The effect of protein source on weight loss, body composition, and substrate oxidation following a 12-week high-protein, ketogenic diet: A randomized trial

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Abstract

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Background: Ketogenic diets, diets high in fat and protein and low in carbohydrates, have been shown to be effective for weight loss. Recently, plant-based diets and protein sources have gained in popularity as they are thought to be a healthier alternative to animal-based protein sources. There is limited evidence as to whether protein source impacts ketogenic dietary outcomes.

Objective: The aim of this study is to investigate how plant- and animal-based protein supplementation impact weight loss, body composition, and substrate oxidation following a 12-week high-protein, hypocaloric, ketogenic diet in adults with obesity.

Methods: Adults with obesity were recruited and randomized (N= 35) to receive a 12-week high-protein ketogenic diet which included plant- or animal-based protein supplements. Body composition was assessed through dual energy x-ray absorptiometry (DEXA) and computed tomography (CT). Substrate oxidation was assessed via indirect calorimetry before and after the intervention.

Results: Both the plant-based and animal-based groups saw significant reductions in overall weight, fat mass, and fat-free mass ($p<0.001$ for all). The plant-based group saw a significant reduction in carbohydrate oxidation ($p=0.037$), a trend to suggest an increase in lipid oxidation ($p=0.054$), and a trend to suggest a decrease in respiratory exchange ratio ($p=0.057$). There were

no differences in any body composition variables nor resting energy expenditure following the intervention for either group.

Conclusion: Our results indicate that regardless of protein source, people who followed a 12-week high-protein ketogenic diet saw significant loss of weight, fat mass, and fat-free mas. Following a 12-week plant-based high-protein ketogenic diet may lead to a reduction in carbohydrate oxidation and an increase in lipid oxidation, but this may be due to differences in baseline dietary composition and further research is needed to determine the validity of the proposed conclusion.

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Author Contribution

As the primary author, I was responsible for recruitment of participants, protocols, statistical analyses, and writing of this thesis. Anjalee I. Wanasinghe was also responsible for recruitment of participants and protocols but was not involved in the preparation of this thesis. As the principal investigator, Dr. Santosa was responsible for the conceptualization, funding acquisition, and supervision of the study presented as well as the editing of this thesis.

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Abbreviations

2-D: 2-dimensional

3-D: 3-dimensional

BMI: body mass index

CCER: Comité central d'éthique de la recherche

CHO_{ox}: carbohydrate oxidation

CT: computed tomography

DEXA: dual energy x-ray absorptiometry

FAT_{ox}: lipid oxidation

FFM: fat-free mass

FM: fat mass

HU: Hounsfield units

MCT: medium-chain triglyceride

NFH: Nutritional Foundations of Health Inc.

REE: resting energy expenditure

RER: respiratory exchange ratio

SD: standard deviation

VAT: visceral adipose tissue

V_{CO2}: volume of carbon dioxide

V_{O2}: volume of oxygen

1. Background and literature review

1.1 Obesity

Obesity and the associated health issues remain a large concern for Canadians. The prevalence of obesity in Canada has increased three-fold from 1.1–6.0% since 1985.[1] Obesity is most commonly diagnosed according to a body mass index (BMI) of ≥30kg/m^2 and is characterized by abnormal or excessive adipose tissue accumulation that can impair health.[2] This prevalent, complex, progressive, and relapsing chronic disease[1,3,4] is a notable risk factor for other comorbidities such as cardiovascular disease, type 2 diabetes, and hypertension[5] and as such, it is imperative to find effective methods to treat and prevent obesity in order to avoid disease progression including the development of such comorbidities.

Weight loss remains the main approach to obesity treatment. Though there are many approaches by which weight loss is achieved, the response to changes in body composition varies. Regardless of the type of intervention, weight loss results in the loss of fat mass as well as fat-free mass. Fat-free mass preservation remains an issue when it comes to overall weight loss and weight loss maintenance.[6] Fat-free mass is metabolically active and accounts for approximately 60–70% of the resting metabolic rate of an individual[7,8]; therefore, it is important to preserve fat-free mass during weight loss interventions to reduce the risk of weight regain. In fact, fat-free mass loss was shown to be a significant predictor of weight regain after 1-year in females with overweight who reached a BMI of <25kg/m^2 through dietary, and dietary and exercise interventions.[9] However, fat-free mass is not the sole determinant of weight gain. Buscemi S *et al*[10] studied the relationship between resting metabolic rate, changes in body weight, and degree of fatness across 12-years in a wide patient population (males and females, aged 18–55 years old with BMI: 17.5–63.4kg/m^2) and reported that a low resting metabolic rate

appears to be associated with body weight gain over time, even when fat-free mass was taken into account. Body composition and energy assessment measurements are needed to evaluate and quantify the key determinants of energy imbalance: fat-free mass preservation and metabolic changes in people with obesity.

