal detail, GP contact detail Medical history form, medication form Screen visit form Food preference questionnaire
Visit 2 Dietary counselling/group meeting 1	
Visit 3 CID1 visit Baseline data collection Beginning of the LED period	Height, weight, waist circumference, hip circumference, BMI, Blood pressure Fasting blood glucose level DEXA scan 4-day food diary (baseline) 24-hour urine sample (not included in the present thesis)
Visit 4 Dietary counselling/group meeting 2	
Visit 5 CID2 visit	Height, weight, waist circumference, hip circumference, BMI, Blood pressure Fasting blood glucose level 4-day food diary 24-hour urine sample (not included in the present thesis)
Visit 6 Dietary counselling/group meeting 3	
Visit 7 Dietary counselling/group meeting 4	
Visit 8 CID3 visit End of LED period	Height, weight, waist circumference, hip circumference, BMI, Blood pressure Fasting blood glucose level DEXA scan 4-day food diary (baseline) 24-hour urine sample (not included in the present thesis)

3.7.3 Detailed study visits

3.7.3.1 screening visit

Participants were contacted 24 to 48 hours before their scheduled screening visit to remind them the date and time, as well as reminding them to be fasted for at least 10 hours (nothing should be consumed except for water since the last meal of the previous day). On the day of the screening visit, after the participants arrived at HNU, they were asked to sit in the lounge and a presentation was given to explain the study in detail, which covers:

- The background of the study
- The aim of the study
- Timeline and scheduled visits of the study
- Tests and assessments involved, blood test, urine collection, food record, body composition scan
- The meal replacements and meal plan involved in the study
- Risks and benefits
- The compensations involved

Participants were then asked if they had read and understood the Participant Information Sheet (PIS) that was previously emailed to them, if there were no questions from reading the PIS, they were asked to sign two copies of the informed consent form (ICF) (one for personal record and the other one for research use). After participants were fully informed and the written consents were obtained, participants were asked to fill out a series of questionnaires including:

- Personal details form
  - Self-identified ethnicity, age, contact number, address, email, emergency contact
- GP contact details
  - Name and address of the healthcare provider, phone number of the GP
- Medical history form
  - Including procedures, implants and diagnosis, both ongoing and past use
- Medication form
  - Including all the ongoing medication and nutrition supplements
- Screen visit form
  - Completed by investigators, including weight, height, blood pressure, waist circumference, hip circumference, and fasting blood glucose measurements
- Food preference questionnaire
  - Covered the preferences and allergies for the food items included in the study, including ingredients in the LED products and in the designed cookbook

The measurements including:

- Body weight
- Height
- Blood pressure
- Waist circumference
- Hip circumference
- Fasting blood glucose

The fasting blood sample was collected by a trained medical professional and analyzed locally suing a Roche Reflotron benchtop analyzer located at HNU, where the fasting plasma glucose (FPG) concentration was measured to screen for diabetes.

- If fasting plasma glucose was < 7.0mmol/L, participant was eligible.
- If FPG was ⩾ 7.0mmol/L, another venous blood sample (3ml) was collected to check for HbA1c.
- If HbA1c was ≥ 50mmol/L, the participant was considered ineligible and her GP was contacted.

The eligible participants were asked to sit in the dining room where they were taught about how to collect a 24-hour urine sample and complete a 4-day food diary. Participants were also provided with a urine collection kit, an empty food diary, and the detailed written instruction sheets for both. A date for the start of the treatment and the upcoming visits were scheduled, and participants were asked to complete both the urine collection and food record and bring along with them when they attend the visits in the research unit.

Ineligible participants were given a $20 voucher as a compensation.

3.7.3.2 (LED Group Counselling Meeting) (Week 0 of LED)

Participants were contacted via phone, text message or email 24 to 48 hours before their scheduled group meetings to remind them the date and time of their meeting, and to bring the completed food diary and urine sample. Upon arrival, participants were weighted and asked if they experienced any changes with their medication since their

last visit. The completeness of food diary and urine sample collection was checked by the phd student and the author of the thesis.

The diet sessions were treatment-specific and were held by the study dietitian, the phd student, and the author. The sessions were 90 minutes in duration with a 10-minute break included. Information was delivered in the form of a presentation. The phd student began the presentation with a brief introduction of the study, including some previous successful weight loss trials taking place at HNU, such as the PREVIEW study; the concept of LED and their use; as well as reminding the participants not to change their physical activity level to prevent any adverse health effect. The study dietitian then discussed the concept of 'weight' and 'health status' with the participants, such as how they felt about their current weight, and if their weight had caused any health problems. With the help of the study dietitian, participants identified and wrote down their own SMART goals (Specific, Measurable, Achievable, Realistic, Timed) (Gardner, Kjolhaug, Linde, Sevcik, & Lytle, 2013) and their individualised coping strategies. The study dietitian also discussed the importance of creating a supportive environment for weight loss, such as involving the support person and removing temptations.

The author then presented on the preparation the breakfast oatmeal and the variable meal, including pictures of the ready meals and video clips of the cooking process.

A participant booklet was given to participant of each group, including the following information:

- Participants' own records of body weight change, personal motivations, SMART goals (Gardner et al., 2013).
- Brief description of each study visits, including the scheduled date and time for each visit, the preparations before attending, and the tests and assessments involved at each study visit.
- General weight loss recommendations and strategies to improve compliance, including increasing fluid intake to reduce hunger, limiting physical activities level to low intensity only, removing temptations in daily life, and pre-planning for occasions such as travelling or dining out.
- Instruction on how to prepare the breakfast oatmeal, including the required ingredients, the product information and the reference cost for each ingredient, the amount required, and the instructions on how to prepare a standard serving.
- Instruction on how to prepare the variable meal. The information provided was treatment-specific, containing the required ingredients, the product information and the reference cost for each ingredient, the amount required, and the instructions on how to prepare a standard serving.
- Safety information on cooking, chilling, cleaning the food ingredients to prevent food poisoning.

3.7.3.3 CID1

Participants were contacted via phone, text message or email before their scheduled screening visit to remind them the date and time of their visit and to be fasted for between 10 to 14 hours before their visits (nothing should be consumed except for

water since the last meal of the previous day). Participants were also reminded to bring the completed food diary and collected urine samples if they missed at the last visit.

Participants were expected to arrive at the research unit at 8:00 am on the day of their CID1 visit. Upon arrival, participants were offered with a glass of water while being asked if they had experienced any physical discomforts or having any change in medication, all the adverse events and change in medications reported were documented in the participant folder. Participants were also asked if they had been fasted for between 10 to 14 hours, if participants were not fasted or not feeling well, they were rescheduled to come back on a different day. Participants handed in their food diary and collected urine sample.

- Before the baseline blood sample was taken, participants were taken to a private room and had their weight, blood pressure, waist and hip circumference measured.
- At 8:30, participants had their fasting blood sample collected by a trained medical professional.
- The completeness of the food diary and urine sample collection was checked by the author and the phd student, further details were probed if required.
- Participants were then taken to Auckland City Hospital for the DEXA scan assessment, a copy of the scan results was given to them for personal use.
- Upon return, participants were given another 4-week supply of LED product. Participants were not allowed to choose the flavour or certain LED product of

their interests, only a certain combination of LED products were given by their treatment groups. However, small alterations were allowed.

3.7.3.4 (LED Group Counselling Meeting) (Week 2 of LED)

Participants were contacted via phone, text message or email 24 to 48 hours before their scheduled group meetings to remind them the date and time of their meeting. The diet sessions were 60 minutes in duration and also treatment specific. The sessions were facilitated by the study dietitian and assisted by the phd student and the author. Participants were weighted and asked to reflect on their progress during the last two weeks. The study dietitian then reviewed each participant's progress and barriers, and subsequently discussed the coping strategies specific for each participant. Finally, the study dietitian emphasised on the importance of portion control and choosing foods that are low in energy density, participants were encouraged to pay more attention to the appetite cues, and to stop eating when they felt comfortably full.

3.7.3.5 CID2

Participants were contacted via phone, text message or email before their scheduled screening visit to remind them the date and time of their visit and to be fasted for between 10 to 14 hours before their visits (nothing should be consumed except for water since the last meal of the previous day). Participants were also reminded to bring the completed food diary, collected urine samples, and the empty packets of their consumed LED products.

Participants were expected to arrive at the research unit at 8:00 am on the day of their CID2 visit. Upon arrival, participants were offered with a glass of water while being asked if they had experienced any physical discomforts or having any change in medication, all the adverse events and change in medications reported were documented in the participant folder. Participants were also asked if they had been fasted for between 10 to 14 hours, if participants were not fasted or not feeling well, they were rescheduled to come back on a different day. Participants handed in their food diary and collected urine sample.

- Before the baseline blood sample was taken, participants were taken to a private room and had their weight, blood pressure, waist and hip circumference measured.
- Participants had their fasting blood sample collected by a trained medical professional.
- The completeness of the food diary and urine sample collection was checked by the author and the phd student, further details were probed if required.
- The number of empty packets of their consumed LED products was recorded and double checked by two separate workers. Explanation required from the participants if there was any inconsistency between the numbers.
- Upon leaving, participants were given another 4-week supply of LED product. Participants were not allowed to choose the flavour or certain LED product of their interests, a certain combination of LED products were given according to their treatment groups. However, reasonable alterations were allowed.

3.7.3.6 (LED Group Counselling Meeting) (Week 4 of LED)

Participants were contacted via phone, text message or email 24 to 48 hours before their scheduled group meetings to remind them the date and time of their meeting, and to bring the completed food diary and urine sample. Upon arrival, participants were weighted and asked if they experienced any changes with their medication since their last visit. The completeness of food diary and urine sample collection was checked by the phd student and the author of the thesis.

The session was again 60 minutes in duration, facilitated by the study dietitian and assisted by the phd student and the author. Participants were asked to reflect on their progress during the last two weeks. The study dietitian then reviewed each participant's progress and barriers, and subsequently discussed the coping strategies specific for each participant. In the end, the study dietitian stressed the effectiveness of each diet treatment and encouraged participants to complete the study.

3.7.3.7 (LED Group Counselling Meeting) (Week 6 of LED)

Same as group counselling meeting at week 4 of LED.

3.7.3.8 CID3

Same as CID1 except no more LED products were provided, and participants were asked to return their used kitchen scales.

3.8 Statistical analysis

For the presentation of participant's characteristics, mean, minimum, maximum, standard deviation and standard error of the mean for participant's age, body weight,

change in body weight, BMI, change in BMI, height, waist circumference, hip circumference, waist and hip ratio, body composition, change in body composition, dietary intake and change in dietary intake were calculated using Microsoft Excel 2010. The statistical analysis including T-tests, analysis of variance (two-way ANOVA), repeated measure one-way ANOVA, Tukey's multiple comparisons test were completed using GraphPad Prism 6 software. Significance generated by GraphPad Prism 6 software as following:

- ns: $P > 0.05$
- *: $P \leqslant 0.05$
- **: $P \leqslant 0.01$
- ***: $P \leqslant 0.001$
- ****: $P \leqslant 0.0001$

The participants used in this sub-study come from the Higher-protein vs Low-carbohydrate Diets for Weight loss study, which investigated a total of 140 individuals. This sub-study only focused on 46 completers in the cohort 1, 2 and 3, so there is less statistical power than the main study. This presenting thesis is completer's only. To be a completer of the sub-study, all data required must be collected, including weight measurement, blood glucose measurement, food record, DeXA measurements.

3.9 The author's role

The author contributed to the designing of the cookbooks for variable meals assigned to each treatment arm and energy plan, working alongside the phd student and other staff members.

During the screening visit, the author took part in performing activities including measuring the body weight, height, blood pressure, waist and hip circumferences, and conducting interviews regarding lifestyle and medical history with the participants. The author also constructed and facilitated the information sessions to eligible participants, including how to take food record and collect urine sample.

During baseline, CID2 and CID3 visits, the author took part in performing activities including measuring the body weight, height, blood pressure, waist and hip circumferences, and conducting interviews regarding medication change and adverse events with the participants. The author also checked the completeness of the food diary with the participants.

The author also facilitated the groups meetings with participants alongside a phd student and a registered dietitian, giving information on the preparations of oatmeal breakfast, LED meal replacement products and variable meals, as well as the allowed items during LED treatment.

Chapter 4. Results

4.1 Participants

Following prescreening via telephone and e-mail, 111 participants were invited to Human Nutrition Unit (HNU) for screening visits for eligibility, 90 met the inclusion criteria, 70 agreed to participate after being fully informed and subsequently randomized into four treatment groups: 17 participants were allocated into the High Protein High Carbohydrate (HPHC) group, 17 into High Protein Low Carbohydrate (HPLC) group, 18 into Normal Protein Normal Carbohydrate (NPNC) group, and 18 into Normal Protein Low Carbohydrate (NPLC) group. During the 8 weeks of LED, 7 participants dropped out between CID1 (week 0) and CID2 (week 4). 63 participants completed the CID2 visit (week 4). 12 participants dropped out after CID2 visit (week 4). After 8 weeks of LED, 51 participants had completed all 3 CID visits.

The drop out reasons are shown in the participant flowchart (Figure 5) and was as following: 1, Inability to attend HNU visit; 2, Inability to comply with meals; 3, Left Auckland; 4, Pregnancy; 5, Lost to contact; 6, Withdrawn consent.

Forty-six participants provided all the data required for the present thesis, which was the food diary data at baseline (CID1, week 0), CID 2 (week 4), and CID3 (week 8). Not all participants who attended 3 CID visits completed their food diaries. For example, 1 participant was unable to provide the food diary recorded between week 4 and week 8, 1 participant was unable to provide the food diary recorded between week 0 and week 4 and between week 4 and week 8, 1 participant was unable to provide quantifiable food diary data recorded at baseline, 1 participant was unable to

provide quantifiable food diary data recorded between week 4 and week 8. As this is a completer's only analysis, the 46 participants with completed food diary data are included in this analysis. The flow diagram for participant information is shown on the following page (see Figure 5).

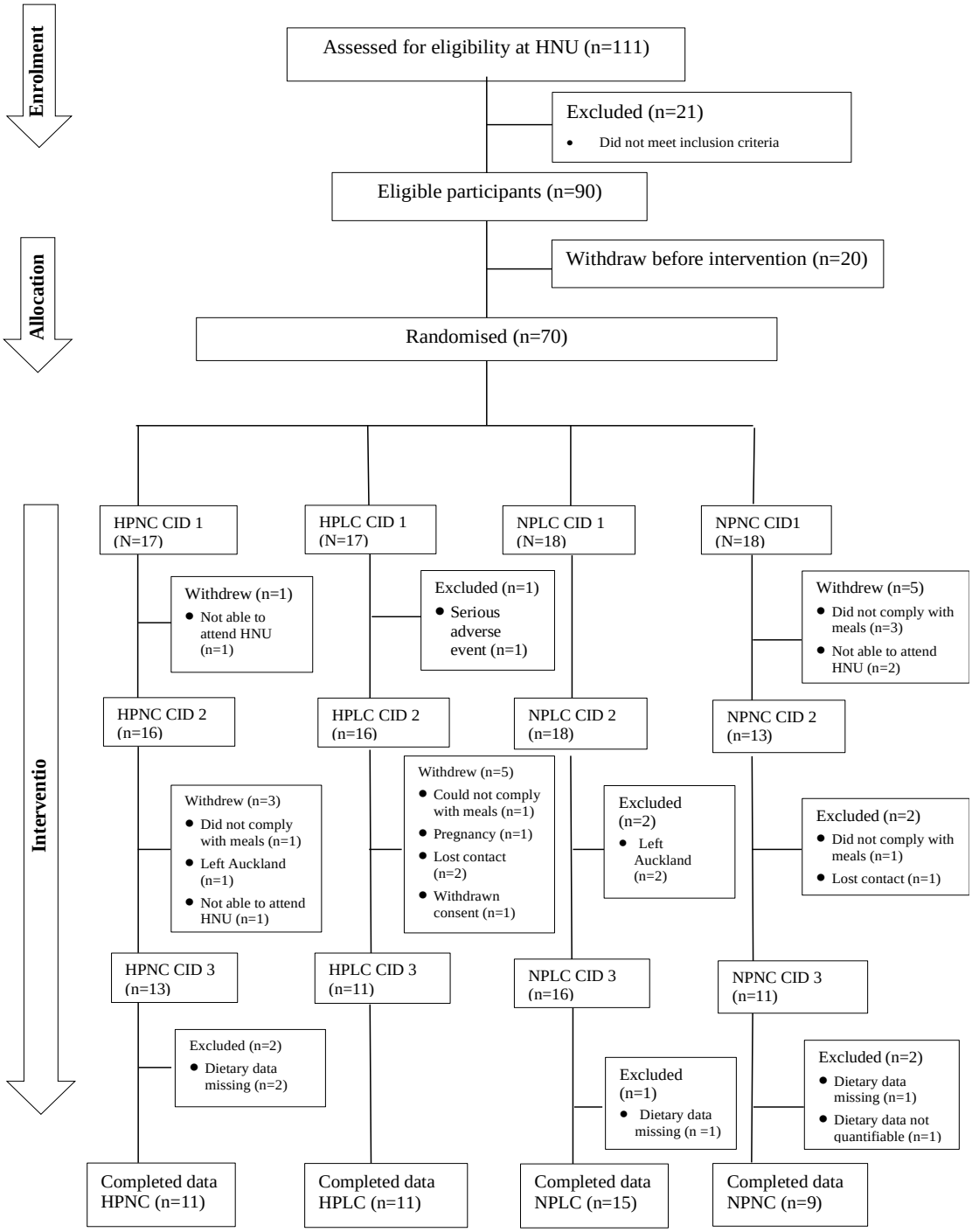

Figure 5: Flow diagram of participant information in the present thesis.

4.2 Participant baseline characteristics collected at baseline (CID1)

The baseline characteristics for the 46 participants who completed the 8-week study with all data collected are shown in Table 9.

Only females were recruited and participated in the study, there was no gender distribution. The median age for all participants was 37.5 years, the mean (SD) age for all participants was 40.0 (11.7) years. When being divided into diet groups, the mean age was 40.9 (9.6) years in NPLC group, 45.1 (15.9) years in NPNC group, 39.4 (8.9) years in HPLC group, 35.4 (12.6) years in HPNC group, no significant difference was found between groups and all of them fell into the eligible age range of the study (18-65 years). 63% participants were between the ages 18-44 years. 37% participants were between 45-65 years (Figure 5a). 37% of participants classified themselves as NZ European, 17% other, 13% Maori, 7% Pacific Island, 11% Indian, and 13% Chinese (Figure 5b).

The mean BMI across the four treatment groups ranged from 33.9 to 35.4 kg/m^2, indicating class I and class II obesity according to the WHO classification of BMI: class I obesity: 30.0-34.9 kg/m^2, class II obesity: 35.0-39.9kg/m^2. The mean fasting glucose across four treatment groups ranged from 5.7 to 6.3 mmol/L, falling into the prediabetes category (6.1-6.9mmol/L).

Table 9, Baseline characteristics for all participants who completed 8 weeks of LED and CID3; and also fulfilled all tasks required at each CID visit

	NPLC (n=15)		NPNC (n=9)		HPLC (n=11)		HPNC (n=11)		P value	Sig
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Age (yr)	40.9	9.6	45.1	15.9	39.4	8.9	35.4	12.6	0.319	NS
Weight (kg)	91.4	9.2	91.5	15.9	91.3	8.6	92.9	12.4	0.9865	NS
Height (m)	1.6	0.1	1.6	0.1	1.6	0.1	1.6	0.1	0.8457	NS
BMI (kg/m^2)	33.9	2.7	34.0	4.6	34.3	3.2	35.4	3.1	0.683	NS
Waist circumference (cm)	97.7	5.6	99.8	13.3	98.4	6.0	102.6	9.0	0.5161	NS
Hip circumference (cm)	119.1	6.9	113.5	12.9	115.9	8.8	119.8	8.1	0.3699	NS
Waist: hip ratio	0.82	0.05	0.88	0.04	0.85	0.07	0.86	0.04	0.0722	NS
Systolic BP (mmHg)	110.1	8.7	112.3	7.7	113.6	7.5	110.9	10.3	0.7541	NS
Diastolic BP (mmHg)	60.0	5.5	62.2	6.2	64.7	6.7	60.1	6.6	0.2308	NS
FPG (mM)	6.2	0.6	5.9	0.4	6.3	1.1	5.7	0.5	0.2143	NS
Total FM (kg)	42.6	5.1	42.5	12.1	40.6	7.4	41.9	7.0	0.9227	NS
Total FM (%)	46.9	2.5	46.0	6.0	44.5	5.2	45.3	3.2	0.5084	NS
Total lean mass (kg)	45.5	4.6	46.1	5.5	47.5	3.9	47.8	6.3	0.6454	NS
Total lean mass (%)	50.3	0.6	51.2	1.9	52.7	1.5	51.8	0.9	0.4835	NS
Android region fat (% BW)	54.5	3.8	52.4	9.6	52.2	5.3	54.2	3.8	0.6893	NS
Gynoid region fat (% BW)	50.3	2.6	47.9	4.3	46.5	7.6	47.9	3.9	0.2579	NS
BMR (MJ)	6.7	0.4	6.9	0.7	6.6	0.3	7.2	0.9	0.1020	NS
BMR * 1.2 (MJ)	8.1	0.5	8.2	0.9	8.0	0.4	8.7	1.1	0.1389	NS
Diet										
Energy (kJ)	9475.0	3338.0	9312.0	2247.0	8258.0	2267.0	8607.0	2468.0	0.6586	NS
Protein (g)	100.6	37.9	90.4	20.9	92.9	28.2	85.2	23.6	0.6209	NS
Protein (kJ)	1679.0	632.4	1510.0	348.8	1551.0	471.3	1423.0	394.5	0.6209	NS

Protein (% TE)	18.2	5.1	17.0	5.9	19.3	4.5	17.0	3.3	0.6564	NS
Carbohydrate (g)	229.9	84.2	233.9	73.8	196.6	64.6	223.1	92.0	0.6956	NS
Carbohydrate (kJ)	3840.0	1406.0	3906.0	1233.0	3283.0	1079.0	3725.0	1537.0	0.6956	NS
Carbohydrate (% TE)	40.4	7.7	41.4	5.8	40.0	7.0	41.8	9.3	0.9408	NS
Fat (g)	94.8	48.1	91.4	28.6	82.5	35.2	84.7	25.7	0.8270	NS
Fat (kJ)	3574.0	1813.0	3446.0	1077.0	3109.0	1327.0	3193.0	968.2	0.8270	NS
Fat (% TE)	37.0	5.9	36.7	6.3	36.7	7.5	37.8	7.4	0.9792	NS

BW: body weight, BMI: body mass index, BMR: basal metabolic rate, FM: fat mass, FPG: fasting plasma glucose; kg: kilograms, SD: standard deviation, TE: total energy, NS: not significant.

One-way ANOVA multiple comparison p-values are between NPLC vs NPNC vs HPLC vs HPNC.

BMR calculation: females between 18 to 30 years, BMR=0.057*WT+1.184*HT+0.411; female between 30 to 60 years, BMR=0.034*WT+0.006*HT+3.530

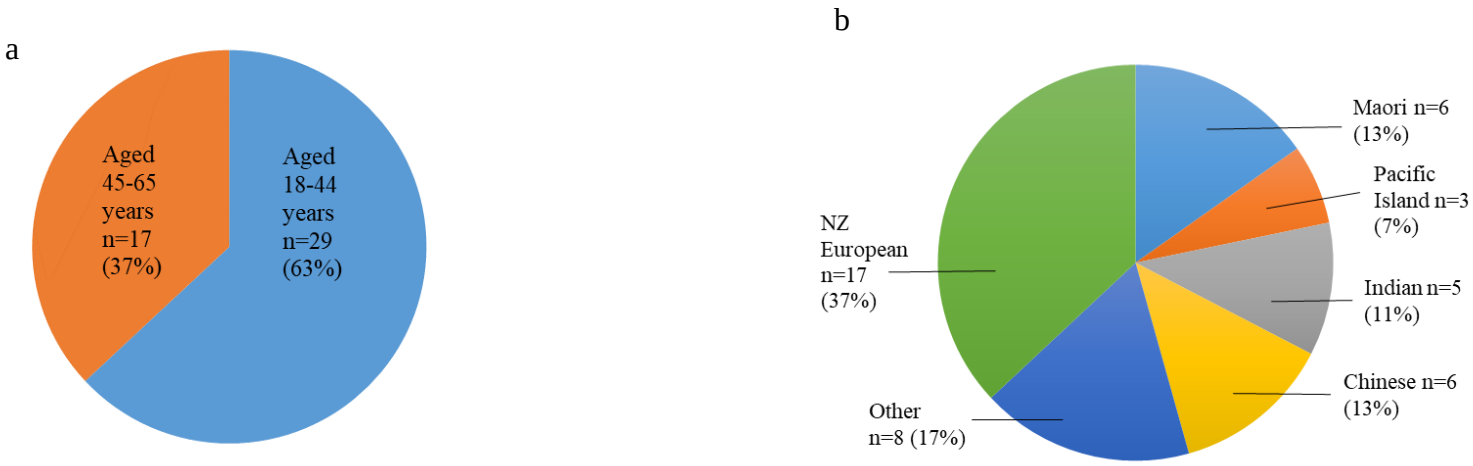

Figure 5, Participant characteristics at baseline. Those who have completed 8 weeks of intervention and CID3 (n=46). a) age distribution. b) ethnicity distribution.

4.3 Outcomes of LED for all participants (N=46)

Body weight and BMI were measured at CID1 (week 0), CID2 (week 4), and CID3 (week 8), body fat and lean mass were measured at CID1 and CID3. Changes in body weight, BMI, body fat and lean mass were seen in all participants between CID1 to CID2, and CID1 to CID3, but not between CID2 to CID3.

4.3.1 Weight loss of all participants after 8-week LED

Table 10, Mean (SEM) body weight (kg) and mean (SEM) body weight change (kg and %) at each CID (Clinical Investigation Day)

CID	*Body weight (kg)*	*P-value*	*Mean body weight change (kg)*	*Mean body weight change (%)*
CID1 (week 0)	91.8 (1.6)	-	-	-
CID2 (week 4)	86.3 (1.6)	<0.0001	-5.5 (0.3)	-6.0 (0.3)
CID3 (week 8)	83.4 (1.7)	<0.0001	-2.9 (0.3)	-3.4 (0.3)
CID 1-3 (week 0-8)	-	<0.0001	-8.4 (0.5)	-9.2 (0.6)

Note: P value calculated through repeated measures one-way ANOVA, comparing body weight (kg) between 3 CIDs; significant change in mean body weight (SEM) from CID1 to CID2, CID2 to CID3, CID1 to CID3 (all p<0.0001).