1.1.1 Body composition measurement in obesity

In the absence of time and equipment to determine body composition, BMI and waist/hip circumference measurements are considered crude markers of adiposity.[11] However, BMI assumes uniform adipose tissue distribution[12] and is largely inadequate for assessing health-risk for people with obesity as BMI is unable to discriminate between fat and lean mass. A study by Romeo-Corral *et al*[13] indicated that using BMI: ≥30kg/m^2 as a cut-off for obesity had poor sensitivity to detect adipose tissue obesity, missing more than half of people with body-fat-percentage-defined obesity which is defined as females with ≥30% body fat and males with ≥25% body fat.[14] As mentioned earlier, the excess accumulation of adipose tissue impairs health and therefore, it is imperative to have an accurate measure of adiposity as a marker of health. When using BMI to diagnose obesity, there is a crucial lack of sensitivity and accuracy of whole-body adiposity. Additionally, while waist and hip circumference measurements have been shown to be well correlated with obesity-related comorbidity,[15] these measurements tend to only explain changes in subcutaneous adipose tissue rather than identifying overall body composition and regional fat distribution.[16] Whole body adiposity is difficult to estimate with these techniques.

Body compositional measurements such as densitometry, air displacement plethysmography, and hydrometry are viable ways to measure fat mass and fat-free mass in individuals with obesity but the gold standard method to measure body composition is dual energy x-ray

absorptiometry (DEXA).[17] Densitometry, air displacement plethysmography, and hydrometry are based on a two-compartment model measuring fat mass and fat-free mass[18,19]; however, this does not account for bone mass. DEXA measures body composition based on a three-compartment model, measuring fat mass, lean mass, and bone mineral content.[18] Advantages of using DEXA are that it is non-invasive and produces highly accurate readings. However, DEXA equipment is expensive, training is needed to conduct the scan, and size and weight restrictions of the equipment do not always allow the full body to be assessed without estimation[20] and this is particularly a problem in participants with higher weights.

The addition of computed topography (CT) imaging alongside DEXA measurement allows investigators to evaluate intraabdominal adipose tissue without needing to measure the entire abdomen.[21] CT imaging uses a technique known as reconstruction to produce a three-dimensional (3D) image from two-dimensional (2D) images in order to assess tissue composition.[22] CT equipment is more costly and specialized than DXA equipment. A trained technician is required to conduct the scan. With CT, a highly reliable measurement of intraabdominal adipose tissue can be obtained.[23] CT can expose people to high levels of x-ray radiation when large areas of the body are assessed. However, Jensen, *et al*[21] reported that one CT slice at the L2-L3 vertebrae in conjunction with DEXA can provide an accurate measurement of intraabdominal adipose tissue without the need for large sections of the abdomen to be assessed which limits radiation exposure.

1.1.2 Resting energy expenditure and respiratory exchange ratio in obesity

Resting energy expenditure may be influenced by various factors including sex, age, weight, body composition, dietary composition, and physical activity[24,25] but fat-free mass preservation

and gradual weight loss is beneficial for minimizing the reduction of resting energy expenditure that commonly accompanies weight loss. Johannsen DL *et al*[26] investigated resting energy expenditure in adults with severe obesity participating in a televised weight loss competition. At week 30, participants showed a 789 kcal/day reduction in resting energy expenditure likely due to the rapid, severe weight loss and loss of fat-free mass (~10.5kg).[26] When resting energy expenditure is reduced, individuals tend to gain weight back through this energy imbalance as resting energy expenditure is estimated to be reduced by approximately 4.4 kcal/day for every kilogram of fat mass loss and 12.7 kcal/day for every kilogram of fat free mass lost in people with obesity.[27] A 12-weeks, energy-reduced (~1700 kcal) dietary intervention led to 2.2 kg weight loss (-1.9% fat mass percentage) and resulted in a moderate reduction (-78 kcal/day) in resting energy expenditure.[28] The loss demonstrated in this study does not align with the approximation of energy changes following fat and fat-free mass lost above but, as mentioned prior, resting energy expenditure is influenced by many factors and this change could be seen depending on external variables.

Respiratory quotient or respiratory exchange ratio provides an indication of relative substrate (carbohydrate or lipid) oxidation.[29] Respiratory quotient and respiratory exchange ratio are the measurements of carbon dioxide production relative to oxygen consumption and can be calculated as V_{CO2}/V_{O2}. Respiratory quotient requires invasive procedures as it is measured at the tissue level while respiratory exchange ratio is measured through breathing.[29,30] Respiratory quotient values typically range between 0.7 and 1.0 and denote a mix of fat, protein, and carbohydrate as energy source used: a value of 0.7 indicates the use of lipids, exclusively, as an energy source and a value of 1.0 indicates the use of carbohydrate, exclusively, as an energy

source.[30] For the purposes of this thesis, we measured respiratory exchange ratio through indirect calorimetry due to the feasibility of this measurement.

Many people with obesity experience reduced rate of fat utilization before and after weight loss. Impairment of fat utilization leads to increased storage of free fatty acids and, in turn, weight regain.[31] Zurlo F *et al*[32] reported that a higher respiratory exchange ratio was directly correlated with subsequent weight gain, independent of low energy expenditure in nondiabetic adults potentially suggesting that weight changes can be influenced by fat oxidation. Interestingly enough, one study demonstrated that people with obesity experience impaired free fatty acid utilization during exercise even following weight loss,[31] suggesting that this problem may be multifaceted and have to do with how weight loss is achieved, not solely due to weight loss in general. An intervention that maintains or increases fat oxidation after weight loss could be a potentially effective treatment option for long-term management of obesity.

1.1.3 Management of obesity

Obesity requires individualized management through weight loss to avoid the development of comorbidities.[33] A review by Ryan and Yockey,[34] concluded that people do not necessarily need to reach a BMI of <25kg/m^2 to experience improvements in health risk: any reduction in weight will provide benefits. They reported that even modest weight reductions of 5% were associated with increased odds of achieving a 0.5% point reduction in HbA1c, 5-mmHg decrease in systolic and diastolic blood pressure, and improved other glycemic outcomes for people with type 2 diabetes.[34]

Weight reduction is achieved through a variety of strategies including bariatric surgery, weight loss medications, and lifestyle changes such as exercise and dietary modifications.[5,35] For

the purpose of this thesis, we will focus on dietary modifications despite the lack of standardized treatment strategy. Diet options such as ketogenic, high-protein, and plant-based diets are among some popular weight loss tactics, but it is unknown what may be the most effective or if there may be some additive effect when combining different diets.

1.2 Ketogenic diets

The ketogenic diet, a diet consisting of very low carbohydrate intake and high fat intake, is commonly used by the public with many different variations.[36] Even though the parameters of the diet are not well defined, a standard ketogenic diet is generally composed of about 5–10% carbohydrates, 30–35% protein, and 55–65% fat.[37] This macronutrient breakdown is effective to induce a physiological state of nutritional ketosis where carbohydrate depletion occurs forcing the body to use lipid as the primary energy source.[38] Additional types of ketogenic diets (reported in Table 1) include the modified Atkins diet: 65% fat, 25% protein, and 10% carbohydrates[39]; the more popular type of ketogenic diet that is defined as <50 g/day of carbohydrates regardless of energy requirements; and a carbohydrate-restrictive diet with <130 g/day carbohydrates regardless of the energy requirements.[40] There is also a 4:1 ratio ketogenic diet described that consisted of 80% fat and 20% carbohydrates and proteins. Given that fat-free mass preservation is believed to be the key to sustained weight loss, ketogenic diets higher in protein may be a preferential weight loss solution as further described below.

Table 1: Ketogenic diet modifications.

Name of diet	Fat composition	Protein composition	Carbohydrate composition
Standard ketogenic diet	55–65%	30–35%	5–10%
Modified Atkins diet	65%	25%	10%
Popularized ketogenic diet			<50 g
Carbohydrate-restrictive diet			<130 g
4:1 ratio ketogenic diet	80%	20%	

1.2.1 The mechanism of ketosis

During periods of starvation, intentional fasting, lactation, and briefly following exercise, the body adapts to the metabolic state known as ketosis (Figure 1).[41] Insulin secretion is stimulated when circulating blood glucose levels rise after periods of carbohydrate intake; yet in periods of limited carbohydrate intake, glucagon, a glucoregulatory peptide is secreted to stimulate the conversion of stored liver glycogen to glucose for energy.[42] Once liver glycogen stores are depleted, the body adapts to use free fatty acids to support the body's energy requirements. Through a process known as beta oxidation, free fatty acids are converted into acetyl-CoA which can be used as energy.[42] As beta oxidation continues, there is an overproduction of acetyl-CoA which are then converted into ketone bodies through a process known as ketogenesis. Ketone bodies are used as an alternate form of energy alongside free fatty acids during ketosis.[42] Ketogenesis and beta oxidation continue to indirectly and directly produce energy from the conversion of ketone bodies and free fatty acids, respectively, until glucose is reintroduced. When carbohydrate intake is reestablished, insulin secretion is stimulated and the body returns to using glucose as the primary energy source.[42]