As shown by Table 10, the 8-week LED successfully resulted in a significant and clinically meaningful weight loss for all participants between CID1 (week 0) and CID3 (week 8) of -8.4 (0.5) kg (P<0.0001) and -9.2 (0.6) % of baseline body weight (P<0.0001). The mean (SEM) baseline body weight at CID1 (week 0) was 91.8 (1.6) kg. The baseline weight for all participants was highly variable, with the highest baseline weight being 116 kg, and the lowest being 71.6 kg.

The mean (SEM) weight at CID2 (week 4) was 86.3 (1.6) kg. The mean (SEM) weight change from baseline to CID2 for all 46 participants was significant, and all participants lost weight during this 4-week period. Between CID1 (week 0) and CID2

(week 4), the mean weight loss was -5.5 (0.3) kg (P<0.0001), which was equivalent to -6% of baseline body weight (p<0.0001).

The mean (SEM) body weight at CID3 (week 8) was 83.4 (1.7) kg. All but 2 of the 46 participants who completed the 8-week of LED lost weight. The mean (SEM) weight change from CID2 to CID3 for all participants was significant, with an average weight loss of -2.9 kg (P<0.0001) and -3.4 % baseline body weight (P<0.0001).

Figure 6a shows the individual weight change (kg) from CID1 (baseline) to CID2 (week 4), and then to CID3 (week 8). The weight loss for all 46 participants displayed a large variability between the individuals, where the weight lost from baseline to CID2 ranged from -8.6 kg to -0.4 kg, the weight lost from CID2 to CID3 ranged from -7.2 kg to -0.6 kg. As for the 8 weeks from baseline to CID3, the minimum weight loss was -3 kg, and the maximum weight loss was -14.6 kg.

When compared with baseline, 2 participants showed no reduction in body weight at CID3 (post LED), where one participant did not experience any change in body weight after 8-week LED (labelled as participant 2), while the other gained +3.5 kg after 8-week LED (labelled as participant 1) (Figure 6c).

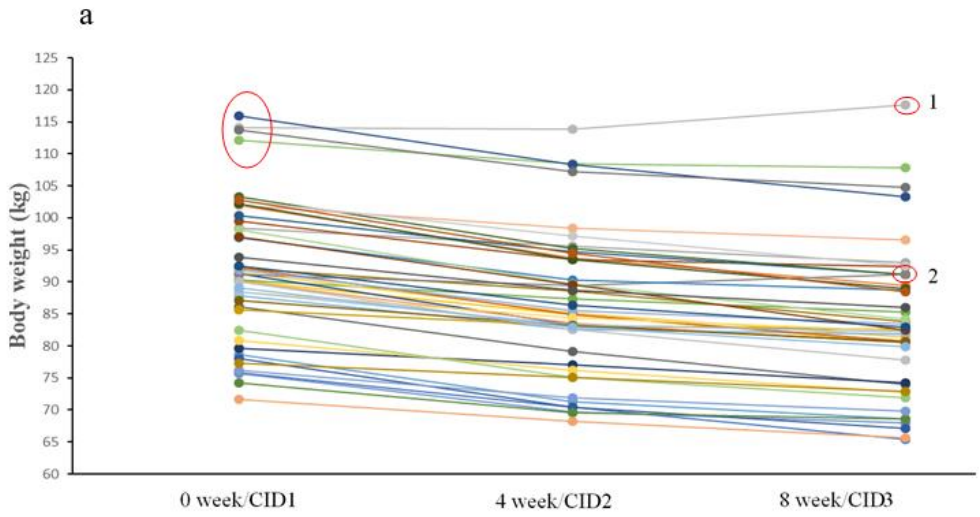

Figure 6, a) Weight changes (kg) from baseline CID1 (week 0), CID2 (week 4), and CID3 (week 8). Each line represents a single participant (n=46). 1: one participant gained weight from CID1 to CID3. 2: one participant didn't experience any change in body weight from CID1 to CID3.

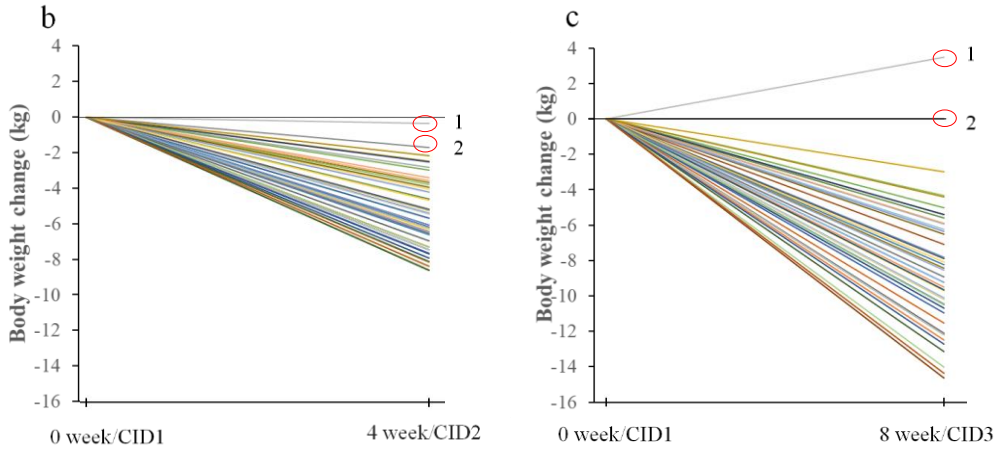

Figure 6, b, c) Weight change (kg) from baseline to CID2 (week 4), and from CID2 (week 4) to CID3 (week 8). Each line represents a single participant (n=46), 1: one participant gained weight from CID1 to CID3. 2: one participant didn't experience any change in body weight from CID1 to CID3.

4.3.2 Body weight change (in %)

Figure 7a shows weight change (%) from CID1 (week 0) to CID2 (week 4). All participants lost weight during this period ranged from -9.8% to -0.3%. Figure 7b shows weight change (%) from CID1 (week 0) to CID3 (week 8), all participants but 2 lost weight during this period ranged from -15.1% to -3.5%. One participant gained 3.0% body weight compared to baseline (labelled as 1), and one participant did not experience any change in body weight compared to baseline (labelled as 2).

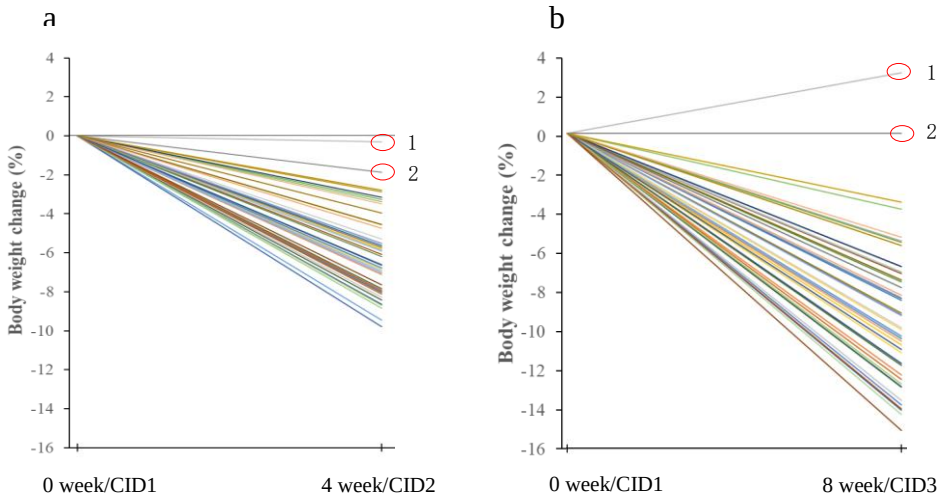

Figure 7, a) Weight change (%) from baseline CID1 (week 0) and CID2 (week 4) for all participants (n=46). Each line represents a single participant (n=46). b) Weight change (%) from CID1 (week 0) and CID3 (week 8) for all participants (n=46). 1: one participant gained weight from CID1 to CID3. 2: one participant didn't experience any change in body weight from CID1 to CID3.

4.3.3 Effect of baseline body weight on weight loss

There is a non-significant effect (P=0.5284) of baseline body weight (kg) on the absolute weight loss in kilograms (Figure 8a) (P=0.5284) or % weight loss (Figure 8b) (0=0.2887) for all 46 participants from baseline to CID3 (week 8). The two participants who did not experience any weight loss during the 8-week LED period were circled and labelled as participant 1 and 2 in Figure 8a and Figure 8b. Participant 1 started with a baseline BW of 114.2kg and gained 3.5kg after 8-week of LED treatment, while participant 2 started with a baseline body weight of 91.2kg and experienced no gain/loss in body weight. However, after excluding these two participants, the association between baseline body weight and absolute weight loss became significant (P=0.0328) (Figure 8c), but the association between baseline BW and % weight loss was still not significant after excluding these two participants (P=0.9250) (Figure 8d).

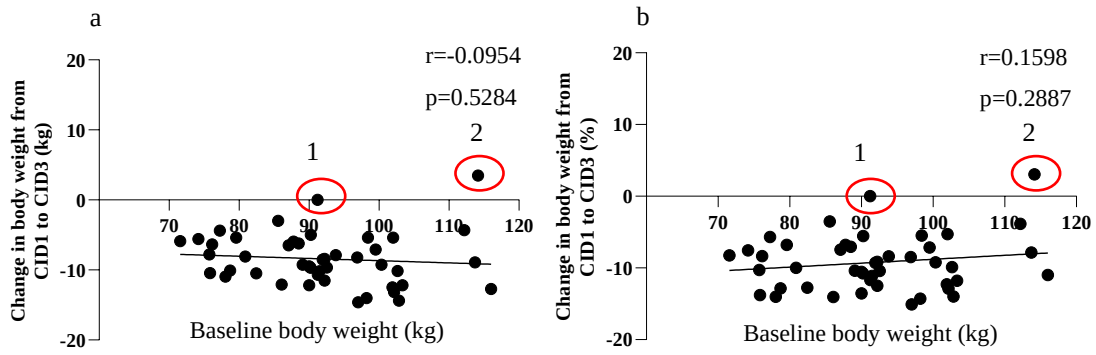

Figure 8, The effect of baseline weight on weight change from CID1 to CID3 in n=46. Each data point represents an individual. a) Absolute weight change CID1-CID3 (P=0.5284); b) percentage weight change CID1-CID3 (P=0.2887).

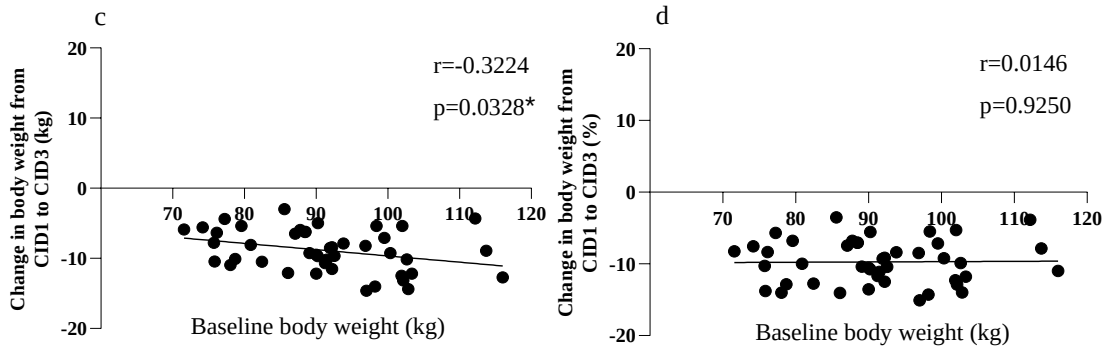

Figure 8, c) Absolute weight change from CID1 to CID3 after excluding the two outliers (P=0.0328); d) Percentage weight change from CID1 to CID3 after excluding the outliers (P=0.9250).

4.3.3 Mean body weight and change in body weight

Figure 9a shows the mean (SEM) body weight for all 46 participants at baseline, CID2 (4-week of LED), and CID3 (end of LED). Interestingly, the reduction in both mean body weight (Figure 9b) and mean % body weight (Figure 9c) appears to lessen over time, the weight reduction experienced between CID2 to CID3 was significantly smaller than what was seen between CID1 to CID2 ($p<0.0001$).

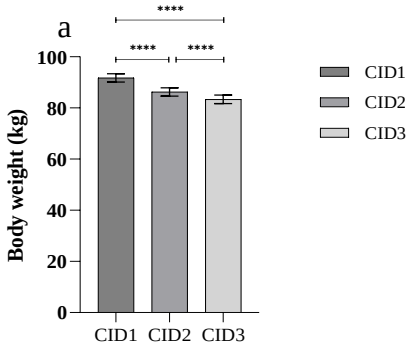

Figure 9, a) Mean (SEM) body weight (kg) at CID1, CID2 and CID3 (n=46). Significant interaction (main effect ANOVA p<0.0001), Tukey's post hoc analysis showed significant decreases from CID1-CID2, CID2-CID3, and CID1-CID3 (p<0.0001).

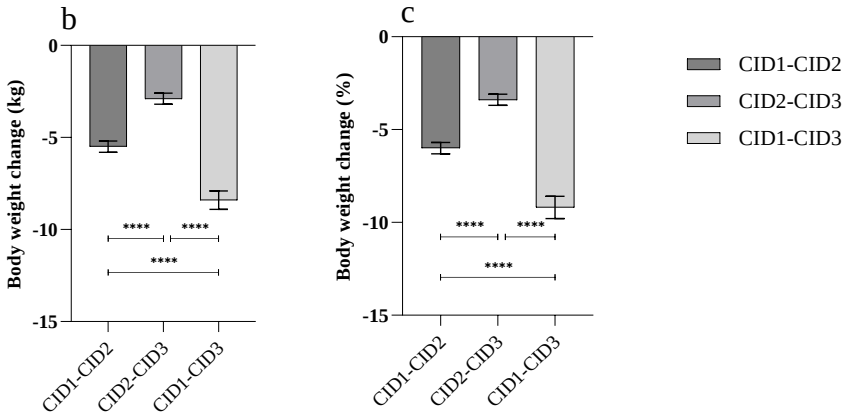

Figure 9, b) Mean (SEM) body weight (kg) from CID1 to CID2, from CID2 to CID3, and from CID1 to CID3. c) Mean (SEM) body weight in % from CID1 to CID2, from CID2 to CID3 and from CID1 to CID3. Significant difference in weight change (kg) and % between 3 study periods in all participants (n=46) (P<0.0001).

4.3.4 BMI change

As shown by Figure 10a, all but two participants had reductions in BMI from CID1 (week 0) to CID3 (week 8). In most participants, the BMI was greatest at CID1, followed by a significant decrease in BMI at CID2 ($p<0.0001$), and a lesser but still significant decrease in BMI at CID3 ($p<0.0001$) (Figure 10c). As noted previously above for body weight, there is one participant experienced an increase in BMI from CID1 to CID3 (labelled as 2), and one participant experienced no change in BMI from CID1 to CID3 (labelled as 1). At baseline, BMI ranged from 29.4 kg/m^2 to 41.8

kg/m^2.

In all participants, the mean (SEM) BMI (kg/m^2) at CID1 was 34.4 (0.5) kg/m^2, at CID2 was 32.3 (0.5) kg/m^2 and at CID3 was 31.2 (0.5) kg/m^2 (Figure 10b). The mean (SEM) change in BMI from CID1 to CID2 was -2.1 (0.1) kg/m^2 and -6.0 (0.3) % of baseline BMI, from CID2 to CID3 was -1.1 (0.1) kg/m^2 and -3.4 (0.3) % of baseline BMI, and from CID1 to CID3 is -3.2 (0.2) kg/m^2 and -9.2 (0.6) % of baseline BMI. Both absolute and % of BMI decreased significantly from CID1 (week 0) to CID2 (week 4) ($p<0.0001$, both), from CID2 (week 4) to CID3 (week 8) ($P<0.0001$, both), and from CID1 (week 0) to CID3 (week 8, both) ($P<0.0001$) (Figure 10c, Figure 10d). Similar to body weight, the decrease in BMI also lessened over time, for both the BMI (Figure 10c) and % baseline BMI (Figure 10d) ($P<0.0001$).

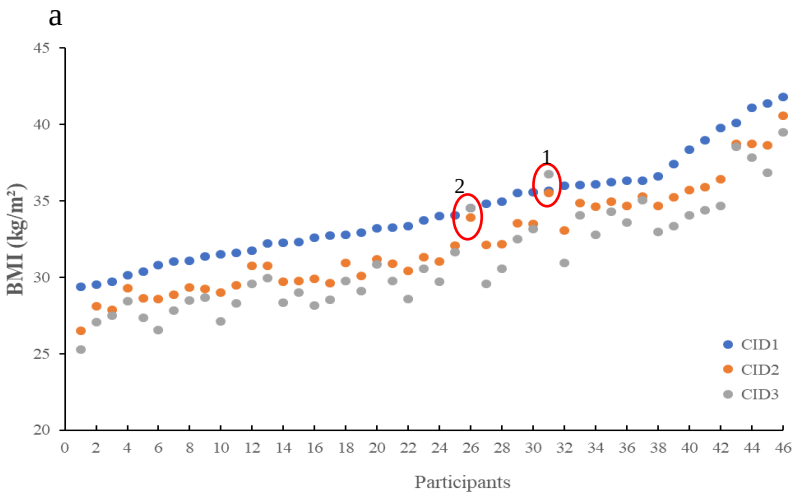

Figure 10, a) Individual BMI (kg/m²) at CID1 (week 0), CID2 (week 4), and CID3 (week 8). Each point represents an individual participant (n=46). 1: there was an increase in BMI from CID1 to CID3 for one participant, 2: there was no change in BMI from CID1 to CID3 for one participant.

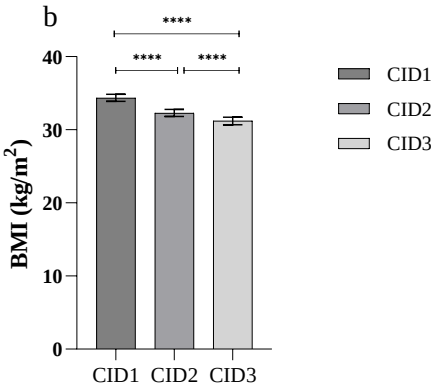

Figure 10, b) Mean (SEM) BMI (kg/m²) at CID1, CID2 and CID3 for all participants (n=46). Significant decreases from CID1 to CID2, CID1 to CID3, CID2 to CID3 (P<0.0001).

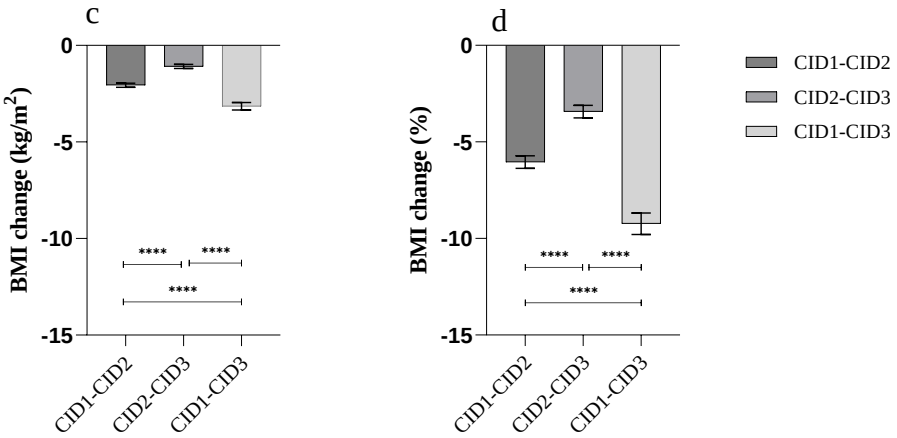

Figure 10, c) Mean (SEM) change in BMI (kg/m²) from CID1 to CID2, from CID2 to CID3, and from CID1 to CID3 of all participants (n=46). d) Mean (SEM) change in BMI (%) from CID1 to CID2, from CID2 to CID3, and from CID1 to CID3 of all participants (n=46). Significant difference in BMI change (kg/m²) and (%) between 3 study periods in all participants (n=46) (P<0.0001).

4.3.5 Body composition change for all participants (N=46)

4.3.5.1 Body fat mass (kg) change

Body fat mass (kg) was shown to be variable between participants. All participants began the study with the highest body fat mass (kg) at CID1 (week 0), followed by a significant decrease in body fat mass (kg) at CID3 ($P<0.0001$) (Figure 11a). At baseline, body fat mass (kg) ranged between 27.2kg and 62.3kg. Interestingly, although the two participants (labelled as participant 1 and participant 2) had not experienced weight loss/decrease in BMI, both still managed to lose body fat mass of -2.1kg (participant 1) and -0.8kg (participant 2) respectively. This is unexpected since the reduction in body fat mass is usually coupled with weight loss. However, bigger sample size is necessary to investigate further.

In all participants, the mean body fat mass decreased significantly from CID1 to CID3 ($p<0.0001$) (Figure 11b). Mean (SEM) body fat mass (kg) at CID1 was 41.9 (1.1) kg; at CID3 was 35.4 (1.1) kg.

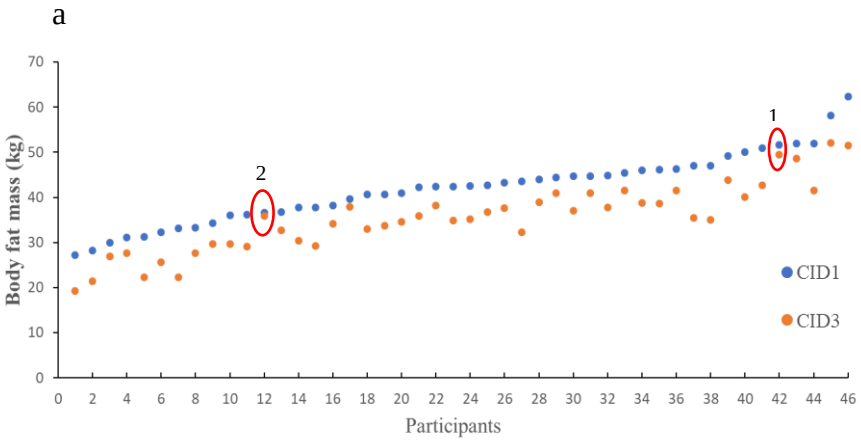

Figure 11, a) Individual changes in body fat mass (kg) from CID1 (week 0) to CID3 (week 8). Each plot represents an individual participant (n=46). 1, 2: participants who didn't experience any reduction in body weight/BMI still experienced decreases in body fat (kg) from CID1 to CID3.

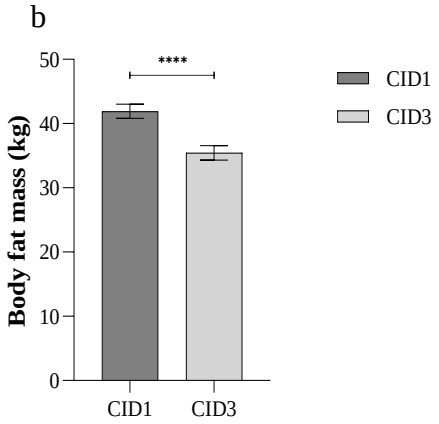

Figure 11, b) Mean (SEM) body fat mass (kg) at CID1 (week 0) and CID3 (week 8) (n=46). Significant decreases from CID1-CID3 (p<0.0001).

4.3.5.2 Body fat (%) change

Similar to the changes observed with body fat mass, all participants (n=46) began the study with the highest body fat (%) at CID1 (week 0), followed by a significant decrease in body fat (%) at CID3 (week 8) (Figure 12a). At baseline, body fat (%) ranged between 36.1% to 53.9%. Although the two participants (labelled as participant 1 and participant 2) had not experienced weight loss/decrease in BMI, both still managed to lose body fat (%) of -2.47% (participant 1) and -0.1% (participant 2) respectively. This is again, unexpected.

In all participants, the mean body fat (%) decreased significantly from CID1 to CID3 ($p<0.0001$) (Figure 12b). Mean (SEM) body fat (%) at CID1 was 45.8 (0.6) %, at CID3 was 42.4 (0.8) %.

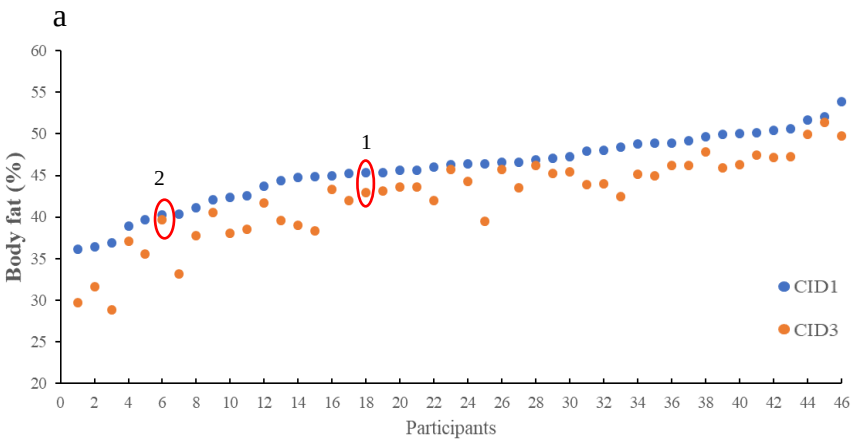

Figure 12, a) Individual changes in body fat (%) from CID1 (week 0) to CID3 (week 8). Each plot represents an individual participant (n=46). 1, 2: participants who didn't experience any reduction in body weight/BMI still experienced decreases in body fat (%) from CID1 to CID3.

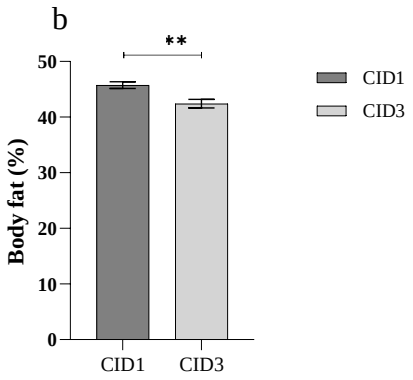

Figure 12, b) Mean (SEM) body fat (%) at CID1 and CID3 (n=46). Significant decreases from CID1-CID3 (p<0.0001).

4.3.5.3 Lean mass (kg) change

Lean mass (kg) was also shown to be variable between the participants. All but three participants lost lean mass from CID1 (week 0) to CID3 (week 8) (labelled as 1,2,3). Interestingly, the participant who gained +3.5 kg body weight experienced a +3.7 kg increase in lean mass (labelled as participant 1), while the participant who

experienced no change in body weight had a +0.2 kg increase in lean mass (labelled as participant 2). It appears that when participants gained body weight from the LED treatment, it is mainly comprised of lean mass instead of fat mass. Again, the number of participants included in the present thesis was too small to draw any conclusions. The participant who lost weight and gained lean mass was labelled as Participant 3 in Figure 13a. This participant lost body weight of -5.9 kg and -8.2%, while being coupled with a +0.3 kg increase in lean mass. Although this is unexpected since weight loss is usually coupled with some loss of lean mass.

Most participants began the study with the highest lean mass (kg) at CID1, followed by a significant decrease in lean mass (kg) at CID3 (Figure 13a). The changes in lean mass (kg) are highly variable between participants, ranging between -4.6kg to +3.7kg. On average, the lean mass (kg) decreased significantly from CID1 to CID3 ($p<0.0001$) (Figure 13b). Mean (SEM) lean mass (kg) at CID1: 46.7 (0.7) kg, at CID3: 44.9 (0.9) kg.

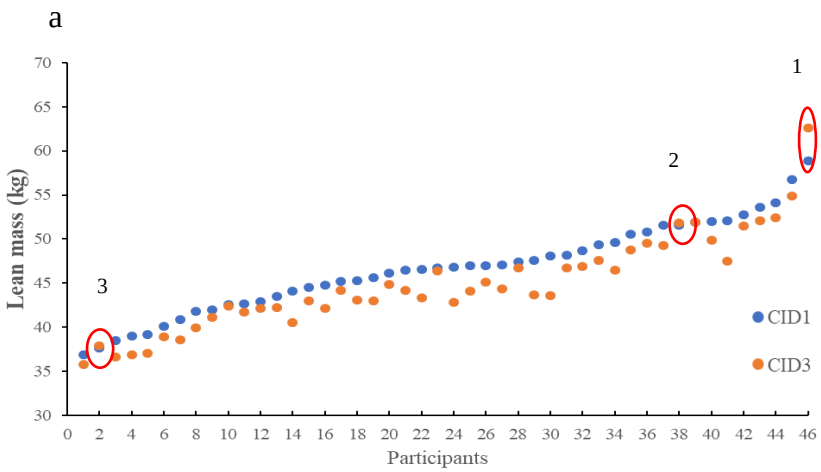

Figure 13, a) Individual changes in body lean mass (kg) from CID1 to CID3. Each plot represents an individual participant (n=46). 1,2,3: three participants experienced increases in lean mass (kg) from CID1 to CID3. 1,2: two participants did not experience any decrease in body weight and BMI.

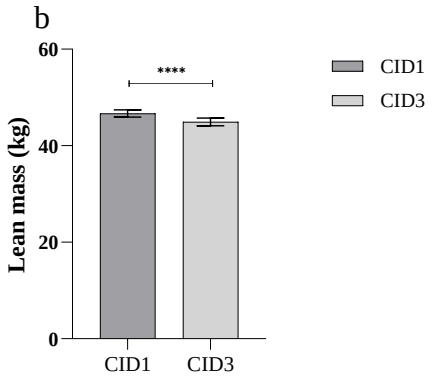

Figure 13, b) Mean (SEM) body lean mass (kg) at CID1 and CID3 (n=46). Significant decreases from CID1-CID3 (p<0.0001).

4.3.5.4 Lean mass (%) change

As opposed to the trend observed with changes in lean mass (kg), all participants gained % lean mass from CID1 (week 0) to CID3 (week 8) (Figure 14a), possibly due to the significant decrease in body weight during this period. Participant 1 and 2 experienced an increase in % lean mass 2.5% and 0.6%, respectively.

All participants began the study with a lower lean mass (%) at CID1, followed by a significant increase in lean mass (%) at CID3 (Figure 14b). The changes of lean mass

(%) are highly variable between participants, ranged between +7.5% and +0.2%.

On average, the lean mass (%) increased significantly from CID1 to CID3 (P<0.0001) (Figure 14b). Mean (SEM) lean mass (%) at CID1: 51.4 (0.6) %, at CID3: 54.5 (0.7) %.

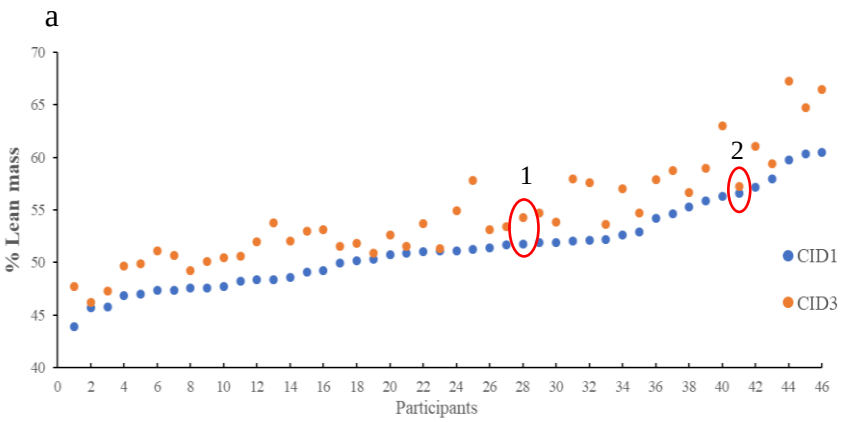

Figure 14, a) Individual changes in lean mass (%) from CID1 to CID3. Each plot represents an individual participant (n=46). 1, 2: the two participants who didn't experience decreases in body weight/BMI still gained body lean mass % from CID1 to CID3.

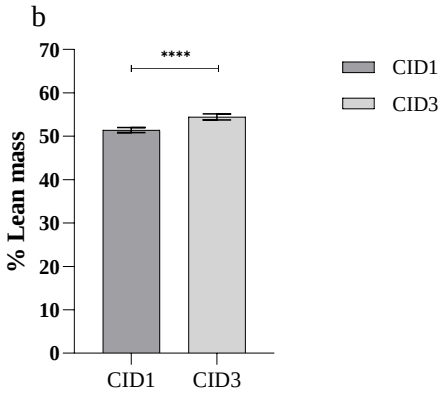

Figure 14, b) Mean (SEM) lean mass (%) at CID1 and CID3 (n=46). Significant decreases from CID1-CID3 (p<0.0001).

4.3.5.5 Contribution of fat mass loss and lean mass loss to total body weight loss

After 8-week of LED, the mean (SEM) body weight loss was -8.4 (0.5) kg, mean (SEM) fat mass loss was -6.5 (0.4) kg, mean (SEM) lean mass loss -1.7 (0.2) kg. The majority of the weight lost was from body fat loss (77.4%), followed by lean mass loss (20.2%) (Figure 15).

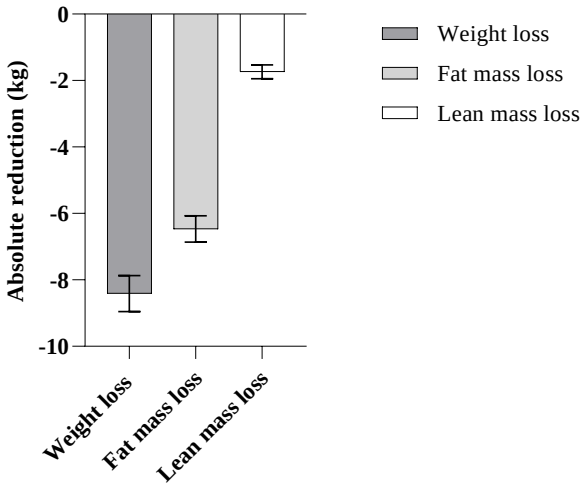

Figure 15, Mean (SEM) reduction in body weight, body fat mass, and lean mass (kg) in all participants. Mean (SEM) weight loss was -8.4 (0.5) kg, mean (SEM) fat mass loss was -6.5 (0.4) kg, mean (SEM) lean mass loss -1.7 (0.2) kg.

4.4 Outcomes of LED in the four treatment groups (NPLC vs. NPNC vs. HPLC vs. HPNC)

4.4.1 Change in body weight

Table 11a presents the body weight of participants of the four treatment groups at each CID. The body weight of the four treatment groups was not statistically significantly different at CID1 (week 0), which is expected from the protocol, since dietary treatments were randomly assigned to participants by chance. However, it is notable that there was also no significant difference in mean body weight between

treatments at any of the following CIDs (CID1 week 0: P=0.9864; CID2 week 4: P=0.9592; CID3 week 8: P=0.7985).

As expected, participants lost a significant amount of weight at the end of the weight loss period irrespective of dietary treatment (One Sample T-test, P<0.0001 for NPLC, NPNC, HPLC group; P=0.0017 for HPNC group) (Figure 16a). When participants were grouped based on the dietary treatment they received, the dietary treatment that differs in macronutrient composition did not affect the weight change differently across the weight loss period (treatment*time interaction, P=0.9997).

However, of some interest, there still was a trend for variation in the absolute body weight change during the 8-week LED across treatment groups, where NPNC lost the most body weight of -9.6 (1.1) kg, followed by HPLC of -9.2 (0.7) kg, NPLC of -8.5 (0.8) kg, and HPNC lost the least body weight of -6.5 (1.5) kg, yet this difference was not statistically significant, as shown by Tukey's pairwise multiple comparison test (Figure 16b). Similarly, NPNC lost the most % body weight while HPNC lost the least % body weight, but again with no statistically significant difference (Figure 16c).

Overall, all treatment groups significantly lost body weight during the 8-week LED, yet there was no significant effect of different diet groups and hence no support in this analysis of a sub-cohort of the full study that macronutrient composition altered weight loss trajectory and success during LED-driven acute weight loss.

Table 11, a) Mean (SEM) body weight (kg) at each CID visits for four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC. b) Mean (SEM) change in body weight (kg) and (% baseline body weight) from CID1 to CID2 and from CID1 to CID3 for four treatment groups

a

	Mean body weight (SEM) kg				
CID	**NPLC (n=15)**	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P value**
1 (week 0)	91.4 (2.4)	91.5 (5.3)	91.3 (2.6)	92.9 (3.7)	0.9864 [a]
2 (week 4)	85.9 (2.1)	85.6 (4.8)	85.7 (2.7)	87.9 (4.1)	0.9592
3 (week 8)	82.9 (2.0)	81.9 (4.7)	82.2 (2.9)	86.4 (4.6)	0.7985
[a] *P value showing the significance when comparing mean body weight (kg) between four treatment groups at week 0, 4, and 8.*					

b

CIDs	**NPLC (n=15)**	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P-value**
Body weight change (SEM) (kg)					
1→2	-5.5 (0.5) P<0.0001 [b]	-6.0 (0.7) P<0.0001 [b]	-5.7 (0.6) P<0.0001 [b]	-5.0 (0.8) P<0.0001 [b]	0.7824 [a]
1→3	-8.5 (0.8) P<0.0001 [b]	-9.6 (1.1) P<0.0001 [b]	-9.2 (0.7) P<0.0001 [b]	-6.5 (1.5) P=0.0017 [b]	0.2358 [a]
Body weight change (SEM) (%)					
1→2	-6.0 (0.4)	-6.4 (0.6)	-6.3 (0.7)	-5.6 (0.9)	0.8506 [a]
1→3	-9.2 (0.7)	-10.4 (0.9)	-10.2 (0.9)	-7.4 (1.7)	0.2565 [a]
Note: [a] *P value showing the significance in body weight change (kg, %) when comparing four treatment groups (NPLC vs NPNC vs HPLC vs HPNC), Tukey's multiple comparisons from repeated measures 2-way ANOVA indicating no significant difference between four treatment groups from CID1→CID2 and from CID1→CID3 (P>0.05, all).* [b] *P value showing the significance in body weight change (kg, %) within the same treatment group over time. One sample T-test comparing the Weight change (kg) and weight change (%) from null.*					

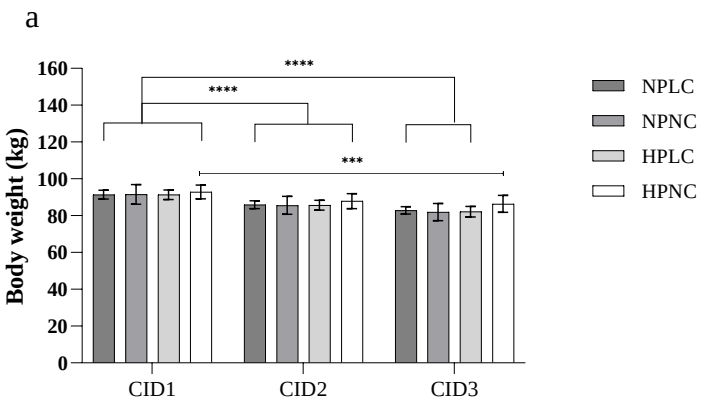

*Figure 16, a) Mean (SEM) body weight (kg) at each CID visits for four treatment groups: NPLC vs NPNC vs HPLC vs HPNC. Two-way ANOVA (treatment*time interaction, P=0.9997), (time effect, P=0.0025).*

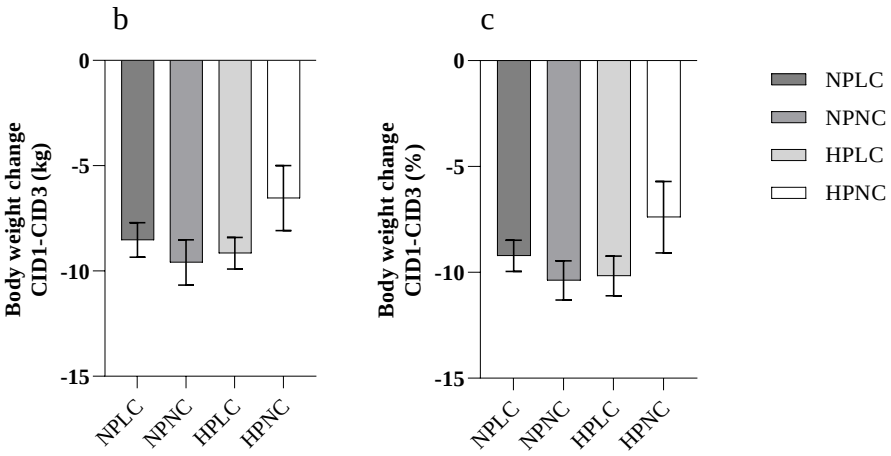

Figure 16, b) Mean (SEM) change in body weight (kg) between CID1 to CID3 in four treatment groups. c) Mean (SEM) change in body weight (%) between CID1 to CID3 in four treatment groups.

4.4.2 Change in BMI

Similar to body weight, a significant reduction in BMI was seen in all treatment groups at the end of the 8-week study period (T-test P<0.0001 for NPLC, NPNC, HPLC group; P=0.0012 for HPNC group) (Figure 17a). Again, when participants were grouped based on the dietary treatment they received, the dietary treatment that differs in macronutrient composition did not affect the change in BMI differently across the weight loss period (treatment*time interaction, P=0.9995) (Figure 17b).

Table 12, a) Mean (SEM) BMI (kg/m^2) at each CID visits for four treatment groups: NPLC vs NPNC vs HPLC vs HPNC. b) Mean (SEM) change in BMI (kg/m^2) and (% BMI) from CID1 to CID2 and from CID1 to CID3 for four treatment groups

a

	Mean BMI (SEM) kg/m2				
CID	**NPLC (n=15)**	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P value**
1 (week 0)	33.9 (0.7)	34.0 (1.5)	34.3 (1.0)	35.4 (0.9)	0.6827 [a]
2 (week 4)	31.8 (0.6)	31.8 (1.4)	32.2(1.1)	33.4 (1.0)	0.6330 [a]
3 (week 8)	30.7 (0.6)	30.5 (1.3)	30.9 (1.1)	32.8 (1.2)	0.3706 [a]
[a] *P value showing the significance when comparing mean BMI (kg/m2) between four treatment groups at week 0, 4, and 8. Tukey's multiple comparisons showed significant difference in BMI (kg/m2) between CID1 to CID3 in NPLC group (p=0.0338) and in HPLC group (p=0.05).*					

b

CIDs	**NPLC (n=15)**	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P-value**
Mean BMI change (SEM) (kg/m^2)					
1→2	-2.0 (0.2) P<0.0001 [b]	-2.2 (0.2) P<0.0001 [b]	-2.1 (0.2) P<0.0001 [b]	-2.0 (0.3) P<0.0001 [b]	0.8929 [a]
1→3	-3.1 (0.3) P<0.0001 [b]	-3.5 (0.4) P<0.0001 [b]	-3.4 (0.3) P<0.0001 [b]	-2.6 (0.6) P=0.0012 [b]	0.3332 [a]
Mean BMI change (SEM) (%)					
1→2	-6.0 (0.4)	-6.4 (0.6)	-6.3 (0.7)	-5.6 (0.9)	0.8519 [a]
1→3	-9.2 (0.7)	-10.4 (0.9)	-10.2 (0.9)	-7.4 (1.7)	0.2568 [a]
Note: [a] *P value showing the significance in BMI change (kg/m^2, %) when comparing four treatment groups (NPLC vs NPNC vs HPLC vs HPNC). Tukey's multiple comparisons from repeated measures 2-way ANOVA indicating no significant difference between four treatment groups from CID1→CID2, CID2→CID3, and CID1→CID3 (P>0.05, all).* [b] *P value showing the significance in BMI change (kg/m^2, %) within the same treatment group over time. One sample T-test comparing the BMI change (kg/m^2) and BMI change (%) from null*					

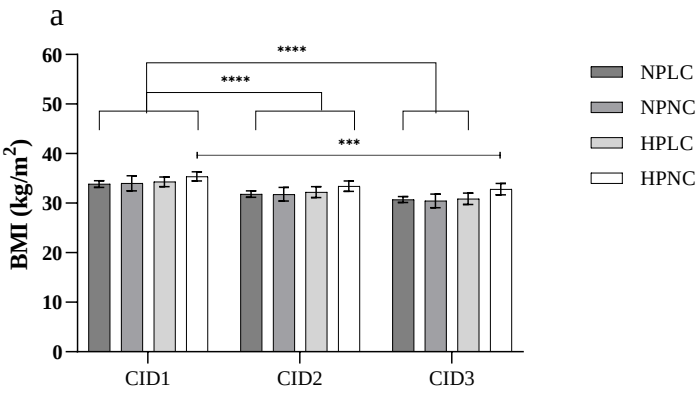

*Figure 17, a) Mean (SEM) BMI (kg/m^2) at each CID visits for four treatment groups: NPLC vs NPNC vs HPLC vs HPNC. Two-way ANOVA (treatment*time interaction, P=0.9995), (time effect, P<0.0001).*

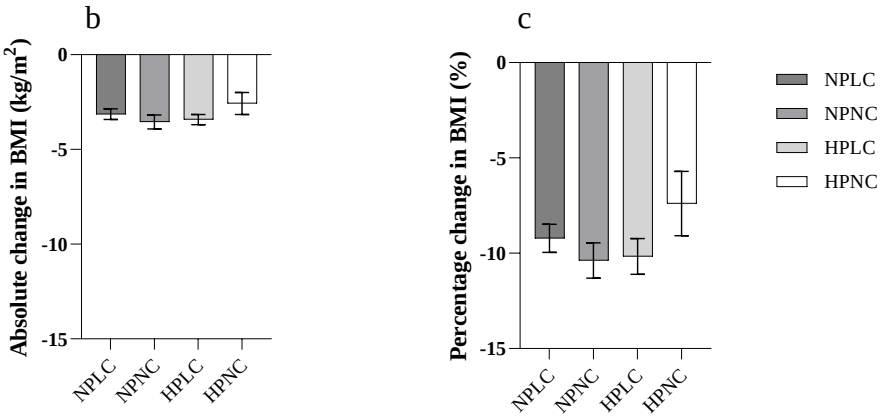

Figure 17, b) Mean (SEM) change in BMI (kg/m^2) between CID1 to CID3 in four treatment groups. c) Mean (SEM) change in BMI (%) between CID1 to CID3 in four treatment groups.

4.4.3 Change in body fat mass (kg) and % body fat

Loss in body fat was observed in parallel with weight loss for all treatment groups after the 8-week LED, as absolute kg and as % body weight (Table 13). In all groups, there was a significant decrease of body fat mass (T-test, P<0.0001 for NPLC, NPNC, HPLC group; P=0.0002 for HPNC group) (Figure 18a) and % body fat (T-test, P<0.0001 for NPLC and HPLC group; P=0.0003 for NPNC; P=0.0006 for HPNC group) (Figure 18b) between CID1 (week 0) and CID3 (week 8), which is expected and desired from the study. However, after participants were grouped based on the

dietary treatment they received, the dietary treatment that differs in macronutrient composition did not affect the change in body fat mass (treatment*time interaction, P=0.9781) (Figure 18c) or % body fat (Figure 18d) (treatment*time interaction, P=0.9723) differently across the weight loss period.

Table 13, Mean (SEM) body fat mass (kg and % body weight) at CID1 and CID3 visits and mean (SEM) change in body fat mass (kg and % body weight) from CID1 to CID3 for four treatment groups: NPLC vs NPNC vs HPLC vs HPNC

CIDs	**Mean body fat (SEM) (kg)**				
	NPLC (n=15)	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P value**
1 (week 0)	42.6 (1.3)	42.5 (4.0)	40.6 (2.2)	41.9 (2.1)	0.9227 [a]
3 (week 8)	36.3 (1.1)	34.9 (3.7)	33.8 (2.4)	36.4 (2.4)	0.8298 [a]
1→3	-6.3 (0.7) P<0.0001 [b]	-7.6 (0.9) P<0.0001 [b]	-6.8 (0.7) P<0.0001 [b]	-5.4 (0.9) P=0.0002 [b]	0.3535 [a]
	Mean body fat (SEM) (%)				
1 (week 0)	46.9 (0.6)	46.0 (2.0)	44.5 (1.6)	45.3 (1.0)	0.5081 [a]
3 (week 8)	44.0 (0.8)	42.0 (2.3)	40.7 (2.0)	42.4 (1.2)	0.4508 [a]
1→3	-2.9 (0.4) P<0.0001 [b]	-4.0 (0.7) P=0.0003 [b]	-3.7 (0.6) P<0.0001 [b]	-2.9 (0.6) P=0.0006 [b]	0.4022 [a]

Note:

[a] P value showing the significance in body fat change (kg, %) when comparing four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).Tukey's multiple comparisons from repeated measures 2-way ANOVA indicating no significant difference between four treatment groups from CID1→CID3 (P>0.05, all).

[b] P value showing the significance in body fat change (kg, %) within the same treatment group over time. One sample T-test comparing the change in body fat mass (kg) and percentage body fat (%) from null.

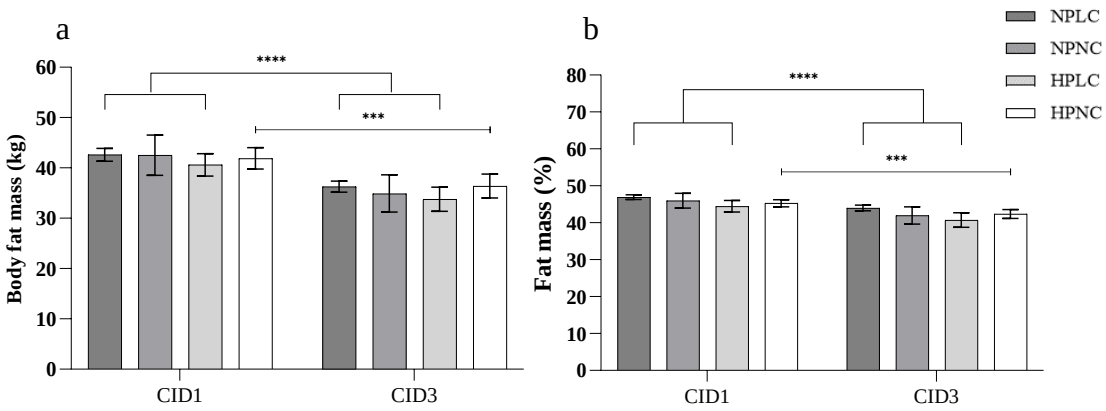

*Figure 18, a) Mean (SEM) body fat mass (kg) at each CID visits for four treatment groups: NPLC vs NPNC vs HPLC vs HPNC. Two-way ANOVA (treatment*time interaction, P=0.9781), (time effect, P=0.0001). b) Mean (SEM) percentage body fat (%) at each CID visit for four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC. Two-way ANOVA (treatment*time interaction, P=0.9723), (time effect, P=0.001).*

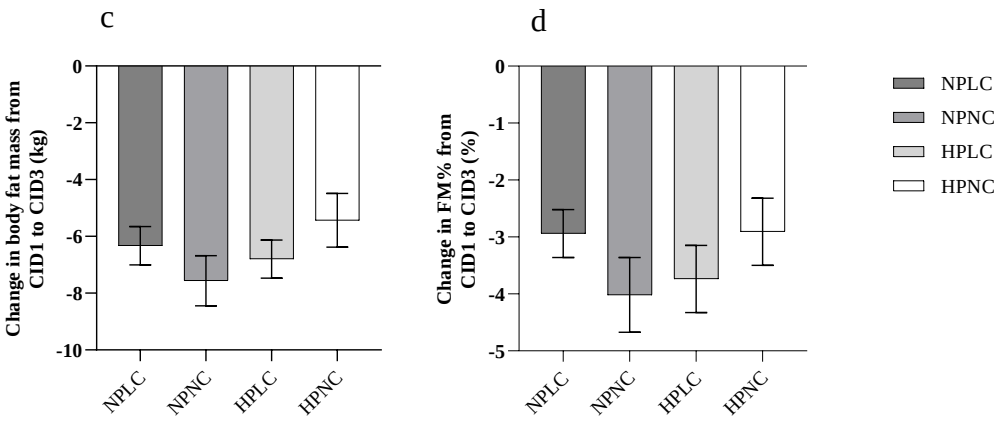

Figure 18, c,d) Mean (SEM) change in body fat mass (kg) and % body fat (%) between CID1 to CID3 in four treatment groups.