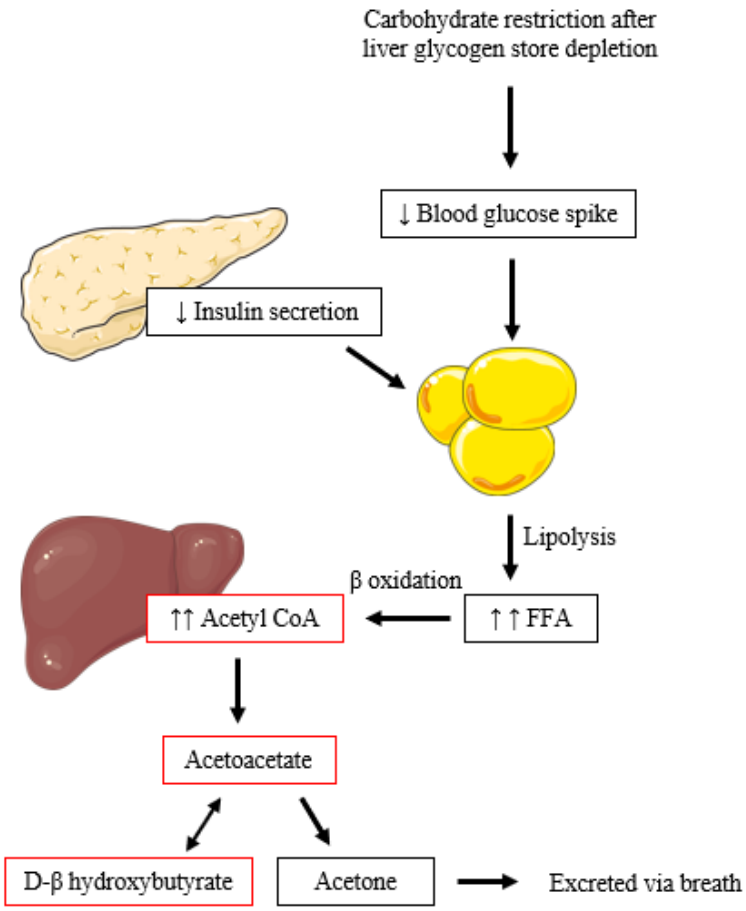

Figure 1: Mechanism of ketosis once liver glycogen stores are depleted. Red boxes represent energy sources during ketosis.

There are three ketone bodies produced by the liver when blood glucose and liver glycogen stores are reduced: acetoacetate, D-β hydroxybutyrate, and acetone.[42] Acetoacetate is a necessary precursor used for cholesterol synthesis and an important cofactor for lipogenesis.[43,44] Acetoacetate can be metabolized into the two other primary ketone bodies: D-β hydroxybutyrate and acetone.[37] D-β hydroxybutyrate is the most abundant of ketone bodies; circulating D-β hydroxybutyrate concentrations increase faster than circulating acetoacetate can be replenished.[41,45] D-β hydroxybutyrate is understood to have cellular signalling activity while also

being used as an energy metabolite during periods of ketosis.[41] Acetone is a byproduct of the break down of acetoacetate and is reduced quickly after being formed.[45] Acetone is volatile and commonly excreted through waste or in the breath during periods of build-up.[45] Ketone bodies synthesized during periods of natural or nutritional ketosis are used for energy by the heart, muscle tissue, kidneys, and brain when necessary.[37] It is hypothesized that ketosis was a metabolically adaptive pathway for survival during times of extreme starvation early in human history.[46]

Ketone bodies serve important functions for other metabolic pathways such as fatty acid oxidation, tricarboxylic acid cycle, gluconeogenesis, de novo lipogenesis, and the biosynthesis of sterols and therefore, circulating ketone levels vary throughout the day regardless of blood glucose concentration or diet composition.[44,46] Adult blood ketone body concentrations range from <0.1 mM in the fed state to 6 mM in the fasted state[47] but to classify the human body in a state of ketosis, blood ketone body concentrations should range from ~0.2 mM to >3.0 mM regardless of feeding state.[48]

Ketosis is a catabolic state[37] used therapeutically for decades to help treat diseases such as epilepsy, cancer, diabetes, and non-alcoholic fatty liver disease[38,40,49,50] and like any dietary change, can result in side effects but is considered well-tolerated and safe. Commonly occurring side effects of ketosis include fatigue, nausea, low exercise tolerance, and dizziness.[37,38] Ketogenic diets of up to 24-weeks have been shown to be well tolerated and showed no significant side effects.[51] Davidson *et al*[52] even suggested that ketone bodies may have a protective effect against diet-induced cognitive impairment associated with obesity.