4.4.4 Change in lean mass (kg)

In four treatment groups, body lean mass decreased in parallel with weight loss from CID1 (week 0) to CID3 (week 8), with the changes being significant in NPLC (P<0.0001), NPNC (P=0.0032), and HPLC (P=0.0003), but not in HPNC group (P=0.0610) (Figure 19a). This is unexpected, but the possibility for HPNC being able to preserve lean mass better than other groups is unlikely, since the change in lean

mass between groups displayed no significant difference (One-way ANOVA: P=0.6668) (Figure 19b).

Overall, the dietary treatment that differs in macronutrient composition did not affect the change in lean mass (treatment*time interaction, P=0.9974) differently across the weight loss period.

Table 14, Mean (SEM) lean mass (kg) at CID1 and CID3 visits and mean (SEM) change in lean mass (kg) from CID1 to CID3 for four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC

CIDs	**Mean lean mass (SEM) (kg)**				
	NPLC (n=15)	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P value**
1 (week 0)	45.5 (1.2)	46.1 (1.8)	47.5 (1.2)	47.8 (1.9)	0.6453 [a]
3 (week 8)	43.6 (1.1)	44.3 (1.7)	45.7 (1.3)	46.5 (2.3)	0.5471 [a]
1→3	-2.0 (0.3) P<0.0001 [b]	-1.8 (0.4) P=0.0032 [b]	-1.9 (0.3) P=0.0003 [b]	-1.3 (0.6) P=0.0610 [b]	0.6668 [a]

Note:

[a] *P value showing the significance when comparing mean lean mass (kg) and their changes (kg) between four treatment groups at week 0, 4, and 8.*

[b] *P value showing the significance in lean mass change (kg) within the same treatment group over time.*

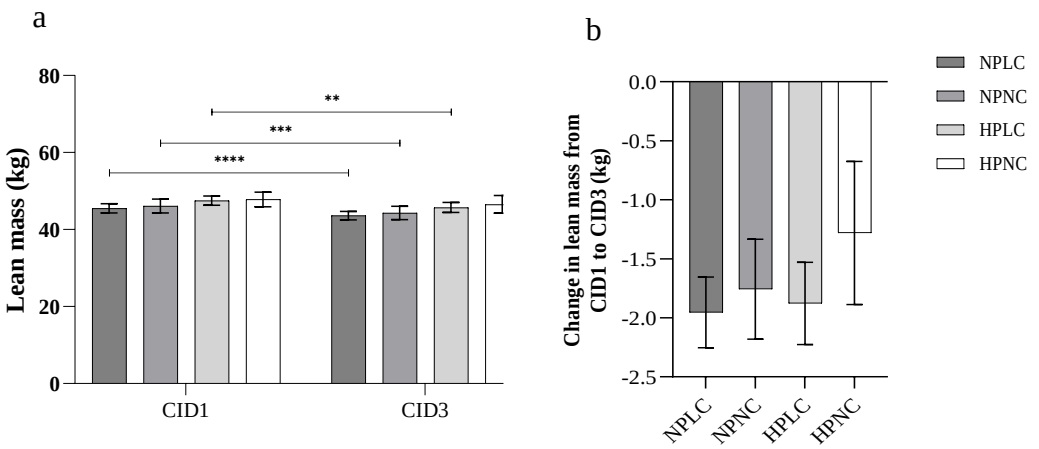

*Figure 19, a) Mean (SEM) lean mass (kg) at CID1 and CID3 visits for four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC. Two-way ANOVA (treatment*time interaction, P=0.9974), (time effect, P=0.1309). b) Mean (SEM) change in lean mass (kg) between CID1 to CID3 in four treatment groups.*

4.4.5 Change in lean mass (%)

Interestingly, opposing to what was observed with lean body mass, there was a significant increase for % lean mass in all groups after the 8-week LED treatment (NPLC: P<0.0001; NPNC: P=0.004; HPLC: P=0.0001; HPNC: P=0.0007). It is possibly due to the greater decrease in body fat mass and an overall decrease in body weight. However, no significant difference was found between groups at any CID (CID1: P=0.4834; CID3: P=0.4134) (Figure 20a), and the change in % lean mass was not significantly different between groups (One-way ANOVA: P=0.4035) (Figure 20b).

Overall, the dietary treatment that differs in macronutrient composition did not affect the change in percentage lean mass (treatment*time interaction, P=0.9709) (Figure 20a) differently across the weight loss period.

Table 15, Mean (SEM) lean mass (%) at CID1 and CID3 visits and mean (SEM) change in lean mass (%) from CID1 to CID3 for four treatment groups: NPLC vs NPNC vs HPLC vs HPNC

CIDs	**Mean lean mass (SEM) %**				
	NPLC (n=15)	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P value**
1 (week 0)	50.3 (0.6)	51.2 (1.9)	52.7 (1.5)	51.8 (0.9)	0.4834
3 (week 8)	52.9 (0.7)	54.9 (2.2)	56.1 (1.8)	54.5 (1.1)	0.4134
1→3	2.7 (0.4) P<0.0001 [b]	3.7 (0.6) P=0.004 [b]	3.5 (0.6) P=0.0001 [b]	2.7 (0.6) P=0.0007 [b]	0.4035

Note:

[a] P value showing the significance in lean mass change (%) between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC), Tukey's multiple comparison from repeated measures 2-way ANOVA indicating no significant difference between four treatment groups from CID1→CID3 (P>0.05, all).

[b] P value showing the significance in lean mass change (%) within the same treatment group over time. One sample T-test comparing the change in lean mass (%) from null

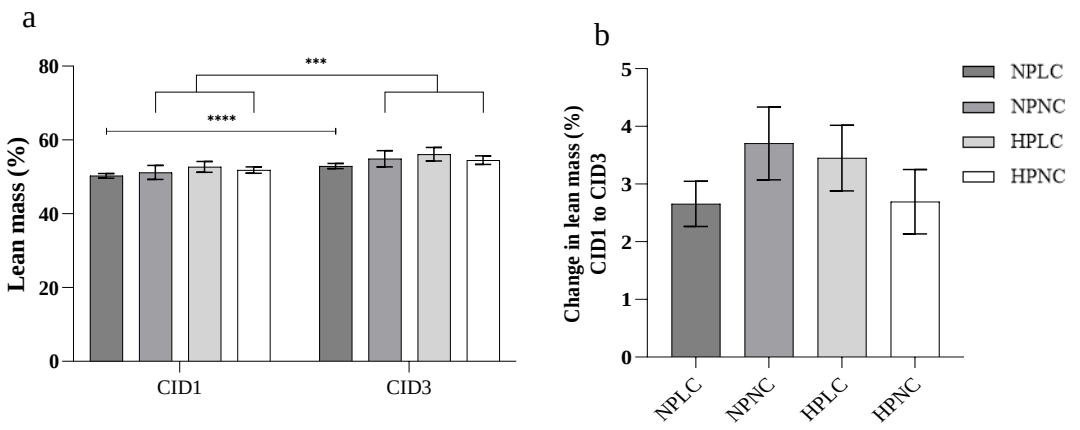

*Figure 20, a) Mean (SEM) lean mass (%) at CID1 and CID3 visits for four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC. Two-way ANOVA (treatment*time interaction, P=0.9709), (time effect, P=0.0012). b) Mean (SEM) change in lean mass (%) between CID1 to CID3 in four treatment groups.*

4.5 Energy intake for four treatment groups (NPLC vs NPNC vs HPLC vs HPNC)

4.5.1 Total energy intake

As shown by Table 16, there was no significant difference in energy intake (EI) between four treatment groups at any CID (CID1: P=0.6585, CID2: P=0.3255, CID3: P=0.7467).

At baseline, participants in all groups achieved similar EI (NPLC: 9475 (861.6) kJ, NPNC: 9312 (748.8) kJ, HPLC: 8258 (683.6) kJ, HPNC: 8607 (744) kJ), which is expected from the protocol since participants were randomly assigned into treatment groups. By comparing the EI to the BMR×1.2, which was a cut off for under-reporting, all groups were presumed to have correctly reported at baseline (EI> BMR×1.2). No comparison was made for under reporting at CID2 (week 4) and CID3 (week 8), since all participants restricted their EI during this period according to protocol.

At CID2 and CID3, no group achieved significantly lower EI than the other groups, which is contrary to my hypothesis, indicating that no diet treatment was more advantageous in promoting weight loss by reducing total EI. When comparing the EI of four treatment groups, all groups significantly reduced total EI from baseline to CID2 ($P<0.0001$ for all groups), and from baseline to CID3 ($P<0.0001$ for all groups), but not from CID2 to CID3 (Figure 21a). This difference is again, expected from the study protocol, since participants were on energy restriction from baseline to CID3 (week 8), and the dietary treatment was the same at both CID2 and CID3.

The target total EI based on the protocol was 4043kJ for HPNC group, 3957kJ for HPLC group, 3995kJ for NPNC group, and 3946kJ for NPLC group. As shown by Figure 21b, the actual EI was not significantly different from the target EI intended for all four groups ($P=0.1923$), which is the desired outcome and indicates that participants were compliant to study protocol.

Table 16, Mean (SEM) total energy intake (KJ) at CID1, CID2 and CID3 visits and the BMR*1.2 in four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC

CIDs	**Mean total energy intake (SEM) (kJ)**				
	NPLC (n=15)	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P value**
CID1 (week 0)	9475 (861.9)	9312 (748.8)	8258 (683.6)	8607 (744)	0.6585 [a]
CID2 (week 4)	4097 (92.1)	3824 (147.8)	3854 (116)	4767 (783.7)	0.3255 [a]
CID3 (week 8)	3988 (97.2)	4202 (253.1)	3891 (184.9)	4249 (452.5)	0.7467 [a]
BMR×1.2 [b]	8054 (127.4)	8064 (214.3)	7974 (105.9)	8526 (280.4)	0.1389 [a]

Note:

[a] P value showing the significance in total energy intake (kJ) between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).

[b] cut-off for under-reporting, below the cut off is considered under-reporting

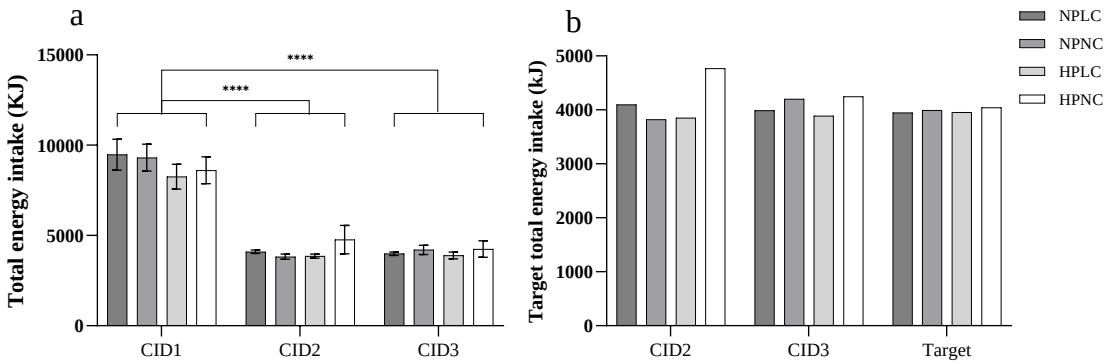

Figure 21, a) Mean (SEM) total energy intake (kJ) at CID1, CID2 and CID3 in four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC. Data represents mean ± SEM. Significant reductions in total energy intake in all four groups from CID1 to CID2, and from CID1 to CID3. b) Mean total energy intake (kJ) at CID2 and CID3, and the target total energy intake in four treatment groups.

4.5.2 Fixed energy (oatmeal breakfast and LED) intake and compliance to treatment

The mean (SEM) EI for fixed meals (oatmeal breakfast and LED) at each CID is shown in Table 17. According to study protocol, participants were asked to consume 3 fixed meals in a day, including a serving of oatmeal breakfast and two LED products. No fixed meals (oatmeal breakfast and LED products) was consumed at baseline.

Participants in all groups achieved similar level of EI from fixed meals (oatmeal breakfast and LED products) at both CID2 (week 4) and CID3 (week 8), indicating all groups were equally compliant to the study protocol (Figure 22a). There was also no difference found between CID2 and CID3 either. This is expected since all participants consumed the same breakfast and LED products at both CIDs.

In terms of compliance, no treatment group achieved a better level of compliance with the fixed meals than any other group. A cut-off for good compliance was made at 85% (Figure 22b). Based on this cut-off, all participants were able to achieve a good level

of compliance with fixed meals, with no reduction in compliance over time.

Table 17, Mean (SEM) fixed (oatmeal breakfast and LED) energy intake (kJ) and mean compliance to fixed (Oatmeal breakfast and LED) energy intake (%) at CID1, 2, 3 for four treatment groups (NPLC vs. NPNC vs. HPLC vs. HPNC)

Mean fixed (oatmeal breakfast and LED) energy intake (SEM) (kJ)					
CID	**NPLC (n=15)**	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P value**
CID1 (week 0)	-	-	-	-	-
CID2 (week 4)	2451 (49.8)	2257 (130.5)	2193 (141.6)	2283 (176.5)	0.4168 [a]
CID3 (week 8)	2430 (43.7)	2214 (121.1)	2227 (110.7)	2201 (192.0)	0.3705 [a]
Mean compliance to the fixed meals (oatmeal breakfast and LED) (SEM) (%)					
CID	**NPLC (n=15)**	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P value**
CID1 (week 0)	-	-	-	-	-
CID2 (week 4)	96.9 (2.0)	89.8 (5.2)	86.7 (5.6)	90.4 (7.0)	0.4340 [a]
CID3 (week 8)	96.1 (1.7)	88.1 (4.8)	88.1 (4.4)	87.1 (7.6)	0.3866 [a]
Note: no fixed meals (oatmeal and LED) was consumed at CID1					
[a,] P value showing significance in fixed energy intake (kg) and the compliance between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).					

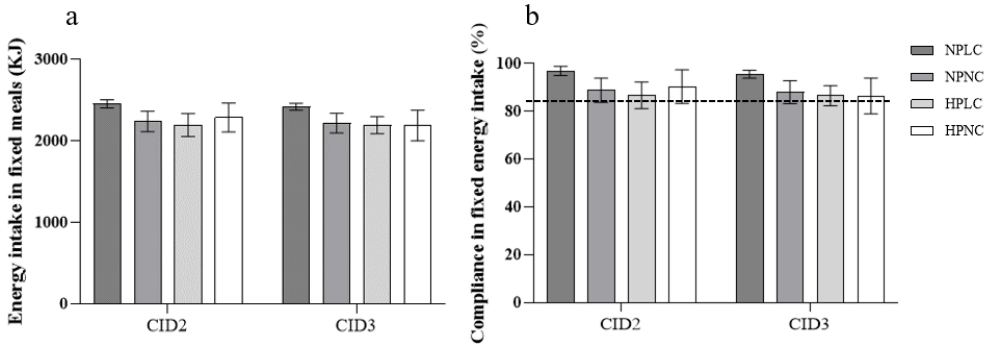

Figure 22, a) Mean (SEM) fixed (oatmeal breakfast and LED) EI (kJ) at CID2 and CID3 in four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC. Data represent mean ± SEM. b) Mean (SEM) compliance in fixed meals (oatmeal breakfast and LED) (%) at CID2 and CID3 for four treatment groups, a cut off for good compliance was made at 85%.

4.5.3 Fixed (oatmeal breakfast and LED) and ad libitum (ad lib) EI

According to the study protocol, participants were asked to consume a serving of oatmeal breakfast, 2 LED products, and an *ad lib* variable meal throughout the day. Table 18 displays the mean (SEM) energy intake in four treatment groups at CID2 (week 4) and CID3 (week 8), which was subdivided into four categories (oatmeal breakfast, LED products, *ad lib*, and total EI). No significant difference in EI from oatmeal breakfast or LED products was found between groups at CID2 (week 4) and CID3 (week 8). Surprisingly, EI from *ad lib* meals was also not significantly different between treatment groups at either CID (Figure 23c), indicating no diet treatment was more advantageous in promoting weight loss by reducing *ad lib* EI, which is contrary to my hypothesis.

When comparing the *ad lib* EI across CIDs (Figure 23c), the mean *ad lib* energy EI reduced significantly from CID1 to CID2 ($P<0.0001$), and from CID1 to CID3 ($P<0.0001$), but not from CID2 to CID3 ($P>0.05$). This was expected based on the protocol since EI at baseline was entirely a*d lib* (no energy-restricting treatment was applied at this stage), and all participants were then on energy restriction at both CID2 (week 4) and CID3 (week 8).

Interestingly, there was a pattern for a higher *ad lib* energy intake (kJ) at CID2 with a large variation (SEM) in the HPNC group, yet no significance was found. This is possibly due to the two participants who received 4.6MJ energy treatment in HPNC group, where the target EI from *ad lib* meals was higher than participants receiving 3.9 MJ energy treatment (2.2MJ vs. 1.6MJ).

Table 18, Mean (SEM) energy intake (KJ) at CID2 and CID3 visits four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC

N=46 (total)	NPLC (n=15)	NPNC (n=9)	HPLC (n=11)	HPNC (n=11)	P value
Oatmeal breakfast energy intake, Mean (SEM) (kJ)					
CID2 (week 4)	774.8 (47.4)	748.5 (68.4)	693.9 (66.7)	825.7 (85.4)	0.5782 [a]
CID3 (week 8)	743.8 (42.2)	680.9 (99.9)	634.9 (87.3)	629.2 (121.6)	0.7260 [a]
LED energy intake, Mean (SEM) (kJ)					
CID2 (week 4)	1676 (25.2)	1485 (69.8)	1499 (85.6)	1458 (131.4)	0.1736 [a]
CID3 (week 8)	1672 (25.9)	1533 (152.0)	1554 (99.6)	1555 (140.5)	0.7285 [a]
Ad libitum energy intake, mean (SEM) (kJ)					
CID1 (week 0)	9475 (861.9)	9312 (748.8)	8258 (683.6)	8607 (744)	0.6585 [a]
CID2 (week 4)	1647 (68.2)	1590 (113.3)	1661 (161.4)	2483 (900.2)	0.4504 [a]
CID3 (week 8)	1572 (87.58)	1988 (280.7)	1703 (164.8)	1686 (508.3)	0.5491 [a]
CID1→CID3	7950 (1.2) P<0.0001 [b]	7337 (2.8) P<0.0001 [b]	6594 (1.8) P<0.0001 [b]	6561 (2.3) P<0.0001 [b]	

Note:

[a] P value showing the significance in oatmeal, LED, Ad lib energy intake (kJ) between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).

[b] P value showing the significance in oatmeal, LED, Ad lib energy intake (kJ) within the same treatment group over time.

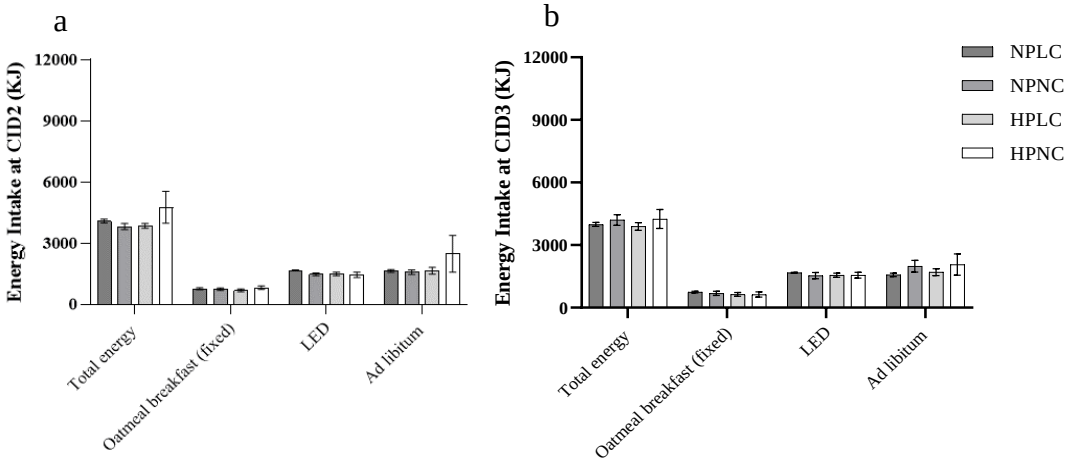

Figure 23, a, b) Mean (SEM) fixed (oatmeal breakfast and LED), and ad libitum energy intake (kJ) at CID2 and CID3 for four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC.

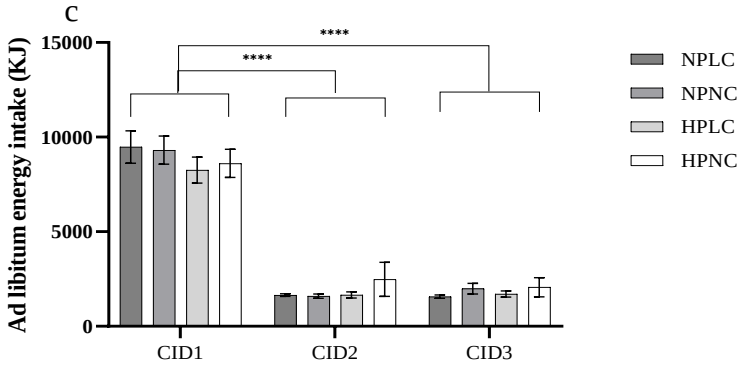

Figure 23, c) Mean (SEM) ad libitum energy intake (kJ) at CID1, CID2, and CID3 for four treatment groups. CID1-CID2 (P<0.0001) for all treatment groups, CID1-CID3 (P<0.0001) for all treatment groups.

4.6 Protein intake for four treatment groups (NPLC vs. NPNC vs. HPLC vs. HPNC)

4.6.1 Total intake of protein (g) and (% EI) at CID1, 2, 3.

The total protein intake throughout the day in the four treatment groups is shown in Table 19, both NP groups reduced their intake from CID1 (week 0) to CID3 (week 8), with the reduction being significant in the NPLC group (P=0.0207). While both HP groups tended to increase their intake from CID1 to CID3, yet not significantly (HPLC: P=0.4230, HPNC: P=0.3990). As for % EI protein intake, statistically significant increases were seen in all groups from CID1 to CID3 (NPNC: P=0.0003; NPLC, HPLC, and HPNC: P<0.0001).

No significant difference in protein intake was seen between the four groups at baseline, both absolute (P=0.6192) and % EI (P=0.6573). However, at both CID2 and CID3, there is a significant difference in the protein intake between groups, both absolute (CID2: P=0.0007, CID3: P=0.0004) and % EI (CID2: P=0.0049, CID3: P<0.0001). This significance was largely generated when comparing the HPLC with other groups. For example, at CID3, Tukey's multiple comparison test showed that

HPLC group consumed significantly more % protein than two NP groups (HPLC vs. NPLC: P<0.0001, HPLC vs. NPNC: P<00001), which is the desired outcome and was expected from the study protocol (Figure 24b). However, despite receiving the high protein treatment, HPNC group did not generate sufficient differences with two NP groups in terms of protein intake. Tukey's multiple comparison test showed no significant difference in % EI protein intake between the HPNC group and NP groups at CID3 (HPNC vs. NPNC: P= 0.5953; HPNC vs. NPLC: P= 0.2864) (Figure 24b).

Table 19, Mean (SEM) total protein intake (g/day and % EI) at CID1, CID2 and CID3 visits four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC

N=46 (total)	NPLC (n=15)	NPNC (n=9)	HPLC (n=11)	HPNC (n=11)	P value
Total protein intake, Mean (SEM) (g/day)					
CID1 (week 0)	100.6 (9.8)	90.4 (7.0)	92.9 (8.5)	85.2 (7.1)	0.6192 [a]
CID2 (week 4)	82.3 (1.8)	80.7 (1.2)	98.8 (5.0)	102.8 (6.8)	0.0007 [c]
CID3 (week 8)	79.3 (1.7)	84.0 (2.6)	104.8 (6.9)	92.3 (7.7)	0.0049 [d]
CID1→CID3	-21.3 (9.5) P=0.0407 [b]	-6.6 (7.8) P=0.4230 [b]	11.9 (6.1) P=0.0783 [b]	7.0 (8.0) P=0.3990 [b]	
Total protein intake, Mean (SEM) (% EI)					
CID1 (week 0)	18.2 (1.3)	17.0 (2.0)	19.3 (1.4)	17.0 (1.0)	0.6573 [a]
CID2 (week 4)	33.6 (0.5)	35.6 (1.1)	42.8 (1.7)	39.8 (2.5)	0.0004 [e]
CID3 (week 8)	33.5 (0.5)	34.3 (1.9)	44.9 (1.8)	37.1 (1.9)	<0.0001 [f]
CID1→CID3	15.4 (1.2) P<0.0001 [b]	17.3 (2.8) P<0.0001 [b]	25.6 (1.8) P<0.0001 [b]	20.1 (2.3) P<0.0001 [b]	

Note:

[a] P value showing the significance in total protein intake (g/day, %EI) between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).

[b] P value showing the significance in total protein intake (g/day, %EI) within the same treatment group over time.

[c] Significance found between NPLC vs HPLC, NPNC vs HPLC, NPNC vs HPNC (P<0.05), and between NPLC vs HPNC (P<0.0001).

[d] Significance found between NPNC vs HPLC (P<0.05), and between NPLC vs HPLC (P<0.0001).

[e] Significance found between NPLC vs HPNC, NPNC vs HPLC (P<0.05), and between NPLC vs HPLC (P<0.0001).

[f] Significance found between HPLC vs HPNC (P<0.05), and between HPLC vs NPLC, HPLC vs NPNC (P<0.0001).

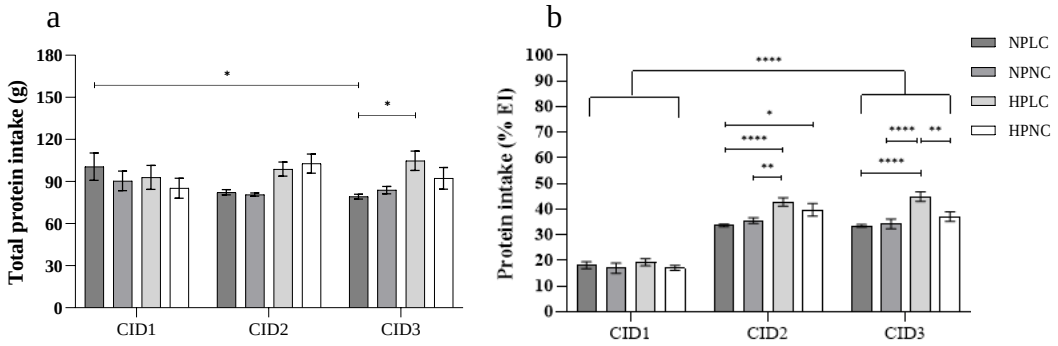

Figure 24, a,b) Mean (SEM) total protein intake (g) and % EI at CID1, CID2, and CID3 for four treatment groups: NPLC vs. NPNC vs. HPLC vs. HPNC, data represent mean ± SEM.

4.6.2 Fixed (oatmeal breakfast and LED) and ad libitum protein intake at CID1, 2, 3

The mean (SEM) protein intake (% EI) for fixed meals (oatmeal breakfast and LED products) and for *ad lib* meals is shown in Table 20. No significant difference was observed in the oatmeal protein intake between groups at both CIDs, indicating good compliance to the study protocol since all treatment groups were asked to consume a serving of oatmeal porridge for breakfast with the same macronutrient composition. However, a significant difference was seen in LED protein intake at CID3 (P=0.0299), where the intake was higher in HP groups than NP groups (Figure 25c). This is again, expected from the study protocol, with the target protein intake from LED products being higher in HP groups (HPLC: 16% EI; HPNC: 16% EI) than NP groups (NPNC: 13% EI; NPLC: 14.3% EI).

At baseline, the mean *ad lib* protein intake was not significantly different between groups (P=0.6573). This was expected from the study protocol since no fixed meals were consumed at this time, all meals were considered *ad lib*. Interestingly, all but HPLC group reduced their *ad lib* protein intake from baseline to CID3. A small increase in *ad lib* protein intake was seen in HPLC group with no significance identified (P=0.0953).

At both CID2 and CID3, a significant difference in *ad lib* protein intake was observed when comparing HPLC with other groups. At CID3, Tukey's multiple comparison test showed that HPLC group consumed significantly more % *ad lib* protein than two NP groups (HPLC vs. NPLC: P<0.0001, HPLC vs. NPNC: P=0.0394), which is the

desired outcome and was expected from the study protocol. However, HPNC group did not generate sufficient differences with two NP groups in terms of *ad lib* protein intake, which was undesirable. Tukey's multiple comparison test showed no significant difference in % EI protein intake between the HPNC and any NP group at CID3 (HPNC vs. NPNC: P= 0.9843; HPNC vs. NPLC: P= 0.6327) (Figure 25c).

Table 20, Mean (SEM) ad libitum protein intake (% EI) at CID1, 2, 3 for four treatment groups (NPLC vs. NPNC vs. HPLC vs. HPNC)

N=46 (total)	**NPLC (n=15)**	**NPNC (n=9)**	**HPLC (n=11)**	**HPNC (n=11)**	**P value**
Oatmeal breakfast protein intake, Mean (SEM) (% EI)					
CID1 (baseline)	-	-	-	-	-
CID2 (week 4)	6.8 (0.3)	7.4 (0.7)	6.8 (0.6)	7.6 (1.1)	0.8062 [a]
CID3 (week 8)	6.9 (0.5)	6.4 (1.1)	6.1 (0.9)	5.8 (1.1)	0.8158 [a]
LED protein intake, Mean (SEM) (% EI)					
CID1 (baseline)	-	-	-	-	-
CID2 (week 4)	12.9 (0.4)	11.8 (0.4)	15.1 (1.0)	14.0 (1.7)	0.1703 [a]
CID3 (week 8)	12.9 (0.5)	11.2 (1.1)	16.6 (1.5)	15.4 (1.8)	0.0299 [a]
***Ad libitum* protein intake, Mean (SEM) (% EI)**					
CID1 (baseline)	18.2 (1.3)	17.0 (2.0)	19.3 (1.4)	17.0 (1.0)	0.6573 [a]
CID2 (week 4)	13.9 (0.2)	16.3 (1.3)	21.0 (2.1)	18.2 (1.6)	0.0034 [c]
CID3 (week 8)	14.0 (0.5)	16.9 (1.0)	22.2 (1.9)	16.0 (1.7)	0.0002 [d]
CID1→CID3	-4.5 (1.4) P=0.0058 [b]	-0.4 (2.4) P=0.8659 [b]	2.9 (1.6) P=0.0953 [b]	-1.2 (2.1) P=0.5844 [b]	

Note: No oatmeal breakfast or LED products was consumed at baseline

[a] P value showing the significance in oatmeal, LED, Ad lib protein intake (%EI) between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).

[b] P value showing the significance in in oatmeal, LED, Ad lib protein intake (%EI) within the same treatment group over time.

[c] Significance found when comparing HPLC vs NPLC and between NPLC vs HPNC (P<0.05).

[d] Significance found when comparing HPLC vs NPNC, HPLC vs HPNC (P<0.05), and between NPLC vs HPLC (P<0.0001).

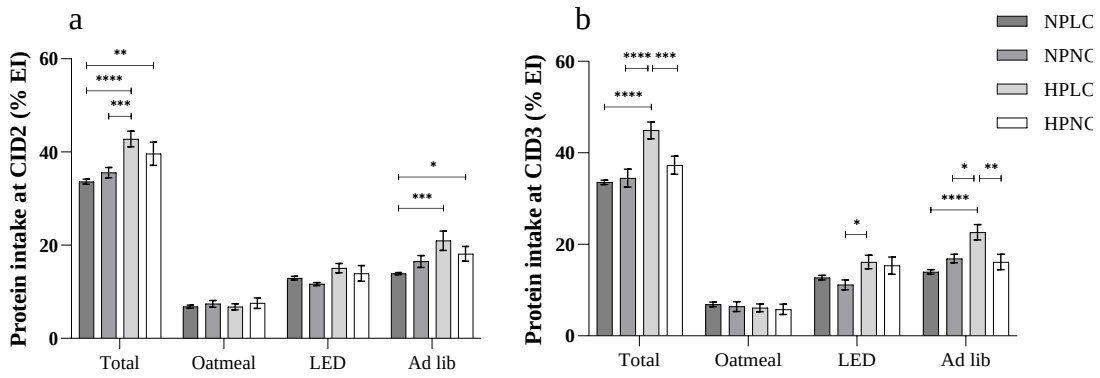

Figure 25,a,b) Mean (SEM) protein intake (% EI) at CID2 and CID3, categorized into eating occasions, total, oatmeal, LED and Ad lib

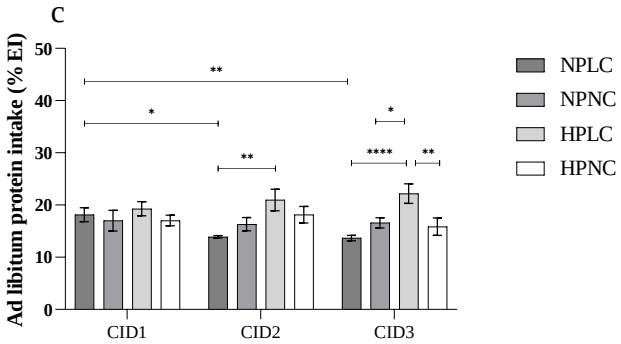

Figure 25, c) Mean (SEM) ad libitum protein intake (% EI) at CID1, CID2, and CID3.

4.7 Carbohydrate intake for four treatment groups (NPLC vs. NPNC vs. HPLC vs. HPNC)

4.7.1 Total intake of carbohydrate at CID1, 2, 3.

In terms of CHO intake, all groups significantly decreased their absolute intake from CID1 (week 0) to CID2 (week 4), and to CID3 (week 8) (P<0.0001) as per protocol (Figure 26a). As for % EI from CHO, all groups reduced their ratio from CID1 to CID3 with only two of the LC groups being significant (NPLC: P=0.0002; HPLC: P=0.0045). This is expected from the study protocol, since LC groups were expected to restrict their CHO intake while NC groups were expected to maintain a 'normal' CHO ratio.

There was no significant difference in baseline CHO intake between treatment groups,

both absolute (P=0.6960) and % EI (P=0.9413). However, a significant difference was seen at CID2 and CID3, both absolute and % EI. Tukey's multiple comparison test showed significant differences between the LC groups and the NC groups, which was obviously the desired outcome and indicated good compliance to the study protocol (Figure 26b).

Table 21, Mean (SEM) total carbohydrate intake (g and % EI) at CID1, 2, 3 for four treatment groups (NPLC vs NPNC vs HPLC vs HPNC)

N=46 (total)	NPLC (n=15)	NPNC (n=9)	HPLC (n=11)	HPNC (n=11)	P value
Total CHO intake, Mean (SEM) (g/day)					
CID1 (baseline)	229.9 (21.7)	233.9 (24.6)	196.6 (19.5)	223.1 (27.8)	0.6960 [a]
CID2 (week 4)	68.7 (1.3)	85.5 (4.8)	69.3 (3.3)	111.8 (21.7)	0.0198 [a]
CID3 (week 8)	68.6 (1.4)	90.3 (5.5)	67.0 (4.9)	98.3 (11.7)	0.0020 [a]
CID1→CID3	-161.3 (21.8) P<0.0001 [b]	-143.6 (25.7) P<0.0001 [b]	-129.6 (21.2) P<0.0001 [b]	-124.8 (31.1) P<0.0001 [b]	
Total CHO intake, Mean (SEM) (% En)					
CID1 (baseline)	40.4 (2.0)	41.4 (1.9)	40.0 (2.1)	41.8 (2.8)	0.9413 [a]
CID2 (week 4)	28.1 (0.4)	37.2 (0.8)	30.1 (1.1)	38.2 (0.8)	<0.0001 [c]
CID3 (week 8)	29.1 (0.7)	36.2 (1.3)	29.0 (1.7)	38.3 (1.5)	<0.0001 [d]
CID1→CID3	-11.4 (2.3) P=0.0002 [b]	-5.2 (2.9) P=0.1099 [b]	-11.1 (3.0) P=0.0045 [b]	-3.5 (3.6) P=0.3520 [b]	

Note:

[a] P value showing the significance in total CHO intake (g/day, %EI) between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).

[b] P value showing the significance in total CHO intake (g/day, %EI) within the same treatment group over time.

[c] Significance found when comparing NPLC vs NPNC, NPNC vs HPLC, HPLC vs HPNC (P<0.05), and between NPLC vs HPNC (P<0.0001).

[d] Significance found between comparing NPLC vs NPNC, NPNC vs HPNC, HPLC vs HPNC, and NPLC vs HPNC (P<0.05).

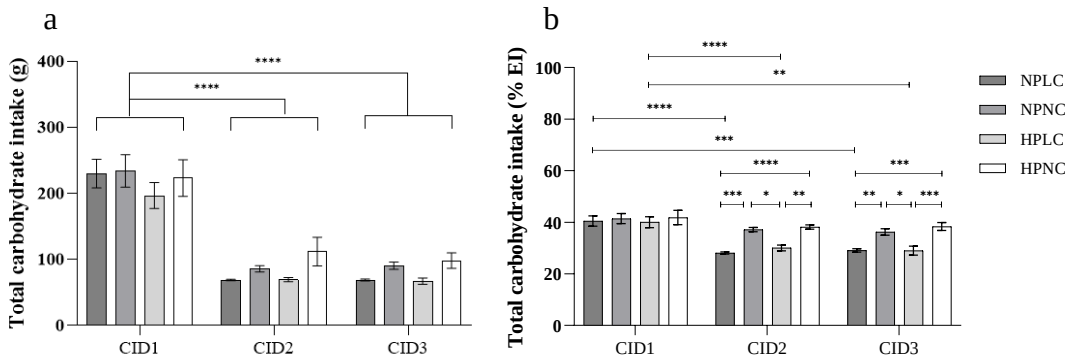

Figure 26, a,b) mean (SEM) total CHO Intake (g) and % EI at CID1, CID2 and CID3 for four treatment groups: NPLC vs NPNC vs HPLC vs HPNC, data represents mean ± SEM.

4.7.2 Fixed (oatmeal breakfast and LED) and ad libitum CHO intake at CID1, 2, 3

The mean (SEM) CHO intake (% EI) for fixed meals (oatmeal breakfast and LED products) and for *ad lib* meals is shown in Table 22. No significant difference was observed in the CHO intake from oatmeal breakfast between groups at any CID. However, in terms of CHO intake from LED products, there was a trend for higher intake in NPNC group, yet not significant. This is again, expected from the study protocol, where the target CHO intake from LED products was higher in NPNC (21.5% EI) than the other three groups (HPNC: 18.5% EI; HPLC: 18.5% EI; NPLC: 18.1% EI).

In terms of CHO intake from *ad lib* meals, significant decreases were observed from CID1 (week 0) to CID2 (week 4), and from CID1 to CID3 (week 8) in all groups (HPNC: P=0.0002; NPLC, NPNC, HPLC: P<0.0001). This was expected from the study protocol since no fixed meals were consumed at baseline, all meals were considered *ad lib.* When comparing across treatment groups, a significant difference was observed at both CID2 and CID3 (CID2: P=0.0009, CID3: P=0.0003), which was mainly generated when comparing NC with LC groups. At CID3, Tukey's multiple comparison test showed that HPNC group consumed significantly more % *ad lib* CHO than two LC groups (HPNC vs. NPLC:P=0.0003, HPNC vs. HPLC: P=0.0110). As for NPNC group, there was a trend for a greater intake of % *ad lib* CHO than the two LC groups, yet not significant (NPNC vs. NPLC: P=0.0694, NPNC vs. HPLC: P=0.3758). This is again expected. According to protocol, the target CHO intake from *ad lib* meals was much higher in NC groups (HPNC: 13.3% EI; NPNC: 10.3% EI)

than LC groups (HPLC: 0.4% EI; NPLC: 0.9% EI).

Table 22, Mean (SEM) ad lib CHO intake (% EI) at CID1, 2, 3 for four treatment groups (NPLC vs NPNC vs HPLC vs HPNC)

N=46 (total)	NPLC (n=15)	NPNC (n=9)	HPLC (n=11)	HPNC (n=11)	P value
***Ad lib* CHO intake, Mean (SEM) (% EI)**					
At CID1 (baseline)	40.4 (2.0)	41.4 (2.0)	40.0 (2.1)	41.8 (2.8)	0.9413 [a]
At CID2 (week 4)	2.4 (0.3)	8.6 (1.3)	6.3 (1.9)	13.9 (3.4)	0.0009 [c]
At CID3 (week 8)	2.8 (0.3)	10.0 (1.8)	5.0 (2.4)	14.3 (2.8)	0.0003 [d]
CID1→CID3	-37.7 (2.0) P<0.0001 [b]	-31.4 (2.3) P<0.0001 [b]	-35.1 (3.6) P<0.0001 [b]	-27.6 (4.7) P<0.0001 [b]	
Oatmeal breakfast CHO intake, Mean (SEM) (% EI)					
At CID1 (baseline)	-	-	-	-	-
At CID2 (week 4)	7.9 (0.6)	8.2 (0.8)	7.1 (0.7)	8.5 (1.1)	0.6295 [a]
At CID3 (week 8)	7.8 (0.4)	7.0 (1.2)	6.2 (0.9)	6.7 (1.4)	0.6702 [a]
LED CHO intake, Mean (SEM) (% EI)					
At CID1 (baseline)	-	-	-	-	-
At CID2 (week 4)	17.8 (0.6)	20.3 (0.9)	16.7 (1.1)	15.8 (1.9)	0.0891 [a]
At CID3 (week 8)	18.5 (0.6)	19.2 (2.2)	17.7 (1.7)	17.4 (2.1)	0.8697 [a]

Note:

[a] P value showing the significance in oatmeal, LED, Ad lib CHO intake (%EI) between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).

[b] P value showing the significance in in oatmeal, LED, Ad lib CHO intake (%EI) within the same treatment group over time. No fixed meals were consumed at baseline, all intake was considered as ad lib.

[c] Significance found when comparing NPLC vs NPNC, NPNC vs HPNC, and HPLC vs HPNC (P<0.05), and between NPLC vs HPNC (P<0.0001).

[d] Significance found when comparing NPLC vs NPNC, and HPLC vs HPNC (P<0.05), and between NPLC vs HPNC (P<0.0001).

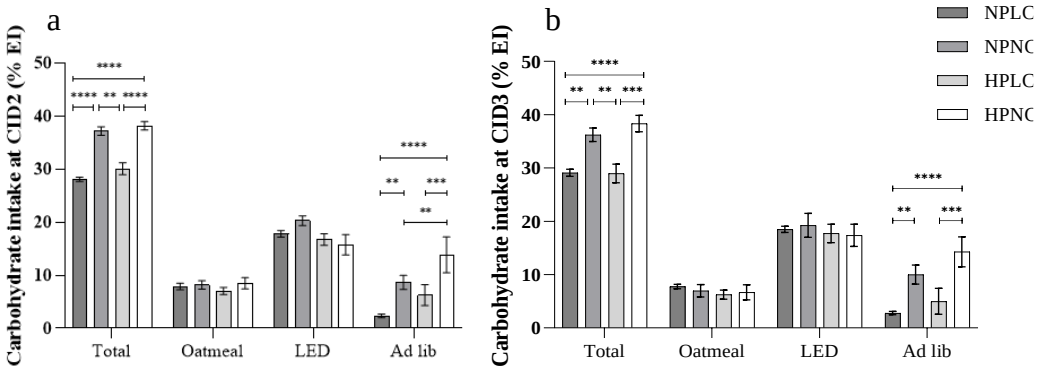

Figure 27,a,b) Mean (SEM) CHO intake (% EI) at CID2 and CID3, categorized into eating occasions, total, oatmeal, LED and Ad libitum.

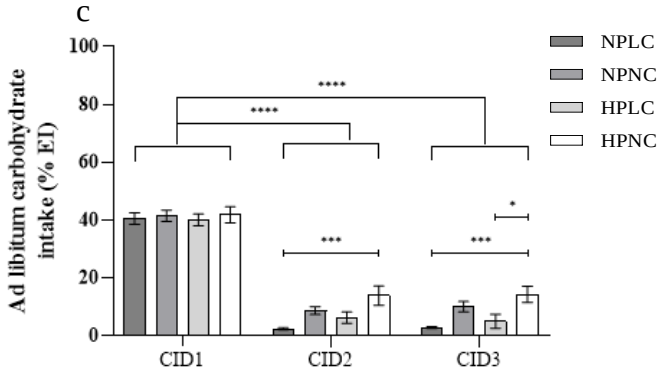

Figure 27, c) Mean (SEM) ad libitum CHO intake (% EI) at CID1, CID2, and CID3

4.8 Fat intake for four treatment groups (NPLC vs. NPNC vs. HPLC vs. HPNC)

4.8.1 Intake of total fat (g and % EI) at CID1, 2, 3.

As shown in Table 23, all groups showed significant reduction in absolute fat intake from CID1 (week 0) to CID3 (week 8) (NPLC: P=0.0003; NPNC: P=0.0001; HPLC: P=0.0003; HPNC: P<0.0001). Interestingly, significant reduction in % fat intake was seen in all but NPLC group (NPLC: P=0.1668; NPNC: P=0.0038; HPLC: P=0.0002; HPNC: P=0.0001), which is expected from the study protocol. According to the study protocol, the target total fat intake was designed to be the highest in NPLC group (38% EI), followed by NPNC (25% EI), HPLC (22% EI) and HPNC (10% EI). Therefore, the change required to achieve target fat intake was the least significant for the NPLC group.

There was no significant difference in baseline fat intake between the four groups, for both absolute (P=0.8273) and % EI (P=0.9792). However, at both CID2 and CID3, there is a significant difference in the fat intake between groups, both absolute (CID2: P=0.0007, CID3: P=0.0004) and % EI (CID2: P=0.0049, CID3: P<0.0001). Tukey's multiple comparison test showed that the significance was mainly generated when

comparing NPLC and the other three groups, where a descending order of % EI fat intake from NPLC to NPNC, HPLC, HPNC groups was generated (NPLC vs NPNC: P=0.0003; NPLC vs HPLC: P<0.0001; NPLC vs HPNC: P<0.0001). This is again, expected, and desired from the study protocol.

Table 23, Mean (SEM) total fat intake (g and % EI) at CID1, 2, 3 for four treatment groups (NPLC vs. NPNC vs. HPLC vs. HPNC)

N=46 (total)	NPLC (n=15)	NPNC (n=9)	HPLC (n=11)	HPNC (n=11)	P value
Total fat intake, Mean (SEM) (g/day)					
At CID1 (baseline)	94.8 (12.4)	91.4 (9.5)	82.5 (10.6)	84.7 (7.7)	0.8273 [a]
At CID2 (week 4)	38.0 (1.5)	23.2 (1.4)	23.4 (1.4)	24.6 (8.7)	0.0367 [a]
At CID3 (week 8)	36.1 (1.6)	28.2 (3.8)	22.2 (1.6)	22.5 (4.5)	0.0021 [a]
CID1→CID3	-58.7 (12.4) P=0.0003 [b]	-63.2 (9.0) P=0.0001 [b]	-60.2 (10.9) P=0.0003 [b]	-62.2 (7.0) P<0.0001 [b]	
Total fat intake, Mean (SEM) (% EI)					
At CID1 (baseline)	37.0 (1.5)	36.7 (2.1)	36.7 (2.3)	37.8 (2.2)	0.9792 [a]
At CID2 (week 4)	34.8 (0.9)	22.7 (0.8)	22.9 (1.2)	16.7 (1.9)	<0.0001 [c]
At CID3 (week 8)	34.2 (0.9)	24.5 (2.3)	21.4 (1.0)	19.1 (1.9)	<0.0001 [d]
CID1→CID3	-2.8 (1.9) P=0.1668 [b]	-12.3 (3.0) P=0.0038 [b]	-15.3 (2.7) P=0.0002 [b]	-18.7 (2.3) P=0.0001 [b]	

Note:

[a] P value showing the significance in total fat intake (%EI) between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).

[b] P value showing the significance in total fat intake (%EI) within the same treatment group over time.

[c] Significance found when comparing NPLC vs NPNC, NPLC vs HPLC, NPLC vs HPNC (P<0.0001), and between HPLC vs HPNC (P<0.05).

[d] Significance found when comparing NPLC vs HPLC, NPLC vs HPNC (P<0.0001), and NPLC vs NPNC (P<0.05).

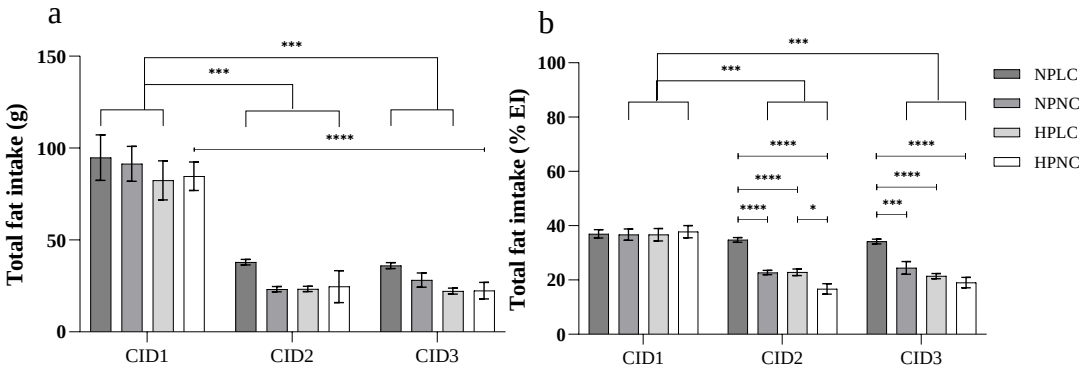

Figure 28, a,b) mean (SEM) total fat Intake (g) and % EI at CID1, CID2 and CID3 for four treatment groups: NPLC vs NPNC vs HPLC vs HPNC, data represents mean ± SEM.

4.8.2 Fixed (oatmeal breakfast and LED) and ad libitum fat intake at CID1, 2, 3

The mean (SEM) fat intake (% EI) for fixed meals (oatmeal breakfast and LED products) and for *ad lib* meals is shown in Table 24. As expected from the protocol, no significant difference was observed in the fat intake from oatmeal breakfast between groups at both CIDs. However, significance was seen regarding the fat intake from LED products ($P<0.0001$), where the intake was significantly higher in NPLC group than the other three groups ($P<0.0001$ for all comparisons). This is expected from the study protocol, the target fat intake from LED products was designed to be the highest in NPLC group (9.2% EI) (HPNC: 6.4% EI; HPLC: 6.4% EI; NPNC: 6.4% EI).

In terms of *ad lib* fat intake, a significant difference was seen between groups at CID2 and CID3. Tukey's multiple comparison test displayed a descending order of % EI fat intake from NPLC to NPNC, HPLC, HPNC groups, where NPLC achieved significantly more % EI intake than HPLC (21.1 (1.3) % vs 12.8 (1.1) %, $P=0.0085$) and HPNC groups (21.1 (1.3) % vs 10.4 (2.6) %, $P=0.0005$). This is consistent with the study protocol, where the target *ad lib* fat intake was designed to be the highest in the NPLC group (66.3% EI), followed by NPNC (43.9% EI), HPLC (32.9% EI), and HPNC group (4.4% EI).