1.2.2 Ketogenic diet and weight loss

The rationale behind a ketogenic diet as an effective weight loss treatment option is derived from the Carbohydrate-Insulin model of obesity (Figure 2).[53] This model is based on the understanding that consuming carbohydrates is directly correlated to insulin production which leads to energy stored as fat. As reported in 1985 by Rodin J *et al,*[54] insulin production promotes increased hunger, increased food intake, and heightened perceived pleasantness of sweetness. By reducing insulin stimulation, we can reduce energy intake and increased storage of energy, most commonly within the adipose tissue. The Carbohydrate-Insulin model also suggests that elevated insulin decreases energy expenditure by shuttling metabolic fuels into storage rather than using as energy.[53] Based on the theory of the Carbohydrate-Insulin model, inducing a ketogenic state can be considered a viable weight loss strategy due to limited stimulation of insulin secretion stimulated by carbohydrate intake and the consequential effects.

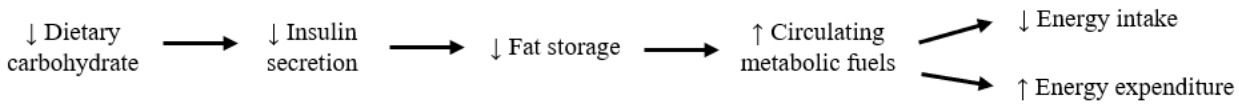

Figure 2: Carbohydrate-Insulin model of obesity.

Ketogenic diets have shown long term weight loss efficacy when compared to other diets[55–57], though there is a common view that rapid initial weight loss induced by the ketogenic diet is mostly attributable to losses in water weight. The initial water weight loss is thought to be because the low carbohydrate composition of the diet results in greater losses of glycogen which is the storage form of carbohydrates in the body. For every gram of carbohydrates, there are four grams of water associated and as carbohydrate intake is reduced, less glycogen is stored which is

accompanied by water weight loss.[7,37] However other dietary strategies may also result in equal water weight loss. Johnstone A *et al*[55] reported that over 4 weeks, a ketogenic diets lead to ~2kg more weight loss compared to Mediterranean diet. Importantly, there were no differences in water loss between the two diets indicating that the greater weight loss observed on the ketogenic diet was likely due to changes in tissue mass. Despite the belief that weight loss within a ketogenic diet is due to water weight loss, ketogenic diets have been shown to lead to significant weight loss over prolonged duration. Shai I *et al*[56] reported that people with obesity following a low-carbohydrate diet lost significantly more weight after 2-years when compared to those who followed a low-fat or Mediterranean diet. Another study showed that people following a low-carbohydrate diet maintained a ~1.5 kg greater weight loss at 1-year compared to people following a traditional Mediterranean diet though this difference did not reach significance.[57] It is important to consider that the aforementioned study was completed in people with overweight and diabetes and therefore, these results may not be representative of all populations.

Low-fat diets have long been considered the most effective strategy for weight loss but more recently, low-carbohydrate diets have also shown efficacy in weight loss.[58] Brinkworth GD *et al*[59] reported that people following a low-carbohydrate diet lost ~3kg more than people following a low-fat diet. A caveat, however, is that in this study, the low-fat group consumed about ~30% fat which is considered more moderate than low-fat; low fat diets are generally considered to be ~20% fat.[60] Also of note, the difference of weight lost between groups was considered nonsignificant as the study duration of this trial was 12-months. Despite a statistically nonsignificant weight loss, this study demonstrates the efficacy of weight loss using a low-carbohydrate diet. Quite similarly, a study completed by Stern L *et al*[61] demonstrated ~2 kg greater weight loss at 1-year in people following a low carbohydrate diet when compared to a

low-fat diet.[61] This study was conducted in a generally unobserved, outpatient group with monthly counseling sessions and establishes that low-carbohydrate diets are both efficacious and effective for weight loss. As demonstrated by the literature, when it comes to overall weight loss, it seems carbohydrate restriction may be moderately superior to fat restriction.[58–62]

Several other studies have shown that ketogenic diets are efficacious in promoting weight loss, primarily through fat-mass loss and improve substrate oxidation. Gomez-Arbelaez D *et al*[17] conducted a prospective study and reported that people with obesity who followed a ketogenic diet lost ~20 kg after 4-months. Furthermore, the investigators determined that only ~15% of overall weight loss was attributable to fat-free mass loss and approximately 85% of the weight loss demonstrated was fat mass[17], indicating that the lost weight was primarily due to losses in fat with preservation of fat free mass. As discussed previously, fat-free mass preservation is a crucial aspect of long-term weight loss management. Most studies assessing body composition in the context of a ketogenic diet do so in people who exercise regularly[63–67] and thus, there remains a need for further evidence to support the findings from this study.

In a study conducted by Paoli A *et al*,[68] individuals with overweight demonstrated a greater reduction of body fat percentage and lower respiratory exchange ratio following a 3-week ketogenic Mediterranean diet compared to a standard Mediterranean diet. Despite this seemingly beneficial increase in fat oxidation, this is to be expected as we know that respiratory exchange ratio is directly correlated with dietary composition. This is confirmed in a study completed by Hall K *et al*[69] where after following a restricted-carbohydrate diet, the respiratory exchange ratio was reduced by -0.055 points yet following a restricted-fat diet, increased +0.004 points. The greater shift exhibited when following a restricted-carbohydrate diet is likely because traditionally, carbohydrates account for the majority of dietary intake for individuals so when

restricting carbohydrate intake to even ~140 g/day[69], this would cause a greater metabolic shift than when restricting fat which accounts for less of the dietary intake to begin with. However, an interesting conclusion seen by Paoli A *et al*[68] was that both groups saw a significant decrease in body fat mass yet there were no significant changes in resting energy expenditure. As discussed earlier, resting energy expenditure tends to decrease with weight loss; however, these results suggest a ketogenic diet may be able to conserve energy expenditure even following weight loss, likely due to fat-free mass preservation. This similar phenomenon has been demonstrated in a few other studies as well. A study completed by Rubini A *et al*[70] showed that after 20 days of a ketogenic diet, resting energy expenditure remained relatively unchanged despite loss of fat-free mass. A four-week isocaloric ketogenic diet demonstrated a small increase in energy expenditure in males with overweight or obesity (n=17) compared to a four-week habitual baseline diet.[71] The increase in resting energy expenditure cannot be confirmed however as the habitual baseline diet was not controlled and, as discussed earlier, resting energy expenditure can be influenced by dietary composition.[24] Further research is needed to determine if this effect can be seen after a 12-week intervention.

Ketogenic diets have demonstrated efficacy in weight loss, fat mass loss, fat-free mass preservation, and have shown increased lipid oxidation and metabolic processes yet high-protein diets have also shown favourable outcomes. The incorporation of specific sources of increased protein in a ketogenic diet remains understudied even though there is potential to result in more efficient and sought after weight loss outcomes, especially in the potential to retain fat free mass.

1.3 High-protein ketogenic diet and weight loss

Protein plays an imperative role in diet to ensure proper bodily function. The recommended dietary allowance of protein for Canadian adults is 0.80 g/kg/day and about 10-35% of total energy.[72] A high-protein diet is clinically difficult to define, yet for the purposes of this proposal, we will consider a diet to be high in protein if the diet comprises at least 20% protein since the standard western diet typically contains ~15% protein despite the dietary recommendations provided by the Canadian goverment.[73]

There are limited and conflicting results when it comes to the role of protein in weight loss diets. The belief that protein supports weight loss and weight maintenance was supported Westerterp-Plantenga MS *et al,*[74] where weight regain was 50% lower when people were consuming a diet consisting of 18% protein when compared to a diet with 15% protein. Despite studying weight regain rather than weight loss, this study indicates even slight increases in dietary protein intake can have long-term effects on weight management. A meta-analysis of 23 trials (n=1063) showed that high-protein diets (diets comprising of anywhere between 15%-32% protein) resulted in increased weight loss, increased fat mass loss, and increased preservation of fat-free mass compared to standard protein diets (0.5-0.82 g/kg/day protein).[75] Two 12-week high-protein diet studies showed that people with overweight exhibited contradictory results of overall weight loss and fat-free mass preservation when compared to a standard western diet.[76,77] Clifton *et al*[76] exposed 215 research participants to either a high-protein (27% protein) or standard protein (16% protein) hypocaloric diet for 12 weeks and reported that both a high-protein or a standard protein hypocaloric 12-week diet were efficacious weight loss strategies; however, the high-protein 12-week hypocaloric diet demonstrated increased weight loss and increased fat loss when compared to the standard protein diet. Conversely, a study by Luscombe

et al[77] reported that after 16 weeks, there was no effect of diet composition on body weight reduction between a high-protein hypocaloric or low-protein hypocaloric diet, suggesting that total energy intake is the main determinant for weight loss, not the macronutrient composition. Unfortunately, Luscombe *et al*[77] did not analyze the changes in body composition so we are unable to determine if protein quantity had any impact on fat-free mass preservation between the groups. The studies completed by Clifton *et al* and Luscombe *et al* involved participants with high triacylglycerol levels and hyperinsulinemia, respectively, and therefore may explain the contradictory results within these studies and represent population variability rather than results generalizable to people with obesity.