Table 24, Mean (SEM) ad libitum fat intake (% EI) at CID1, 2, 3 for four treatment groups (NPLC vs. NPNC vs. HPLC vs. HPNC)

N=46 (total)	NPLC (n=15)	NPNC (n=9)	HPLC (n=11)	HPNC (n=11)	P value
Oatmeal breakfast fat intake, Mean (SEM) (% EI)					
At CID1 (baseline)	-	-	-	-	-
At CID2 (week 4)	2.6 (0.1)	2.7 (0.3)	2.7 (0.3)	3.0 (0.4)	0.7821 [a]
At CID3 (week 8)	2.7 (0.1)	2.6 (0.4)	2.2 (0.3)	2.1 (0.4)	0.4661 [a]
LED fat intake, Mean (SEM) (% EI)					
At CID1 (baseline)	-	-	-	-	-
At CID2 (week 4)	9.5 (0.3)	5.8 (0.2)	6.0 (0.4)	5.8 (0.7)	<0.0001 [c]
At CID3 (week 8)	10.5 (0.5)	5.7 (0.6)	6.4 (0.6)	6.4 (0.8)	<0.0001 [d]
***Ad lib* fat intake, Mean (SEM) (% EI)**					
At CID1 (baseline)	37.0 (1.5)	36.7 (2.1)	36.7 (2.3)	37.8 (2.2)	0.9792 [a]
At CID2 (week 4)	22.7 (0.9)	14.2 (1.2)	14.1 (1.5)	8.0 (2.7)	<0.0001 [e]
At CID3 (week 8)	21.1 (1.3)	16.2 (3.0)	12.8 (1.1)	10.4 (2.6)	0.0019 [f]
CID1→CID3	-16.2 (2.0) P<0.0001 [b]	-20.5 (2.3) P<0.0001 [b]	-24.0 (3.6) P<0.0001 [b]	-27.3 (4.7) P<0.0001 [b]	

Note:

[a] P value showing the significance in oatmeal, LED, Ad lib fat intake (%EI) between the four treatment groups (NPLC vs NPNC vs HPLC vs HPNC).

[b] P value showing the significance in in oatmeal, LED, Ad lib fat intake (%EI) within the same treatment group over time

[c] Significance found when comparing NPLC vs NPNC, NPLC vs HPLC, and NPLC vs HPNC (P<0.0001).

[d] Significance found when comparing NPLC vs NPNC, NPLC vs HPLC, and NPLC vs HPNC (P<0.0001).

[e] Significance found when comparing NPLC vs NPNC, NPNC vs HPNC (P<0.05), and between NPLC vs HPLC, NPLC vs HPNC (P<0.0001).

[f] Significance found when comparing NPLC vs HPLC and NPLC vs HPNC (P<0.0001), and between NPLC vs NPNC and NPNC vs HPNC (P<0.05).

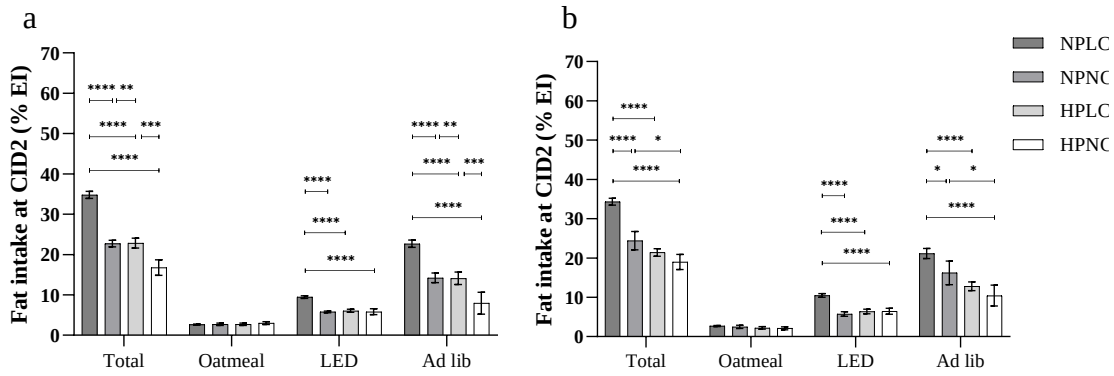

Figure 29, a,b) mean (SEM) fat intake (% EI) at CID2 and CID3, categorized into eating occasions, total, oatmeal, LED and Ad lib

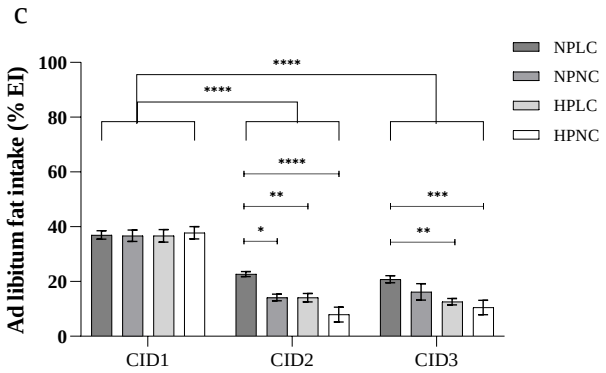

Figure 29, c) mean (SEM) ad libitum Intake (% EI) at CID1, CID2 and CID3 for four treatment groups: NPLC vs NPNC vs HPLC vs HPNC, data represents mean ± SEM.

Chapter 5: Discussion

5.1 Overall Findings

The primary finding is that most of the participants in this sub-study achieved a significant weight loss after completing the 8-week LED treatment, as well as showing significant changes in BMI, body fat mass, percentage body fat, lean mass, and percentage lean mass. A total of 46 participants were randomized into four dietary treatment groups. After randomisation, changes in body weight and body composition were still significant in all diet groups, indicating that irrespective of diet group, significant changes in body weight and body composition occurred.

My primary hypothesis was that an 8-week higher-protein LED treatment may result in significantly greater body weight loss among obese women when compared to a normal-protein LED treatment independent of carbohydrate content. However, when comparing changes in body weight and body composition between diet groups, no significant difference was observed, indicating the weight loss and change in body composition after an 8-week LED program were not influenced by the diet plan (high

protein vs normal protein) received by participants, at least not observed in the present sub-study, indicating that high protein diet is not superior in promoting weight loss and improving body composition than lower protein diet regardless of the CHO content of the diet, which is contradictory to my primary hypothesis.

5.2 LED outcomes

5.2.1 Weight loss post LED

For the 46 participants who completed the 8-week LED treatment, the mean (SEM) body weight at baseline was 91.8 (1.6) kg and 83.4 (1.7) kg post LED, which gives a significant weight loss of -8.4 (0.5) kg. Due to the great variability in body size among participants at baseline, percentage weight loss and BMI change were also used along with the absolute weight loss in the result section.

Weight change showed great variability among individuals (-14.6 to +3.5kg). In all 46 participants, no relationship was found between baseline body weight and the weight change during the 8-week LED program. However, after removing the two participants displaying abnormal weight loss trajectory, significance occurred, indicating that a higher baseline body weight was associated with greater absolute weight loss induced by the 8-week LED program. This is consistent with past research showing that during weight loss, heavier people tend to achieve a higher absolute body weight reduction (Henry, 2005). Body weight is a main contributing factor in predicting basal metabolic rate and energy needs in weight loss studies, when the same level of energy restriction was applied, heavier individuals were able to achieve a greater energy deficit than those with lower body weight due to a higher BMR,

which results in a greater weight loss (Schofield, 1985). In the present sub-study, a similar level of energy restriction was applied (~40% of daily energy requirement), those with higher baseline body weight inevitably resulted in greater weight loss.

Overall, all but two participants were able to achieve significant weight loss after the 8-week of LED treatment, which is consistent with many previous studies involving LED. (Flynn & Walsh, 1993; Rössner & Flaten, 1997a; Wadden & Stunkard, 1986).

When comparing with some previous studies with similar duration and energy composition, the present study was successful. The pragmatic randomized controlled trial conducted by Christensen et al (2011) applied an 8-week LED (810 kcal/day) to 96 obese but otherwise healthy participants, the study showed a mean reduction in body weight of 10.7 kg and in BMI of 3.9 kg/m^2 at week 8 (Christensen, P. et al., 2011a). Another study by Rossner and Flaten et al (1997) assigned 17 obese but otherwise healthy participants into 880 kcal energy treatment for 6 weeks and observed a mean of 12.9 kg weight loss (Rössner & Flaten, 1997b). Foster et al (1992) assigned 24 obese females to an 800kcal/day liquid formula diets and observed a mean of 7.2 kg weight loss at 5 weeks and a mean of 12.2 kg weight loss at 9 weeks (Foster, G. D. et al., 1992). Although these three studies have a similar level of energy restriction and study duration to the present sub-study, they started with a higher baseline body weight, which might explain the greater absolute weight loss observed in them. For example, baseline body weight was 113.8kg in the study by Rossner and Flaten et al (1997), compared with 91.8kg in the present sub-study.

However, the absolute weight loss presented by the current study seemed lower than some other studies with similar study duration and participant characteristics. Ryttig et al (1997) an 18.9 kg weight loss at 2 months in 54 obese but otherwise healthy participants (Ryttig, Flaten, & Rössner, 1997). Stenius et al (2000) reported a 14.2 kg/14.5% weight loss at 8 weeks (Brita Stenius-Aarniala, Tuija Poussa, Johanna Kvarnström, Eeva-Liisa Grönlund, Mikko Ylikahri, Pertti Mustajoki, 2000). Lantz et al (2003) observed a 14.9 kg weight loss at week 8 in 334 obese participants (Lantz, Peltonen, Ågren, & Torgerson, 2003). The greater weight loss observed in the three studies above are possibly from a lower energy treatment, where they provided VLEDs with an energy content of less than 3.3MJ/day. VLED refers to a hypo-caloric diet containing 3.35MJ or less per day, in comparison with the 3.9MJ/day used in the present sub-study. Although in theory, VLED can induce greater weight loss than LED by having a lower energy content, they are more from the loss of lean mass rather than body fat mass. Meanwhile, more frequently reported side effects were also found with the use of VLED, such as bad breath (Christensen, P. et al., 2011a). There are increasing evidence showing that VLED might generate greater weight loss than LED initially, but this superiority diminishes over time as VLED is associated with more weight regain in the long term (Foster, G. D. et al., 1992; Rössner & Flaten, 1997b; Ryttig et al., 1997).

The data demonstrated by the present sub-study, along with many previous trials, all supported the capability of LED in inducing significant weight loss in the short term. The weight loss using LED/VLED were shown to be beneficial in treating obesity and

obesity-related comorbidities, including T2DM, osteoarthritis (Christensen, P., Bliddal, Riecke, Leeds, Astrup, & Christensen, 2011b; Christensen, Pia et al., 2013), obstructive sleep apnoea (Johansson et al., 2011), psoriasis (Jensen et al., 2013), and secondary coronary prevention (Mulholland, Nicokavoura, Broom, & Rolland, 2012; Parretti et al., 2016). LED is considered relatively safe as a part of weight control program under proper medical supervision in patients with moderate and severe obesity, with better efficiency seen when being incorporated with behavioural modification, exercise, nutritional education, weekly group meetings, and when involving a multi-disciplinary team comprised of physicians, dietitians, behavioural therapists (Very low-calorie diets. national task force on the prevention and treatment of obesity, national institutes of health.1993).

5.2.2 Body composition post LED

Participants were scanned by DEXA in the body composition laboratory at Auckland Hospital at both baseline and CID3 (post-LED). All 46 participants experienced significant reductions in both % body fat (-3.4%) and absolute body fat mass (-6.5kg) after the 8-week LED. Change in lean mass was also significant, where lean body mass decreased by 1.8kg whereas % lean mass increased by 3.1% compared with baseline.

Interestingly, the two participants who showed no decreases in body weight, circled and labelled as 1 and 2 (1: gained 3.5 kg, 2: lost 0 kg), reduced body fat mass of -2.1kg and -0.7kg respectively. Meanwhile, both of them gained lean body mass of 3.7kg and 0.2kg respectively. This is unexpected as LED typically induces losses in

body weight, body fat, and lean tissue. However, it was shown that exercise triggers the conservation of muscle tissue, with and without the combination of a VLED (Saris, 2001). It is possible that these two participants introduced more exercise into their daily routine against our recommendation of having minimal exercise.

In comparison with some previous studies involving LED/VLED, the present sub-study is relatively successful. In a study by Christensen et al (2011), an 8-week LED program (810kcal/day) induced a mean of -1.2kg reduction in lean body mass and -9.8kg reduction in FM (Christensen, P. et al., 2011b). In a similar study by Ryttig et al (1997), they reported a mean reduction of -12.9kg in body FM after treating participants with VLED (420kcal/day) for 8 weeks (Ryttig et al., 1997). As for studies with a longer duration, Foster et al (1992) reported a mean of -3.1kg reduction in FFM and -17.9kg reduction in FM after a 21-week VLED treatment ranging from 420 to 800kcal/day in energy (Foster, G. D. et al., 1992). In another study by Foster et al (1990), a 24-week VLED treatment (500kcal/d) induced an 8.5% reduction in FM and a 3.6% reduction in body lean mass (Foster, G. D. et al., 1990). Similarly, Lantz et al (2003) reported a mean reduction of -15.6kg in FM and -4.53kg in FFM after 24-week of VLED (450kcal/day) (Lantz et al., 2003).

It is important to note that in addition to variations in study design, study duration and energy composition within the studies discussed above, there is also inconsistency with the methods of assessing body composition. For example, same as the present sub-study, DEXA was used in the study by Christensen et al (2011) (Christensen, P. et al., 2011a). While Ryttig et al (1997) used bio-impedance in their study (Ryttig et

al., 1997), Foster et al (1992) used densitometry (Foster, G. D. et al., 1992), and Lantz et al (2003) used a mixed of DEXA and TBK (total body potassium) in their studies to assess body composition (Lantz et al., 2003).

5.2.3 Lean and fat mass ratio

It was estimated that approximately 25-30% of the excess weight is composed of FFM in obese individuals, therefore it is reasonable to expect that 25% of lost body weight would be from lean tissue (Foster, Gary D. et al., 1988). In the present study, 77% of the weight lost was FM while 20% being lean mass. This ratio is very close to what was reported previously with the active uses of VLED and LED, such as the study by Lantz et al (2003), where they reported 22.5% of lost body weight as lean mass after a 24-week of VLED (450kcal/day) (Lantz et al., 2003). Torgerson et al (1999) reported a 25:75 ratio for lost body weight as lean mass and fat mass respectively after a 24-week of VLED (456kcal/day) (Torgerson, Ågren, & Sjöström, 1999). Similarly, a 28:72 ratio was reported by Wadden et al (1994) after a 16-week of VLED (420kcal/day) (Wadden et al., 1994).

However, there are some studies showing a better ability of preserving lean body mass during LED/VLED induced weight loss, such as the study by Christensen et al (2011), where 17% of weight lost being lean body mass was reported after an 8-week LED program (810kcal/day) (Christensen, P. et al., 2011). An 82:17 ratio for lost body weight as lean mass and fat mass respectively was reported by another study involving a 6-month VLED (420kcal/day) (Barrows & Snook, 1987). Foster et al

(1992) reported an even lower percentage of only 14% of lost weight as lean mass (Foster, G. D. et al., 1992).

5.3 Primary hypothesis

The primary aim of this thesis was to investigate whether higher protein diet results in greater weight loss independent of carbohydrate content, under conditions of negative energy balance achieved through the use of LED over 8 weeks, by assessing several significant indicators of weight loss and self-reported food intake.

My primary hypothesis was that an 8-week higher-protein LED treatment may result in significantly greater body weight loss among obese women when compared to a normal-protein LED treatment independent of carbohydrate content.

5.3.1 Change in protein intake

At baseline, protein consumption was not significantly different between HP groups and NP groups with a constant CHO, where HPLC group consumed 19.3% protein while NPLC group consumed 18.2% protein, HPNC group consumed 17.0% protein while NPNC group consumed 17.0% protein. This is consistent with a typical New Zealand diet. According to the Clinical Guidelines for Weight Management in New Zealand Adults released in 2017, for the typical New Zealand diet, protein contributes to 15-25% total EI. 2008/09 New Zealand Adult Nutrition Survey estimated an average of 16.5% EI protein intake for New Zealand females. This indicates that the four groups can be considered as representative samples for NZ female population. However, in this survey, dietary intake was assessed using 24-hour diet recall and FFQ (food frequency questionnaire), and it is noteworthy this survey was completed

and published over 10 years ago, which might lack precision and accuracy. This dietary intake is also consistent with the dietary recommendations for New Zealand adults, where 50-55% total energy should come from CHO, 10-15% total energy from protein, and 30-33% total energy from fat (Ministry of Health, 2003).

At 8 weeks, both HP and NP groups had significantly increased their protein intake as expected, where NPLC group increased their protein intake from 18.2% to 33.5% EI while NPNC group increased from 17.0% to 34.3% EI. As for HP groups, HPLC group increased their intake from 19.3% to 44.9% EI while HPNC group increased from 17.0% to 37.1% EI. This indicates compliance to the diet plan, where participants in all treatment groups successfully increased their protein intake from baseline.

When compared with target protein intake, both NP groups were able to achieve the intended protein intake of 35% EI (although slightly lower in NPLC group). HP groups were less successful in meeting the recommended intake of 50% EI, especially HPNC group where protein intake was 37.1%EI compared with the recommended 50%. This was also observed in other studies, where participants were more likely to achieve the target intake when their usual diets were closer to the recommended level (Sacks et al., 2009). In this case, it was more challenging for participants in HP groups to reach the protein goal, where they needed to change their customary level of protein intake a lot more than participants following NP diets.

In the present thesis, dietary intake and compliance was only estimated through the analysis of 4-day food record. Other indicators of dietary compliance such as urinary

nitrogen was not used in the present thesis due to time constraint, which might result in self-reporting error and bias (Bingham, 2003).

5.3.2 HP vs NP, the effect of higher protein intake on weight loss

In this thesis, it was hypothesized that a diet with high protein would promote greater weight loss when compared to a diet with lower protein, regardless of the carbohydrate content. At the end of 8 weeks, all groups lost weight significantly, with NPNC group showing the greatest weight loss of -10.4%, followed by HPLC group of -10.2%, NPLC group of -9.2% and HPNC group of -7.4%. However, Tukeys multiple comparison test showed no significant difference when comparing HP groups and NP groups: HPLC vs. NPLC and HPNC vs. NPNC, indicating that a HP diet was equally successful in weight loss as a NP diet. This finding aligns with many previous studies where HP diets results similar weight loss outcomes compared with diets with lower protein. Larsen et al (2011) found no significant difference in weight loss resulted from a hypo-energetic HP diet (30%en) or an iso-energetic NP diet (15%en) (Larsen, Mann, Maclean, & Shaw, 2011). Noakes et al (2005) also found no significant difference in weight loss after comparing a HP diet (34%en) and a NP diet (17%en) (P=0.29) (Noakes, Keogh, Foster, & Clifton, 2005). In another more recent study, weight loss was similar from a standard protein diet (0.8g/kg/day) and an iso-caloric higher protein diet (1.34g/kg/day) (Campos-Nonato, Hernandez, & Barquera, 2017). However, these results are contradictory to many weight loss studies showing high protein diets were more advantageous in promoting weight loss than lower protein diets (Samaha et al., 2003; Yancy et al., 2004). For example, clinically significant

weight loss was seen in participants who increased protein intake from 15% to 30% EI while their CHO intake remained constant (Weigle et al., 2005). A 3.5 to 1.4 reduction in the CHO to protein ratio was also found having beneficial effects on weight loss and body composition in overweight women (Layman et al., 2003). Overall, no significant difference between HP and NP groups was observed in the present thesis, it might be due to the limited statistical power resulted from the small sample size of only 46 participants. However, it is noteworthy that there was a trend for less weight loss in HPNC group, yet the multiple comparison P value indicated non-significance. This is possibly due to the less-than-target intake of protein in HPNC group, meaning the beneficial effects of high protein intake was not sufficiently shown.

5.3.3 HP vs NP, the effect of higher protein intake on body composition

When further focusing on the change in body composition, in this thesis, all groups experienced significant reduction in % body fat after the 8-week LED treatment. Among all groups, NPNC group experienced the greatest reduction in % body fat of -4.0%, followed by HPLC group of -3.7%, NPLC group of -2.9%, and HPNC group of -2.9%, yet this difference between groups displayed no significance. As for lean mass, all groups showed significant increase in % lean mass from the 8-week LED. NPNC group lost the greatest amount of % lean mass of 3.7%, followed by HPLC group of 3.5%, NPLC group of 2.7%, and HPNC group of 2.7%. The result from this thesis showed that HP diets were not more beneficial in promoting fat loss and

preserving lean mass when compared to NP diets, which is contradictory to the original hypothesis.

A loss of body weight is typically coupled with loss of both body fat mass and lean mass, especially during stages of dietary energy restriction (Ian Janssen et al., 2002). As a main component and predictor of BMR (basal metabolic rate), a loss of FFM is coupled with decreases in energy expenditure. The preservation of FFM lessens the drop in energy expenditure that was induced by weight loss, especially during periods of energy-restriction. Therefore, weight loss diets which promote FM loss while preserving FFM are often desired (Westerterp-Plantenga, M. S. et al., 2009). Evidence showed that high protein diets was more effective in utilising body fat while maintaining lean body mass during weight loss than diets with lower protein, possibly by reducing nitrogen losses during stages of energy restriction (Bistrian, Winterer, Blackburn, Young, & Sherman, 1977; Evans et al., 2012; Layman et al., 2003). This difference was obvious even when the weight loss was the similar between high protein diets and lower protein diets (Wycherley et al., 2012). In the present study, this lack of difference in fat mass and lean mass change is possibly due to the small sample size. Also, the protein intake was calculated based on the self-reported food record, which inevitably results in errors.

However, there were some studies showing the opposite. Study by Johnston et al (2004) showed that two low-fat, energy-restricted diets with 15% and 30% energy as protein were equally effective at weight loss and reducing FM (Johnston, C. S., Tjonn, & Swan, 2004). Similarly, in the study by Luscombe et al (2005), no significant

difference in the change of body weight, fat mass, or lean mass was found between participants consuming either a high protein diet (34% EI as protein) or a standard protein diet (18% EI as protein), the change in resting energy expenditure was not significantly different either (Luscombe et al., 2005). Interestingly, Farnsworth et al (2003) found a higher protein-to-carbohydrate ratio was more beneficial in preserving total lean mass, but not in reducing body weight or total body fat. (Farnsworth et al., 2003).

It is worth mentioning a recent study by Soenen et al (2012), where the study design is very similar to the present sub-study. In their study, 132 participants were randomized into four LED dietary treatments with the following macronutrient compositions: HPLC (protein/CHO/fat of 60/5/35 En%), HPNC (protein/CHO/fat of 60/35/5 En%), NPLC (protein/CHO/fat of 30/5/65 En%), and NPNC (protein/CHO/fat of 30/35/35 En%) for 3 months. The result showed significantly greater weight loss and fat loss in HP diet groups when compared to NP diet groups with the same CHO content (HPLC vs. NPLC and HPNC vs. NPNC), meanwhile no significant difference was found with the change in FFM. Their findings are clearly, contradictory to what was found by the present sub-study. However, in comparison with the present sub-study, Soenen et al (2012) included a bigger sample size of 132 participants, which might explain the greater significance observed in their study (Soenen et al., 2012).

5.3.4 HP vs NP, the effect of higher protein intake on EI

At baseline, the mean (SEM) total EI was 8258 (683.6) kJ in HPLC group when compared with NPLC group of 9475 kJ, while the mean (SEM) total EI was 8607 (744) kJ in HPNC group when compared with NPNC group of 9312 kJ. This is slightly higher than what was estimated by 2008/09 Adult Nutrition Survey, where the mean daily EI ranged from 7205 to 8426kJ in New Zealand females aged between 19-70, indicating that the participants included in the sub-study were customary to the higher-than-average energy intake (again, the survey was completed over 10 years ago and used different dietary assessment methods from the present study). This is expected since all participants were obese at baseline.

It was hypothesized in this thesis that the high protein intake would promote the satiety level, which would ultimately reflect in the total EI. However, Tukey's multiple comparison test did not show any significant difference in total energy intake when comparing HP groups and NP groups: HPLC vs. NPLC and HPNC vs. NPNC at baseline, mid-LED (week 4), or post-LED (week 8). This finding is consistent with some protein studies showing no difference in EI after increasing protein intake. Blatt et al (2011) did not find any difference in the satiety rating or daily energy intake after varying the protein ratio of the meals when consumed ad libitum (Blatt, Alexandria D., MS, RD, Roe, Liane S., MPH, RD, & Rolls, 2011). Another study confirmed the effects of protein in promoting satiety, yet not sufficiently enough to produce significant difference in EI (Lejeune et al., 2006). While Raben et al (2003) showed

that protein-rich diets do not have a significant impact on satiety or EI (Raben, Agerholm-Larsen, Flint, Holst, & Astrup, 2003).

However, this evidence is contradictory to many previous studies supporting the role of high protein diets in promoting satiety and reducing overall energy intake (Due et al., 2004; Skov et al., 1999; Weigle et al., 2005). A sustained decrease in a*d libitum* energy intake was found associated with an increase in dietary protein from 15% to 30% EI at a constant carbohydrate intake (Weigle et al., 2005). Studies where food intake was *ad lib* indicated that increasing protein intake promotes satiety and body weight loss (Noakes et al., 2005; Weigle et al., 2005). In comparison to carbohydrate and fat, protein is proven to be most satiating (Veldhorst et al., 2008; Weigle et al., 2005), which was especially important in maintaining compliance during periods of energy restriction. In the present sub-study, participants were asked to eat their variable meal till they were comfortably full, which created an *ad lib* feeding condition and allowed the food intake to vary between treatment arms. The satiety was reflected in total EI and was measured through the self-reported food records. The results found no association between weight loss, the macronutrient composition of the diet, or with one's energy intake, which indicates high protein diets were not more advantageous in promoting satiety or the degree of weight loss.

However, it is important to note that in most literatures, the diets investigated were not able to control the carbohydrate content, as in the higher protein diets were often low in carbohydrate. Therefore, the effect of low carbohydrate can not be excluded.

Overall, total EI, satiety, change in body weight and body composition were similar between both HP diets (HPLC and HPNC) and NP diets (NPLC and NPNC). The finding from the sub-study proves the hypothesis to be false.

5.4 Other hypothesis

5.4.1 Change in CHO intake

At baseline, CHO consumption was not significantly different between NC groups and LC groups at a constant protein content, where HPLC group consumed 40.0% CHO while HPNC group consumed 41.8% CHO, NPLC group consumed 40.4% CHO while NPNC group consumed 41.4% CHO. This is slightly lower than what was consumed by typical New Zealand females. 2008/09 New Zealand Adult Nutrition Survey estimated the average CHO intake for New Zealand females ranged from 46.2% to 49.3% EI. This indicates that participants in the sub-study were less representative of the typical New Zealand female population as far as the intake of CHO (again, the survey was completed over 10 years ago and used different dietary assessment methods from the present study). Meanwhile, this intake is also lower than the typical dietary recommendations for New Zealand adults, where 50-55% total energy should come from carbohydrate, 10-15% total energy from protein, and 30-33% total energy from fat (Ministry of Health, 2003).