High-protein diets have demonstrated limited efficacy for decreasing fat mass and preserving fat-free mass independent of exercise.[75,78] Belobrajdic DP *et al*[79] reported that a 12-week high-protein diet preserved ~1.2 kg more lean mass than a high-carbohydrate diet. Additionally, a study of older adults (mean age: 62 years old) with overweight or obesity demonstrated that a high-protein diet had no effect on fat-free mass change compared to a standard western diet.[80] Though contradictory, these results may be able to be explained by the age of the sample population. Sarcopenia, the decline of skeletal muscle tissue with age,[81] could explain these results and might suggest that high-protein diets may not be as effective at preserving fat-free mass in this population. However, a meta-regression analysis showed that >12-week diets consisting of >1.05 g/kg/day protein were associated with 1.21 kg more retention of fat-free mass compared with diets with protein intake ≤1.05 g/kg/day.[82] This analysis did not include high-protein ketogenic diets and therefore, further research is needed to determine if consuming limited carbohydrates may influence body composition in the context of a high-protein diet.

There is a lack of research examining the efficacy of high-protein intake in the context of ketogenic diets on weight loss, body composition, and metabolism. Generally, most ketogenic diets limit overall protein intake to prevent gluconeogenesis;[37] however, it is possible that high-protein ketogenic diets may be a viable option for more effective weight loss. When males with obesity (n=20) were offered either a high-protein ketogenic or high-protein non-ketogenic diet ad libitum for four weeks, the high-protein ketogenic diet resulted in reduced hunger and overall lower food intake.[55] The reduced food intake in the high protein ketogenic diet suggests a caloric deficit that would eventually result in weight loss; however, this is unable to be verified as weight was not assessed within the study. Digestion of fat and protein takes longer than carbohydrate digestion and therefore, high-protein diets ketogenic diets may reduce hunger due to satiety provided by protein or fat. Unfortunately, the previously mentioned study did not investigate effects of the diets on body composition, an outcome that future studies should consider. Foster *et al*[83] reported that a hypocaloric ketogenic diet comprised of ~20 g/day carbohydrates, and in line with *Dr. Atkin's New Diet Revolution* handbook, resulted in a ~4.2% greater reduction in body weight at 6-months when compared to a conventional hypocaloric diet with ~60% carbohydrate, 25% fat, 15% protein. Even after 3 months, a low-carbohydrate, high-protein diet showed ~4.1% greater reduction in weight. This study presents evidence that high-protein, low-carbohydrate diets have extended efficacy and are not only effective in short-term studies.

Based on the literature, it appears that a ketogenic diet that is high in protein can support the preservation of fat-free mass and result in effective weight loss, but it is unknown whether the type of protein source has any impact. The question remains: in addition to the quantity of protein, does protein source have an impact on weight loss outcomes?

1.4 Plant-based diet

Plant-based diets are becoming a popular dietary restriction option for people within the western hemisphere. Plant-based diets consist either entirely or largely of plant and grain products[84] but these can take a variety of shapes: vegetarian, and vegan diets are all types of diets that minimize or eliminate overall animal-based product and animal-based byproduct intake.[84,85]

The popularity of plant-based diets is increasing. In 2018, about 2.3 million Canadians considered themselves vegetarian and in 2022, 850,000 Canadians classified themselves as vegan.[86] This is a very lucrative area of current research as the plant-based food market in the United States is worth approximately $8 billion with ~60% of households purchasing plant-based foods in 2022.[87] The popularity of plant-based diets in recent history can be attributed to a number of potential health benefits, cultural relevance, environmental benefits, and media attention. Plant-based diets have been shown to be efficacious in cardiac health improvements, type II diabetes mellitus prevention, and hypertension management.[88] Additionally, studies have shown that increased animal-based product intake may be linked to a greater health risk when compared to increased plant-based product intake.[88,89]

Protein is important for muscle development and preservation; however, not all proteins are created equal and animal-based and plant-based proteins are very different. Complete proteins are proteins that have adequate levels of indispensable amino acids to support human growth and development and are commonly derived from animal-based sources.[90] Animal-based proteins contain all of the essential amino acids needed, in adequate quantities, and are more easily digested compared to plant-based proteins which tend to be deficient in certain essential amino acids and may result in direct urea synthesis rather than being used to build or preserve muscle