At 8 weeks, LC groups had significantly decreased their CHO intake as expected ($P<0.0001$), where NPLC group decreased their CHO intake from 40.4% to 29.1% EI while HPLC group decreased their CHO intake from 40.0% to 29.0% EI. Both LC groups were relatively successful in achieving their target CHO intake of 28% EI.

As for NC groups, NPNC group decreased their CHO intake from 41.4% to 36.2% EI while HPNC group decreased their CHO intake from 41.8% to 38.3% EI, both non-significantly. However, both NC groups consumed less CHO than their target of 40% EI, which is unexpected since those in NC groups were asked to follow a 'normal CHO' diet, and the target CHO intake was very close to their baseline intake. In theory, it should be less challenging for NC groups to achieve their CHO target, since those in LC groups were expected to restrict their CHO to meet the target intake.

And again, the dietary data is mainly based on the 4-day food record, which is subjected to misreporting.

5.4.2 NC vs LC, effect of lower CHO intake on weight loss

It was hypothesized in this thesis that a lower carbohydrate, higher fat diet would promote greater weight loss than a higher carbohydrate, lower fat diet with a constant protein content. Within the HP diet group, the effects on weight loss from LC diet did not differ significantly from NC diet. The mean weight loss of LC diet in the presence of HP component was -9.2 kg compared to NC diet of -6.5 kg. Likewise, no significant difference in weight loss was found between LC and NC groups in the presence of NP component (-8.5 kg vs -9.6 kg). Interestingly, as for the changes in BMI, it appears that the reduction was significant in LC (NPLC and HPLC) but not in NC (NPNC and HPNC) groups. However, Tukeys post hoc analysis showed no significant difference in the change of BMI between four groups, indicating that a lower carbohydrate, higher fat diet was equally beneficial in promoting weight loss

when compared with a higher carbohydrate, lower fat diet regardless of the protein content.

The results prove the hypothesis to be false, and do not align with the evidence from many similar studies, where a lower carbohydrate, higher fat diet is superior to a higher carbohydrate, lower fat diet during short term weight loss (Brehm et al., 2003; Samaha et al., 2003; Yancy et al., 2004). The mechanisms responsible for this difference in weight loss were unknown, but many studies mentioned the possibility of an improved satiety from ketosis following the low intake of CHO (Brehm et al., 2003; Foster, Gary D. et al., 2003). It was also proposed that low carbohydrate diets lead to a greater loss in body water resulted from the depletion of stored glycogen (Brehm et al., 2003).

However, there is increasing evidence showing no advantage in lower CHO diets (Golay et al., 1996; Linda Stern et al., 2004; Petersen et al., 2006). Dansinger et al (2005) compared four popular diets (Atkins, Zone, Weight Watchers, and Ornish) and found no difference in the weight loss outcomes between them, especially between Atkins and Ornish diets, which by their nature were low in carbohydrate and low in fat respectively (Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005). Multiple systematic reviews reported similar results on low CHO diets, where weight loss is only associated with the duration of the diet and the level of EI, but not with the carbohydrate restriction itself (Freedman, King, & Kennedy, 2001; Hu et al., 2012). Overall, the results from the present thesis showed that participants following lower carbohydrate higher fat diets yielded the same success at weight loss as those who

followed higher carbohydrate lower fat diets, independent of protein intake, which is contradictory to the study hypothesis.

5.4.3 NC vs LC, the effect of lower CHO intake on body composition

Within the HP group, significant reduction in % body fat and % lean mass was seen in both LC and NC groups from baseline to CID3, yet no significant difference was found between two groups (HPLC vs HPNC). Similarly, within the NP group, both LC and NC groups experienced significant decreases in % body fat and % lean mass with no significant difference between groups (NPLC vs NPNC) either. The result from this thesis showed that LC diets were not more beneficial in promoting fat loss and preserving lean mass when compared with NC diets, which is contradictory to the hypothesis.

However, there is inconsistent evidence regarding the effect of low CHO diet on body composition. Wal et al (2007) found lower-carbohydrate higher-fat partial meal replacement (PMR) diets were associated with a greater loss in body fat (Vander Wal, Mcburney, Moellering, Marth, & Dhurandhar, 2007). In a different study, low-carbohydrate diets resulted a greater proportional loss in fat mass and a significantly greater proportional preservation in lean mass than low-fat diets (Bazzano et al., 2014). Significant loss in fat mass and gain in lean mass were seen in participants switching from a diet with moderate carbohydrate and fat to a low-carbohydrate, higher fat diet (Volek et al., 2002). Interestingly, in the study by Meckling et al (2004), both diets resulted significant loss in fat mass, but significant loss in lean body mass was only found in the low carbohydrate group (Meckling,

O'Sullivan, & Saari, 2004). In a different study, participants following low carbohydrate diets lost significantly more fat mass and fat free mass than those following low fat diets (McAuley et al., 2005), an observation that was also found by Brehm et al (2003) (Brehm et al., 2003). It has been proposed that low-carbohydrate diets promote a more favourable body composition by limiting the secretion of insulin, which promotes the fat oxidation and inhibits lipogenesis (Volek, Quann, & Forsythe, 2010).

It is important to mention that in previous literatures, the protein content in these diets was often not controlled. Therefore, the effects of protein on body weight and body composition cannot be excluded.

5.5 Strength of this thesis:

There are a number of strengths within this sub-study. Females with a wide range of ethnic groups within Auckland region were recruited, which covers Maori, Pacific, Asian, and New Zealand Europeans. It is representative of a typical New Zealand population since New Zealand is diverse in their cultures and ethnic groups (Khawaja, Boddington, & Didham, 2007).

Another strength of the sub-study is that it separated the high protein and low carbohydrate components of the weight loss diet, a task that many other studies were not able to do (Hwalla Baba et al., 1999; Larsen et al., 2011; Yancy et al., 2004).

In addition, group meetings with a registered dietitian were held regularly throughout the study period, which gave participants opportunities to communicate and share experience with each other. Meanwhile, participants were contacted frequently via

phone, text message or emails to remind them of each visit, group meeting, and the completion of food records. This potentially encouraged participants to continue study and promoted their compliance to the dietary treatment.

5.6 Limitation of the thesis:

An important limitation of the thesis is its small sample size of only 46 participants. Since this is a sub-study of a weight loss trial, and it only included participants enrolled in cohort A, B, and C, which results in a smaller population compared to the whole trial (149 participants). In addition, the thesis used a completers' only analysis, participants were only included in the sub-study after they have completed 3 CID visits and all the measurements, including body weight, height, body composition and food records. Consequently, the final number of participants (46 participants) was low compared with what was enrolled originally (70 participants). As seen in the flow chart (Figure), out of 70 individuals enrolled in the sub-study, only 55 were able to complete all anthropometric measurements. Of those 55 participants, a final number of only 46 provided completed and interpretable food records.

This thesis was also limited by being not able to check the dietary compliance.

As a reliable method of checking dietary compliance, urinary nitrogen testing was not included in the present thesis due to time constraint. Dietary data and compliance were only collected through the use of 4-day food record, which relied entirely on participants' self-reporting. In addition, the use of food record is often associated with high participant burden, and it requires a certain level of literacy and numeracy.

Although oral and written instructions were provided beforehand, there is still a high chance for misreporting and bias, especially within the obese population.

The sub-study was also limited by its lack of subjective measurement of satiety. Subjective assessments of satiety, such as VAS questionnaires, Becke questionnaire, 3FEQ and blood biomarkers for appetite, were not included in the present thesis due to time constraint. In contrast, total EI was used as a substitute measure of satiety in this sub-study. Again, as reported above, the total EI derived from self-reported food records was associated with misreporting and bias.

Another limitation of the thesis is that participants' physical activity levels were not well controlled. Although participants were recommended to maintain a sedentary activity level, it is possible that some of them still introduce some exercise to their daily routine for the purpose of a greater weight loss, such as the two participants who showed abnormal weight loss trajectory.

In addition, there was a lack of accuracy in the analysis of macronutrients using Foodworks® software. There was a limited selection of food items, such as the cultural food known as traditional cuisine for South-East Asian population.

5.7 Conclusion and future directions

In conclusion, the present sub-study has shown that an 8-week LED programme was capable of inducing clinically meaningful and significant changes in body weight and body composition among obese female individuals, regardless of its macronutrient composition. It was hypothesized in this thesis that an 8-week higher-protein LED treatment might result in significantly greater body weight loss among obese women

when compared to a normal-protein LED treatment independent of their carbohydrate (CHO) contents. However, the findings from the sub-study refuted this hypothesis, where a higher-protein LED treatment was equally effective in inducing weight loss as a lower-protein LED treatment independent of their CHO contents, indicating that a successful weight loss diet perhaps should focus more on energy restriction rather than its macronutrient composition.

There were also significant changes in body fat and lean mass within all treatment groups, yet again, on average, a higher protein LED treatment was not significantly better at losing body fat and preserving lean mass than a lower protein LED treatment independent of their CHO contents. The results from this study indicated that losses in both lean mass and fat mass are inevitable with the application of LED. Future weight loss regimen should focus more on exercise rather than macronutrient composition of the diets, in order to yield a better body composition outcome.

In addition, the results from this sub-study have shown that on average, a higher protein LED treatment did not produce a greater satiety and a lower EI than a lower protein LED treatment independent of their CHO contents. Since participants were allowed to eat variably at the dinner meal (*ad lib* meal), the consumption of a higher protein diet is not better at suppressing appetite and improving satiety than a lower protein diet independent of their CHO contents.

Another comparison made by this sub-study was between a lower-CHO higher-fat LED treatment and a higher-CHO lower-fat LED treatment while their protein contents were kept constant. Interestingly, when protein was kept at a constant level, a

lower-CHO higher-fat LED treatment did not induce a greater weight loss or was better at losing body fat while preserving lean mass than a higher-CHO lower-fat LED treatment. This further ruled out the effects of macronutrient composition on LED-induced weight loss.

Overall, an 8-week LED program is capable in producing significant and clinically meaningful weight loss regardless of its macronutrient composition. For future weight loss programs involving LED, it might be helpful of focusing more on other aspects of weight loss, such as light exercise, rather than on the macronutrient composition of the LED treatment.

Chapter 6 References

A. Pietrobelli, C. Formica, Z. Wang, & S. B. Heymsfield. (1996). Dual-energy X-ray absorptiometry body composition model: Review of physical concepts. American Journal of Physiology - Endocrinology and Metabolism, 271(6), 941-951.

Alberti, K. G. M. M, Zimmet, P., & Shaw, J. (2006). Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. Diabetic Medicine, 23(5), 469-480.

André Tchernof, & Jean-Pierre Després. (2013). Pathophysiology of human visceral obesity: An update. Physiological Reviews, 93(1), 359-404.

Aragon, A. A., Schoenfeld, B. J., Wildman, R., Kleiner, S., VanDusseldorp, T., Taylor, L., . . . Antonio, J. (2017). International society of sports nutrition position stand: Diets and body composition. Journal of the International Society of Sports Nutrition, 14(1), 16.

Ardern, C. I., Katzmarzyk, P. T., Janssen, I., & Ross, R. (2003). Discrimination of health risk by combined body mass index and waist circumference. Obesity Research, 11(1), 135-142.

Ashwell, M., Gunn, P., & Gibson, S. (2012). Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: Systematic review and meta-analysis. Obesity Reviews, 13(3), 275-286.

Astbury, N. M., Piernas, C., Hartmann-Boyce, J., Lapworth, S., Aveyard, P., & Jebb, S. A. (2019). A systematic review and meta-analysis of the effectiveness of meal replacements for weight loss. Obesity Reviews, 20(4), 569-587.

Astrup, A. (1999). Dietary approaches to reducing body weight. Best Practice & Research Clinical Endocrinology & Metabolism, 13(1), 109-120.

Astrup, A., Ryan, L., Grunwald, G. K., Storgaard, M., Saris, W., Melanson, E., & Hill, J. O. (2000). The role of dietary fat in body fatness: Evidence from a preliminary meta-analysis of ad libitum low-fat dietary intervention studies. British Journal of Nutrition, 83(S1), S25-S32.

Atkinson, R. L., Dietz, W. H., Foreyt, J. P., Goodwin, N. J., Hill, J. O., Hirsch, J., . . . Hoofnagle, J. H. (1993). Very low-calorie diets. Jama, 270(8), 967-974.

Barrows, K., & Snook, J. T. (1987). Effect of a high-protein, very-low-calorie diet on body composition and anthropometric parameters of obese middle-aged women. The American Journal of Clinical Nutrition, 45(2), 381-390.

Baumgartner, R. N., Heymsfield, S. B., & Roche, A. F. (1995). Human body composition and the epidemiology of chronic disease. Obesity Research, 3(1), 73-95.

Bethesda, (. (1998). Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: The evidence report. (). Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK2003/

Bingham, S. A. (2003). Urine nitrogen as a biomarker for the validation of dietary protein intake. The Journal of Nutrition, 133 Suppl 3(3), 921S-924S.

Bistrian, D. R., Winterer, J., Blackburn, G. L., Young, V., & Sherman, M. (1977). Effect of a protein-sparing diet and brief fast on nitrogen metabolism in mildly obese subjects. The Journal of Laboratory and Clinical Medicine, 89(5), 1030.

Blatt, Alexandria D., MS, RD, Roe, Liane S., MPH, RD, & Rolls, B. J., PhD. (2011). Increasing the protein content of meals and its effect on daily energy intake. Journal of the American Dietetic Association, 111(2), 290-294.

Bosse, J. D., & Dixon, B. M. (2012). Dietary protein in weight management: A review proposing protein spread and change theories. Nutrition & Metabolism, 9(1), 81.

Boyd A Swinburn, Gary Sacks, Kevin D Hall, Klim McPherson, Diane T Finegood, Marjory L Moodie, & Steven L Gortmaker. (2011). Obesity 1: The global obesity pandemic: Shaped by global drivers and local environments. The Lancet, 378(9793), 804. Retrieved from https://search.proquest.com/docview/887130645

Bravata, D. M., Sanders, L., Huang, J., Krumholz, H. M., Olkin, I., Gardner, C. D., & Bravata, D. M. (2003). Efficacy and safety of low-carbohydrate diets: A systematic review. Jama, 289(14), 1837-1850.

Bray, G. A., & Popkin, B. M. (1998). Dietary fat intake does affect obesity. The American Journal of Clinical Nutrition, 68(6), 1157-1173.

Brehm, B. J., Seeley, R. J., Daniels, S. R., & D'Alessio, D. A. (2003). A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. The Journal of Clinical Endocrinology & Metabolism, 88(4), 1617-1623.

Brita Stenius¬Aarniala, Tuija Poussa, Johanna Kvarnström, Eeva¬Liisa Grönlund, Mikko Ylikahri, Pertti Mustajoki. (2000). Immediate and long term effects of weight reduction in obese people with asthma: Randomized controlled study.

Brown, A., & Leeds, A. R. (2019). Very low-energy and low-energy formula diets: Effects on weight loss, obesity co-morbidities and type 2 diabetes remission – an update on the evidence for their use in clinical practice. Nutrition Bulletin, 44(1), 7-24.

Campos-Nonato, I., Hernandez, L., & Barquera, S. (2017). Effect of a high-protein diet versus standard-protein diet on weight loss and biomarkers of metabolic syndrome: A randomized clinical trial. Obesity Facts, 10(3), 238-251.

Cartwright, M. J., Tchkonia, T., & Kirkland, J. L. (2007). Aging in adipocytes: Potential impact of inherent, depot-specific mechanisms. Experimental Gerontology, 42(6), 463-471.

Chiu, M., Austin, P. C., Manuel, D. G., Shah, B. R., & Tu, J. V. (2011). Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. Diabetes Care, 34(8), 1741-1748.

Christensen, P., Bliddal, H., Riecke, B. F., Leeds, A. R., Astrup, A., & Christensen, R. (2011). Comparison of a low-energy diet and a very low-energy diet in sedentary obese individuals: A pragmatic randomized controlled trial. Clinical Obesity, 1(1), 31-40.

Christensen, P., Frederiksen, R., Bliddal, H., Riecke, B. F., Bartels, E. M., Henriksen, M., . . . Christensen, R. (2013). Comparison of three weight maintenance programs on cardiovascular risk, bone and vitamins in sedentary older adults. Obesity, 21(10), 1982-1990.

Christensen, P., Larsen, T. M., Westerterp-Plantenga, M., Macdonald, I., Martinez, J. A., Handjiev, S., . . . Raben, A. (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 Obesity & Metabolism, 20(12), 2840-2851.

Clifton, P. M., Condo, D., & Keogh, J. B. (2013). Long term weight maintenance after advice to consume low carbohydrate, higher protein diets – A systematic review and meta analysis. Nutrition, Metabolism and Cardiovascular Diseases, 24(3), 224-235.

Cotton, J. R., Burley, V. J., Weststrate, J. A., & Blundell, J. E. (2007). Dietary fat and appetite: Similarities and differences in the satiating effect of meals supplemented with either fat or carbohydrate. Journal of Human Nutrition and Dietetics, 20(3), 186-199.

Crovetti, R., Porrini, M., Santangelo, A., & Testolin, G. (1998). The influence of thermic effect of food on satiety. European Journal of Clinical Nutrition, 52(7), 482-488.

Dansinger, M. L., Gleason, J. A., Griffith, J. L., Selker, H. P., & Schaefer, E. J. (2005). Comparison of the atkins, ornish, weight watchers, and zone diets for weight loss and heart disease risk reduction: A randomized trial. Jama, 293(1), 43-53.

de Castro, J. M. (1987). Macronutrient relationships with meal patterns and mood in the spontaneous feeding behavior of humans. Physiology & Behavior, 39(5), 561-569.

Delbridge, E. A., Prendergast, L. A., Pritchard, J. E., & Proietto, J. (2009). One-year weight maintenance after significant weight loss in healthy overweight and obese subjects: Does diet composition matter? The American Journal of Clinical Nutrition, 90(5), 1203-1214.

Deurenberg, P., & Yap, M. (1999). The assessment of obesity: Methods for measuring body fat and global prevalence of obesity. Best Practice & Research Clinical Endocrinology & Metabolism, 13(1), 1-11.

Drewnowski, A., Brunzell, J. D., Sande, K., Iverius, P. H., & Greenwood, M. R. C. (1985). Sweet tooth reconsidered: Taste responsiveness in human obesity. Physiology & Behavior, 35(4), 617-622.

Due, A., Toubro, S., Skov, A. R., & Astrup, A. (2004). Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: A randomised 1-year trial. International Journal of Obesity, 28(10), 1283-1290.

Dumesnil, J. G., Turgeon, J., Tremblay, A., Poirier, P., Gilbert, M., Gagnon, L., . . . Deapés, J. (2001). Effect of a low-glycaemic index–low-fat–high protein diet on the atherogenic metabolic risk profile of abdominally obese men. British Journal of Nutrition, 86(5), 557-568.

Evans, E. M., Mojtahedi, M. C., Thorpe, M. P., Valentine, R. J., Kris-Etherton, P. M., & Layman, D. K. (2012). Effects of protein intake and gender on body composition changes: A randomized clinical weight loss trial. Nutrition & Metabolism, 9(1), 55.

Everhart, J. E. (1993). Contributions of obesity and weight loss to gallstone disease. Annals of Internal Medicine, 119(10), 1029.

Farnsworth, E., Luscombe, N. D., Noakes, M., Wittert, G., Argyiou, E., & Clifton, P. M. (2003). Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. The American Journal of Clinical Nutrition, 78(1), 31-39.

Flynn, T. J., & Walsh, M. F. (1993). Thirty-month evaluation of a popular very-low-calorie diet program. Archives of Family Medicine, 2(10), 1042-1048.

Foster, G. D., Wadden, T. A., Feurer, I. D., Jennings, A. S., Stunkard, A. J., Crosby, L. O., . . . Mullen, J. L. (1990). Controlled trial of the metabolic effects of a very-low-calorie diet: Short- and long-term effects. The American Journal of Clinical Nutrition, 51(2), 167-172.

Foster, G. D., Wadden, T. A., Mullen, J. L., Stunkard, A. J., Wang, J., Feurer, I. D., . . . Gold, J. (1988). Resting energy expenditure, body composition, and excess weight in the obese. Metabolism, 37(5), 467-472.

Foster, G. D., Wadden, T. A., Peterson, F. J., Letizia, K. A., Bartlett, S. J., & Conill, A. M. (1992). A controlled comparison of three very-low-calorie diets: Effects on weight, body composition, and symptoms. The American Journal of Clinical Nutrition, 55(4), 811-817.

Foster, G. D., Wyatt, H. R., Hill, J. O., McGuckin, B. G., Brill, C., Mohammed, B. S., . . . Klein, S. (2003). A randomized trial of a low-carbohydrate diet for obesity. The New England Journal of Medicine, 348(21), 2082-2090.

Freedman, M. R., King, J., & Kennedy, E. (2001). Popular diets: A scientific review. Obesity Research, 9 Suppl 1, 1S.

Funk, M. D., Lee, M., Vidoni, M. L., & Reininger, B. M. (2019). Weight loss and weight gain among participants in a community-based weight loss challenge. BMC Obesity, 6(1), 2.

Gardner, J., Kjolhaug, J., Linde, J. A., Sevcik, S., & Lytle, L. A. (2013). Teaching goal-setting for weight-gain prevention in a college population: Insights from the CHOICES study. Journal of Health Education Teaching, 4(1), 39-49.

Gallagher, D., Heymsfield, S. B., Heo, M., Jebb, S. A., Murgatroyd, P. R., & Sakamoto, Y. (2000). Healthy percentage body fat ranges: An approach for developing guidelines based on body mass index. The American Journal of Clinical Nutrition, 72(3), 694-701.

Golay, A., Allaz, A. F., Morel, Y., de Tonnac, N., Tankova, S., & Reaven, G. (1996). Similar weight loss with low- or high-carbohydrate diets. The American Journal of Clinical Nutrition, 63(2), 174-178.

Goodwin, S. (2002). The practical guide to the identification, evaluation and treatment of overweight and obesity in adults. Clinical Nurse Specialist, 16(3), 164.

Green, S. M., & Blundell, J. E. (1996). Effect of fat- and sucrose-containing foods on the size of eating episodes and energy intake in lean dietary restrained and unrestrained females: Potential for causing overconsumption. European Journal of Clinical Nutrition, 50(9), 625.

Griffin, H. J., Cheng, H. L., O'Connor, H. T., Rooney, K. B., Petocz, P., & Steinbeck, K. S. (2013). Higher protein diet for weight management in young overweight women: A 12-month randomized controlled trial. Diabetes, Obesity and Metabolism, 15(6), 572-575.

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. Obesity Reviews, 10(1), 36-50.

Heymsfield, S. B., van Mierlo, C A J, van der Knaap, H C M, Heo, M., & Frier, H. I. (2003). Weight management using a meal replacement strategy: Meta and pooling analysis from six studies. International Journal of Obesity, 27(5), 537-549.

Hill, J. O., Melanson, E. L., & Wyatt, H. T. (2000). Dietary fat intake and regulation of energy balance: Implications for obesity. The Journal of Nutrition, 130(2S Suppl), 284S-288S.

Hu, T., Mills, K. T., Yao, L., Demanelis, K., Eloustaz, M., Yancy, J., William S, . . . Bazzano, L. A. (2012). Effects of low-carbohydrate diets versus low-fat diets on metabolic risk factors: A meta-analysis of randomized controlled clinical trials. American Journal of Epidemiology, 176 Suppl 7(suppl_7), S44-S54.

Huerta, S., Li, Z., Li, H. C., Hu, M. S., Yu, C. A., & Heber, D. (2004). Feasibility of a partial meal replacement plan for weight loss in low-income patients. International Journal of Obesity, 28(12), 1575-1579.

Hughes, K., Aw, T. C., Kuperan, P., & Choo, M. (1997). Central obesity, insulin resistance, syndrome X, lipoprotein(a), and cardiovascular risk in indians, malays, and chinese in singapore. Journal of Epidemiology and Community Health, 51(4), 394-399.

Huxley, R., James, W. P. T., Barzi, F., Patel, J. V., Lear, S. A., Suriyawongpaisal, P., . . . Woodward, M. (2008). Ethnic comparisons of the cross-sectional relationships between measures of body size with diabetes and hypertension. Obesity Reviews, 9(s1), 53-61.

Huxley, R., Mendis, S., Zheleznyakov, E., Reddy, S., & Chan, J. (2010). Body mass index, waist circumference and waist:Hip ratio as predictors of cardiovascular risk-a review of the literature. European Journal of Clinical Nutrition, 64(1), 16-22.

Hwalla Baba, N., Sawaya, S., Torbay, N., Habbal, Z., Azar, S., & Hashim, S. (1999). High protein vs high carbohydrate hypoenergetic diet for the treatment of obese hyperinsulinemic subjects. International Journal of Obesity, 23(11), 1202-1206.

Ian Janssen, Anne Fortier, Robert Hudson, & Robert Ross. (2002). Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. Diabetes Care, 25(3), 431-438.

Janssen, I., Katzmarzyk, P. T., & Ross, R. (2002). Body mass index, waist circumference, and health risk: Evidence in support of current national institutes of health guidelines. Archives of Internal Medicine, 162(18), 2074-2079.

Janssen, I., Katzmarzyk, P. T., & Ross, R. (2004). Waist circumference and not body mass index explains obesity-related health risk. The American Journal of Clinical Nutrition, 79(3), 379-384.

Jeffery, R. W., Hellerstedt, W. L., French, S. A., & Baxter, J. E. (1995). A randomized trial of counseling for fat restriction versus calorie restriction in the treatment of obesity. International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 19(2), 132.

Jensen, P., Zachariae, C., Christensen, R., Geiker, N. R. W., Schaadt, B. K., Stender, S., . . . Skov, L. (2013). Effect of weight loss on the severity of psoriasis: A randomized clinical study. JAMA Dermatology, 149(7), 795-801.

Johansson, K., Hemmingsson, E., Harlid, R., Trolle Lagerros, Y., Granath, F., Rössner, S., & Neovius, M. (2011). Longer term effects of very low energy diet on obstructive sleep apnoea in cohort derived from randomised controlled trial: Prospective observational follow-up study. Bmj, 342(7809), 1248.

Johnston, B. C., Kanters, S., Bandayrel, K., Wu, P., Naji, F., Siemieniuk, R. A., . . . Mills, E. J. (2014). Comparison of weight loss among named diet programs in overweight and obese adults: A meta-analysis. Jama, 312(9), 923-933.

Johnston, C. S., Tjonn, S. L., & Swan, P. D. (2004). High-protein, low-fat diets are effective for weight loss and favorably alter biomarkers in healthy adults. The Journal of Nutrition, 134(3), 586-591.

Kahn, H. S., MD, & Bullard, K. M., PhD. (2016). Beyond body mass index: Advantages of abdominal measurements for recognizing cardiometabolic disorders. American Journal of Medicine, The, 129(1), 74-81.e2.

Karst, H., Steiniger, J., Noack, R., & Steglich, H. -. (1984). Diet-induced thermogenesis in man: Thermic effects of single proteins, carbohydrates and fats depending on their energy amount. Annals of Nutrition and Metabolism, 28(4), 245-252.

Katzmarzyk, P. T., Bray, G. A., Greenway, F. L., Johnson, W. D., Newton, R. L., Ravussin, E., . . . Bouchard, C. (2011). Ethnic-specific BMI and waist circumference thresholds. Obesity, 19(6), 1272-1278.

Kendall, A., Levitsky, D. A., Strupp, B. J., & Lissner, L. (1991). Weight loss on a low-fat diet: Consequence of the imprecision of the control of food intake in humans. The American Journal of Clinical Nutrition, 53(5), 1124-1129.

Khawaja, M. A., Boddington, B., & Didham, R. A. (2007). Growing ethnic diversity in new zealand and its implications for measuring differentials in fertility and mortality. Wellington, N.Z: Statistics New Zealand.

Kotler, D. P., Burastero, S., Wang, J., & Pierson, J., R N. (1996). Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: Effects of race, sex, and disease. The American Journal of Clinical Nutrition, 64(3 Suppl), 489S-497S.

Lal, A., Moodie, M., Ashton, T., Siahpush, M., & Swinburn, B. (2012). Health care and lost productivity costs of overweight and obesity in new zealand. Australian and New Zealand Journal of Public Health, 36(6), 550-556.

Lantz, H., Peltonen, M., Ågren, L., & Torgerson, J. S. (2003). Intermittent versus on-demand use of a very low calorie diet: A randomized 2-year clinical trial. Journal of Internal Medicine, 253(4), 463-471.

Larsen, R., Mann, N., Maclean, E., & Shaw, J. (2011). The effect of high-protein, low-carbohydrate diets in the treatment of type 2 diabetes: A 12 month randomised controlled trial. Diabetologia, 54(4), 731-740.

Layman, D. K., Boileau, R. A., Erickson, D. J., Painter, J. E., Shiue, H., Sather, C., & Christou, D. D. (2003). A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. The Journal of Nutrition, 133(2), 411-417.

Leader, N. J., Ryan, L., Molyneaux, L., & Yue, D. K. (2013). How best to use partial meal replacement in managing overweight or obese patients with poorly controlled type 2 diabetes. Obesity, 21(2), 251-253.

Leeds, A. R. (2014). Formula food-reducing diets: A new evidence-based addition to the weight management tool box. Nutrition Bulletin, 39(3), 238-246.

Lejeune, M. P., Westerterp, K. R., Adam, T. C., Luscombe, N. D., & Westerterp-Plantenga, M. S. (2006). Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. American Journal of Clinical Nutrition, 83(1), 89-94.

Lemieux, S., Prud'homme, D., Bouchard, C., Tremblay, A., & Després, J. P. (1993). Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. The American Journal of Clinical Nutrition, 58(4), 463-467.

Leveille, G. A., & Cloutier, P. F. (1987). Isocaloric diets: Effects of dietary changes. The American Journal of Clinical Nutrition, 45(1 Suppl), 158-163.

Linda Stern, Nayyar Iqbal, Prakash Seshadri, Kathryn L. Chicano, Denise A. Daily, Joyce McGrory, . . . Frederick F. Samaha. (2004). The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: One-year follow-up of a randomized trial. Annals of Internal Medicine, 140(10), 778.

Lissner, L., Levitsky, D. A., Strupp, B. J., Kalkwarf, H. J., & Roe, D. A. (1987). Dietary fat and the regulation of energy intake in human subjects. The American Journal of Clinical Nutrition, 46(6), 886-892.

Liu, X., Zhang, G., Ye, X., Li, H., Chen, X., Tang, L., . . . Lin, X. (2013). Effects of a low-carbohydrate diet on weight loss and cardiometabolic profile in chinese women: A randomised controlled feeding trial. The British Journal of Nutrition, 110(8), 1444-1453.

Luscombe, N. D., Noakes, M., Wittert, G. A., Keogh, J., Foster, P., Moran, J., & Clifton, P. M. (2005). Carbohydrate-restricted diets high in either monounsaturated fat or protein are equally effective at promoting fat loss and improving blood lipids. American Journal of Clinical Nutrition, 81(4), 762-772.

Marisa Porrini, Roberta Crovetti, Giulio Testolin, Santino Silva. (1995). Evaluation of satiety sensations and food intake after different preloads. Appetite, 25(1), 17-30.

Mauriege, P., Imbeault, P., Langin, D., Lacaille, M., Almeras, N., Tremblay, A., & Despres, J. P. (1999). Regional and gender variations in adipose tissue lipolysis in response to weight loss. Journal of Lipid Research, 40(9), 1559. Retrieved from http://www.jlr.org/cgi/content/abstract/40/9/1559.

Mazess, R. B., Barden, H. S., Bisek, J. P., & Hanson, J. (1990). Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. The American Journal of Clinical Nutrition, 51(6), 1106-1112.

McAuley, K. A., Smith, K. J., Taylor, R. W., McLay, R. T., Williams, S. M., & Mann, J. I. (2006). Long-term effects of popular dietary approaches on weight loss and features of insulin resistance. International Journal of Obesity, 30(2), 342-349.

McAuley, K. A., Williams, S. M., Mann, J. I., Goulding, A., & Murphy, E. (2002). Increased risk of type 2 diabetes despite same degree of adiposity in different racial groups. Diabetes Care, 25(12), 2360-2361.

McManus, K., Antinoro, L., & Sacks, F. (2001). A randomized controlled trial of a moderate-fat, low-energy diet compared with a low fat, low-energy diet for weight loss in overweight adults. International Journal of Obesity, 25(10), 1503-1511.

Mela, D. J. (1990). The basis of dietary fat preferences. Trends in Food Science & Technology, 1, 71-73.

Meseri, R., Ucku, R., & Unal, B. (2014). Waist:Height ratio: A superior index in estimating cardiovascular risks in turkish adults. Public Health Nutrition, 17(10), 2246-2252.

Ministry of Health. (2003). Food and Nutrition Guidelines for Healthy Adults: A background paper. Published online 02 October 2003. Retrieved from http://www.health.govt.nz/publication/food-and-nutrition-guidelines-healthy-adults-background-paper on 28/04/2020.

Ministry of Health. (2017). Clinical guidelines for weight management in new zealand adults. Wellington, New Zealand: Ministry of Health. Retrieved from https://natlib-primo.hosted.exlibrisgroup.com/primo-explore/search?query=any,contains,9918174655302836&tab=catalogue&search_scope=NLNZ&vid=NLNZ&offset=0 on 27/04/2020.

Ministry of Health. (2019). Obesity statistics. Retrieved from https://www.health.govt.nz/nz-health-statistics/health-statistics-and-data-sets/obesity-statistics on 27/04/2020.

Minstry of Health. (2018). Body size. Retrieved from https://www.health.govt.nz/our-work/populations/maori-health/tatau-kahukura-maori-health-statistics/nga-tauwehe-tupono-me-te-marumaru-risk-and-protective-factors/body-size on 27/04/2020.

Mulholland, Y., Nicokavoura, E., Broom, J., & Rolland, C. (2012). Very-low-energy diets and morbidity: A systematic review of longer-term evidence. The British Journal of Nutrition, 108(5), 832-851.

Neema Tiwari. (2016). Pathology of obesity. Era's Journal of Medical Research, 3(2), 53-56. Retrieved from https://search.proquest.com/docview/1963096257

Nelson, K. M., Weinsier, R. L., Long, C. L., & Schutz, Y. (1992). Prediction of resting energy expenditure from fat-free mass and fat mass. The American Journal of Clinical Nutrition, 56(5), 848-856.

Nielsen, S., Guo, Z., Johnson, C. M., Hensrud, D. D., & Jensen, M. D. (2004). Splanchnic lipolysis in human obesity. The Journal of Clinical Investigation, 113(11), 1582-1588.

Noakes, M., Keogh, J. B., Foster, P. R., & Clifton, P. M. (2005). Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. The American Journal of Clinical Nutrition, 81(6), 1298-1306.

Nordmann, A. J., Nordmann, A., Briel, M., Keller, U., Yancy, W. S., Brehm, B. J., & Bucher, H. C. (2006). Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: A meta-analysis of randomized controlled trials. Archives of Internal Medicine, 166(3), 285-293.

Nuttall, F. (2015). Body mass index: Obesity, BMI, and health: A critical review. Nutrition Today, 50(3), 117-128.

Ogden, C. L., Yanovski, S. Z., Carroll, M. D., & Flegal, K. M. (2007). The epidemiology of obesity. Gastroenterology, 132(6), 2087-2102.

Owen, O. E. (1988). Resting metabolic requirements of men and women. Mayo Clinic Proceedings, 63(5), 503-510.

Parretti, H. M., Jebb, S. A., Johns, D. J., Lewis, A. L., Christian-Brown, A. M., & Aveyard, P. (2016). Clinical effectiveness of very-low-energy diets in the management of weight loss: A systematic review and meta-analysis of randomized controlled trials. Obesity Reviews, 17(3), 225-234.

Pavlou, K. N., Steffee, W. P., Lerman, R. H., & Burrows, B. A. (1985). Effects of dieting and exercise on lean body mass, oxygen uptake, and strength. Medicine and Science in Sports and Exercise, 17(4), 466-471.

Petersen, M., Taylor, M. A., Saris, W. H., Verdich, C., Toubro, S., Macdonald, I., . . . Astrup, A. (2006). Randomized, multi-center trial of two hypo-energetic diets in obese subjects: High- versus low-fat content. International Journal of Obesity, 30(3), 552-560.

Poppitt, S. D., & Prentice, A. M. (1996). Energy density and its role in the control of food intake: Evidence from metabolic and community studies. Appetite, 26(2), 153-174.

Poppitt, S. D., McCormack, D., & Buffenstein, R. (1998). Short-term effects of macronutrient preloads on appetite and energy intake in lean women. Physiology & Behavior, 64(3), 279-285.

Powell, J. J., Tucker, L., Fisher, A. G., & Wilcox, K. (1994). The effects of different percentages of dietary fat intake, exercise, and calorie restriction on body composition and body weight in obese females. American Journal of Health Promotion, 8(6), 442-448.

Prentice, A. M., Goldberg, G. R., Jebb, S. A., Black, A. E., Murgatroyd, P. R., & Diaz, E. 0. (1991). Physiological responses to slimming. Proceedings of the Nutrition Society, 50(2), 441-458.

Pritchard, J. E., Nowson, C. A., Strauss, B. J., Carlson, J. S., Kaymakci, B., & Wark, J. D. (1993). Evaluation of dual energy X-ray absorptiometry as a method of measurement of body fat. European Journal of Clinical Nutrition, 47(3), 216.

Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J. J., & Astrup, A. (2003). Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. The American Journal of Clinical Nutrition, 77(1), 91-100.

Ranganathan, P., Pramesh, C., & Aggarwal, R. (2016). Common pitfalls in statistical analysis: Intention-to-treat versus per-protocol analysis. Perspectives in Clinical Research, 7(3), 144-146.

Roriz, A. K. C., Passos, L. C. S., de Oliveira, C. C., Eickemberg, M., Moreira, P. d. A., & Sampaio, L. R. (2014). Evaluation of the accuracy of anthropometric clinical indicators of visceral fat in adults and elderly. PloS One, 9(7), e103499.

Rössner, S., & Flaten, H. (1997). VLCD versus LCD in long-term treatment of obesity. International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 21(1), 22.

Ryttig, K., Flaten, H., & Rössner, S. (1997). Long-term effects of a very low calorie diet (nutrilett®) in obesity treatment. A prospective, randomized, comparison between VLCD and a hypocaloric diet+behavior modification and their combination. International Journal of Obesity, 21(7), 574-579.

Sacks, F. M., Bray, G. A., Carey, V. J., Smith, S. R., Ryan, D. H., Anton, S. D., . . . Williamson, D. A. (2009). Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. The New England Journal of Medicine, 360(9), 859-873.

Samaha, F. F., Iqbal, N., Seshadri, P., Chicano, K. L., Daily, D. A., McGrory, J., . . . Stern, L. (2003). A low-carbohydrate as compared with a low-fat diet in severe obesity. The New England Journal of Medicine, 348(21), 2074-2081.

Saris, W. H. M. (2001). Very-low-calorie diets and sustained weight loss. Obesity Research, 9(11S), 295S-301S.

Schlundt, D. G., Hill, J. O., Pope-Cordle, J., Arnold, D., Virts, K. L., & Katahn, M. (1993). Randomized evaluation of a low fat ad libitum carbohydrate diet for weight reduction. International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 17(11), 623.

Schofield, W. N. (1985). Predicting basal metabolic rate, new standards and review of previous work. Human Nutrition. Clinical Nutrition, 39 Suppl 1, 5.

Seidell, J. C. (2010). Waist circumference and waist/hip ratio in relation to all-cause mortality, cancer and sleep apnea. European Journal of Clinical Nutrition, 64(1), 35-41.

Shah, M., McGovern, P., French, S., & Baxter, J. (1994). Comparison of a low-fat, ad libitum complex-carbohydrate diet with a low-energy diet in moderately obese women. The American Journal of Clinical Nutrition, 59(5), 980-984.

Sheppard, L., Kristal, A. R., & Kushi, L. H. (1991). Weight loss in women participating in a randomized trial of low-fat diets. The American Journal of Clinical Nutrition, 54(5), 821-828.

Silva, V., Stanton, K. R., & Grande, A. J. (2013). Harmonizing the diagnosis of metabolic Syndrome—Focusing on abdominal obesity. Metabolic Syndrome and Related Disorders, 11(2), 12-108.

Skov, A., Toubro, S., Rønn, B., Holm, L., & Astrup, A. (1999). Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. International Journal of Obesity, 23(5), 528-536.

Smeets, A. J., Soenen, S., Luscombe Marsh, N. D., Ueland, O., & Plantenga, M. S. (2008). Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch. Journal of Nutrition, 138(4), 698-702.

Sours, H. E., Frattali, V. P., Brand, C. D., Feldman, R. A., Forbes, A. L., Swanson, R. C., & Paris, A. L. (1981). Sudden death associated with very low calorie weight reduction regimens. The American Journal of Clinical Nutrition, 34(4), 453-461.

Stijn Soenen, Eveline A P Martens, Ananda Hochstenbach-Waelen, Sofie G T Lemmens, & Margriet S Westerterp-Plantenga. (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 Mass1,2. The Journal of Nutrition, 143(5), 591.

Swinburn, B. A., Craig, P. L., Daniel, R., Dent, D. P., & Strauss, B. J. (1996). Body composition differences between polynesians and caucasians assessed by bioelectrical impedance. International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 20(10), 889.

Tentolouris, N., Pavlatos, S., Kokkinos, A., Perrea, D., Pagoni, S., & Katsilambros, N. (2008). Diet-induced thermogenesis and substrate oxidation are not different between lean and obese women after two different isocaloric meals, one rich in protein and one rich in fat. Metabolism, 57(3), 313-320.

Thom, G., & Lean, M. (2017). Is there an optimal diet for weight management and metabolic health? Gastroenterology, 152(7), 1739-1751.

Torgerson, J., Ågren, L., & Sjöström, L. (1999). Effects on body weight of strict or liberal adherence to an initial period of VLCD treatment. A randomised, one-year clinical trial of obese subjects. International Journal of Obesity, 23(2), 190-197.

Tsai, A. G., & Wadden, T. A. (2006). The evolution of very-low-calorie diets: An update and meta-analysis. Obesity, 14(8), 1283-1293.

Ulijaszek, S. J. (2003). Obesity: Preventing and managing the global epidemic. report of a WHO consultation. WHO technical report series 894. pp. 252. (world health organization, geneva, 2000.) SFr 56.00, ISBN 92-4-120894-5, paperback. Journal of Biosocial Science, 35(4), 624-625.

Van Gaal, L. F., Mertens, I. L., & Ballaux, D. (2005). What is the relationship between risk factor reduction and degree of weight loss? European Heart Journal Supplements, 7(suppl_L), L21-L26.

Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., . . . Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. Physiology & Behavior, 94(2), 300-307.

Volek, J. S., Quann, E. E., & Forsythe, C. E. (2010). Low-carbohydrate diets promote a more favorable body composition than low-fat diets. Strength and Conditioning Journal, 32(1), 42-47.

Waaler, H. T. (1984). Height. weight and mortality the norwegian experience. Acta Medica Scandinavica, 215(S679), 1-56.

Wadden, T. A., & Stunkard, A. J. (1986). Controlled trial of very low calorie diet, behavior therapy, and their combination in the treatment of obesity. Journal of Consulting and Clinical Psychology, 54(4), 482-488.

Wadden, T. A., Foster, G. D., & Letizia, K. A. (1994). One-year behavioral treatment of obesity: Comparison of moderate and severe caloric restriction and the effects of weight maintenance therapy. Journal of Consulting and Clinical Psychology, 62(1), 165.

Weigle, D. S., Breen, P. A., Matthys, C. C., Callahan, H. S., Meeuws, K. E., Burden, V. R., & Purnell, J. Q. (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. The American Journal of Clinical Nutrition, 82(1), 41.

Westerterp, K. R., Wilson, S. A., & Rolland, V. (1999). Diet induced thermogenesis measured over 24h in a respiration chamber: Effect of diet composition. International Journal of Obesity, 23(3), 287-292.

Westerterp-Plantenga, M. S. (2007). How are normal, high- or low-protein diets defined? British Journal of Nutrition, 97(2), 217-218.

Westerterp-Plantenga, M. S., Nieuwenhuizen, A., Tome, D., Soenen, S., & Westerterp, K. R. (2009). Dietary protein, weight loss, and weight maintenance. Annual Review of Nutrition, 29(1), 21-41.

Whitehead, J. M., McNeill, G., & Smith, J. S. (1996). The effect of protein intake on 24-h energy expenditure during energy restriction. International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 20(8), 727.

Williams, R. L., Wood, L. G., Collins, C. E., & Callister, R. (2015). Effectiveness of weight loss interventions – is there a difference between men and women: A systematic review. Obesity Reviews, 16(2), 171-186.

World Health Organisation. (2020a). Body mass index - BMI. Retrieved from http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi on 26/04/2020.

World Health Organisation. (2020b). Obesity and overweight. Retrieved from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight on 26/04/2020.

Wycherley, T. P., Brinkworth, G. D., Clifton, P. M., & Noakes, M. (2012). Comparison of the effects of 52 weeks weight loss with either a high-protein or high-carbohydrate diet on body composition and cardiometabolic risk factors in overweight and obese males. Nutrition & Diabetes, 2(8), e40.

Yancy, J., William S, Olsen, M. K., Guyton, J. R., Bakst, R. P., & Westman, E. C. (2004). A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: A randomized, controlled trial. Annals of Internal Medicine, 140(10), 769.

Yu-Poth, S., Zhao, G., Etherton, T., Naglak, M., Jonnalagadda, S., & Kris-Etherton, P. M. (1999). Effects of the national cholesterol education program's step I and step II dietary intervention programs on cardiovascular disease risk factors: A meta-analysis. The American Journal of Clinical Nutrition, 69(4), 632-646.

Appendices

Appendix 1

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND

NUTRITION STUDY PARTICIPANTS WANTED

HNU

Diets for weight loss:
Is there any difference between high-protein and low-carb diets?

We would be happy to hear from you if you are:

- female
- available for an 8-week weight loss study
- body weight not more than 130kg
- between BMI 30 – 45 kg/m^2
- between 18 – 65 years of age
- otherwise healthy

You must be able to attend Human Nutrition Unit (HNU) at Mt Eden for this study.
A gratuity and travel expenses will be paid.

This study has received ethics approval (**18/CEN/238**).

Contact for more information
Email: diets.hnu@gmail.com
Phone: 09 630 1162

OR use your phone camera to scan this QR code for registration>>>

Appendix 2

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND

School of Biological Sciences and
Department of Medicine

Human Nutrition Unit
The University of Auckland
18 Carrick Place, Mt Eden
Auckland, 1024.
Phone: +64 9 630 1162

Higher-protein vs lower-carbohydrate diets for weight loss.

PARTICIPANT INFORMATION SHEET

We invite you to participate in a clinical study where we aim to compare the effects of higher-protein and lower-carbohydrate diets on eating behaviour, weight loss and body composition.

Your participation in the study is entirely voluntary (your choice). If you do agree to take part, you are free to withdraw from the study at any time, without having to give a reason. You may take as much time as you need to consider whether or not you would like to participate.

Who can take part?

We will invite 140 female participants to participate in this study. You can participate if you are female, between 18 - 65 years of age, have a Body Mass Index (BMI = weight / height^2) between 30-45kg/m^2, body weight no more than 130kg, and are healthy. You should not have any significant diseases (such as liver or kidney disease, diabetes, cardiovascular disease, cancer, digestive disease, etc.), should not be a smoker or have given up smoking for less than 6 months, and have not undergone bariatric surgery for weight loss.

You must be available to attend a **screening visit** at the University of Auckland Human Nutrition Unit (HNU) in Mt Eden, Auckland**,** which will take approximately one hour, to assess whether you are suitable to participate in this study. After that, you must be able to adhere to an assigned **weight-loss diet for 8 weeks,** during which time you will come to HNU on 6 occasions to meet with our Research Nurse and dietary counsellors. This will comprise **three morning study visits** (Week 0, Week 4 and Week 8) where we will collect your blood samples and other information. Additionally, you will also attend four **evening/weekend group counselling meetings** (Week 0, Week 2, Week 4, and Week 6) where you will receive the dietary support you need to stay on this weight-loss program.

Appendix 3

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND

School of Biological Sciences and
Department of Medicine

Human Nutrition Unit
The University of Auckland
18 Carrick Place, Mt Eden
Auckland, 1024.
Phone: +64 9 630 1162

Higher-protein vs lower-carbohydrate diets for weight loss.

CONSENT FORM

I have read and I understand the Patient Information Sheet dated *8th November 2018* and wish to take part in the research entitled "***Higher-protein vs lower-carbohydrate energy-restricted diets: effects on eating behaviour, weight loss and body composition***".

I have had the opportunity to discuss this research with the investigator. I am satisfied with the answers I have been given.

1. I have had the opportunity to use support from a family (whanau) member or a friend to help me ask questions and understand the research.
2. I understand that taking part in this research is voluntary (my choice), and that I may withdraw from the research at any time and this will in no way affect my future or continuing health care.
3. I understand that my participation in this research is confidential and that no material which could identify me will be used in any reports on this research. I understand that the sponsor of the research, others working on the sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current research and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published.
4. I agree for my blood samples to be processed and analysed for appetite-related biomarkers at The University of Adelaide, Australia.
5. I understand that if I consent to such analysis, no rights will be created for the researcher to get my genetic information.
6. I understand that the treatment, or investigation, will be stopped if it should appear harmful to myself.
7. I understand the compensation provisions for this research.
8. I have had time to consider whether to take part.
9. I know whom to contact if I have any side effects from the research.
10. I know whom to contact if I have any questions about the research.
11. I agree not to restrict the use of any data or results that arise from this research provided such a use is only for scientific purposes.

Participant to complete: *Please circle as appropriate*			Participant Signature:
I consent to participate in this study.	Yes	No	
I consent to having blood samples sent to The University of Adelaide, Australia for the analysis of appetite-related biomarkers.	Yes	No	
I consent to having a DXA scan.	Yes	No	
I consent to participate in the Meal Challenge, with cannulation and repeated blood sampling.	Yes	No	

Appendix 4

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

Participants Booklet

High-Protein vs Low-Carbohydrate Diets for Weight Loss

Funded by the Riddet Institute

Supported by the Cambridge Weight Plan NZ and Nutratech Ltd

Appendix 5

Date Started: _____/_____/_____ Study ID _____

Date Ended : _____/_____/_____ CID _____

You are receiving these instructions because the research study you are participating requires that you keep a food record for 4 days - 3 consecutive weekdays and 1 weekend day; either Saturday or Sunday (e.g. Wednesday, Thursday, Friday, Saturday OR Sunday, Monday, Tuesday, Wednesday. A food record is simply a "food journal" you carry with you throughout the day. It is where you will write, with as much detail as possible, the descriptions of all the food and beverages you eat over the 4 days.

Important information:

† Do not start your food diary if you are feeling unwell

† Please do not change you eating habits while keeping this food record

† Record food as you eat, do not wait to record later. It is important that you record what you actually eat. So please take this into account any leftovers before filling in the amount in the diary

† Fill in, all details, as explained, each time you consume a drink, a meal or a snack. Describe everything that you drink or/and eat at the time

† A detailed description of the food/drink should include: brand name, amount consumed, how the item was cooked/prepared (e.g. fried, baked, steamed), and importantly if anything was added to the food (e.g. type of condiment, salt, sugar)

Note you cannot be too precise with the description:

e.g. Bread – white bread, wholemeal, baguette, roll

Spreading fat - butter, margarine made with olive or sunflower oil

Milk – Full, semi-skimmed, skimmed

Cheese – cheddar, cottage cheese, reduced fat (30%)

Method of cooking – raw, boiled, fried, baked

Packaging and processing – fresh, frozen, tinned

Brand name – Heinz, Watties, Select

† If possible please weigh all food and drink using an electronic scale and record weights in your diary. However, this may not be possible and so you could describe as accurately as possible using household measures [*See description below*]

† For home-made recipes, record the raw ingredients in the diary along with the weight of the total amount cooked and the cooked portion served. There are blank pages in the back of the diary for you to write your recipes. Alternatively you could print and staple them to your diary

† If you use a lunch pack, each item must be weighed and recorded in the diary. It may be a good idea to prepare your pack the day before. Remember that any leftovers must be brought back home and subtracted from the initial amounts and the FINAL amount recorded in the diary the same day

† For pre-packaged meals it would be helpful to save the label for us, as well as record the description and weight eaten in the food diary.