due to reduced digestibility.[91–93] Essential amino acids can be supplemented from various sources within the diet but deficiency in these crucial cellular building blocks remains an issue for those unaware of the protein differences between difference sources.[94] Whey protein, an animal-based protein, is considered a complete protein[90] while pea protein, a plant-based protein, is an incomplete protein as it contains limited amounts of methionine, one of the nine essential amino acids.[94] Despite being an incomplete protein, pea protein remains a high-quality protein. Lupine protein is also considered an incomplete protein but when combined with pea protein, constitute a complete protein and provide all essential amino acids.[95] Additional plant-based protein sources lack lysine and sulfur-containing amino acids, methionine and cysteine, respectively.[90] Generally, plant-based proteins tend to be low in leucine as well which inhibits anabolic properties as leucine is hypothesized to have a greater stimulatory effect on skeletal muscle protein synthesis than other essential amino acids.[92] Minimizing the intake of animal-based protein could result in essential amino acid deficiency which negatively impacts cellular repair, and growth and development.[84] Interestingly enough, animal-based diets have demonstrated improved muscle growth and preservation of muscle performance compared to plant-based diets[91]; however, for the purposes of this study, we are focusing on body composition in terms of fat-free mass preservation rather than muscle gain or function.

Plant-based diets consist primarily of foods with a low energy density and have been shown to be effective for weight loss. In fact, a low-fat plant-based diet was associated with significant weight loss despite no limits on portion size or overall energy intake.[96] A systematic review from 2020 suggested that plant-based diets may improve weight loss and BMI in people with overweight and obesity but there is a lack of evidence indicating whether source of protein impacts weight loss outcomes across all diet types as most studies have been focused on low-fat

diets compared to standard western diets.[97] Further evidence is needed to identify if there are any differences associated with the use of a plant-based compared to animal-based proteins for weight loss, fat mass loss, fat-free mass preservation and energy use in high-protein ketogenic diets.

2. Rationale

Ketogenic diets appear to be a viable option for quick and effective weight loss for people with obesity. However, the composition of ketogenic diets that may provide increased weight loss, increased fat loss, and reduced fat-free mass loss has not been well-established. Optimally, the ketogenic diet would preserve fat-free mass and enhance energy expenditure to support weight loss maintenance. Recently, plant-based diets have become increasingly popular. While plant-based diets have been shown to increase weight loss in low-fat diets, the protein quality and composition of plant-based protein may impact fat-free mass preservation and have not been yet been investigated. Despite evidence that supports plant-based and ketogenic diets individually for weight loss, there is a lack of scientific evidence examining whether the incorporation of plant-based proteins in a ketogenic diet impacts weight loss and changes in body composition, and substrate oxidation and energy usage. This study aims to fill these gaps by investigating how weight loss via a plant-based vs animal-based high-protein ketogenic diet affects body composition, energy expenditure, and substrate oxidation. Understanding how protein source affects ketogenic diet outcomes will ultimately result in improved recommendations for weight loss approaches to the public and in clinical settings.

3. Objective

The objective of this study is to compare how a 12-week hypocaloric, high-protein ketogenic diet with either plant- or animal-based supplemental protein impacts overall weight loss, body composition, and substrate oxidation in otherwise healthy adults with obesity.

4. Hypothesis

It is hypothesized that, after 12 weeks, adults with obesity consuming a hypocaloric ketogenic diet with plant-based protein supplementation will experience greater weight loss compared to the animal-based protein supplementation group. It is also hypothesized that both groups will achieve comparable fat-free mass preservation, and substrate oxidation adaptations.

5. Materials and Methods

5.1 Participants

Sixty adults (age: 18-60 years of age) male and females with obesity (BMI: ≥30kg/m^2) from the Montreal and surrounding areas were recruited to participate in this study. Participants were recruited through advertising and word-of-mouth. Participants who reported recent past or present use of nicotine and/or marijuana products, following a vegan diet, and any history of chronic disease were excluded from the study. Participants were also excluded if they had any chronic metabolic conditions, renal impairment, uncontrolled hypothyroidism, or use of any other medications that could affect study outcomes. Females who were pregnant, breastfeeding, or were intending to get pregnant during the study period were also excluded. All participants provided written, informed consent prior to participating in the study. The study is approved by the Comité Central d'Éthique de la Recherche (CCER) of Quebec and was registered through clinicaltrials.gov (ID registration: NCT06461806).

5.2 Experimental design

All study visits were completed at Concordia's School of Health in Montreal, Quebec. This study was part of a larger parent study that was a single-blind, longitudinal 12-week intervention study. Prior to the weight loss period, participants underwent a 2-week weight stabilization period where they were weighed twice weekly to ensure their weight did not fluctuate >2% in order to conclude that any weight changes during the study were due to the exposure variable. Participants were randomized to a plant- or animal-based protein group by card choice to consume either a plant-based protein (pea protein/lupine protein mix) or an animal-based protein (whey protein) shake twice daily. The remaining caloric intake was satisfied by an individualized ketogenic meal based on a target 10% weight loss across the 12 weeks. Measurements were collected at two study visits: prior to and at the end of the 12-week weight loss period.

For each study visit, participants arrived after an overnight fast (≥8-hours of water only). Weight and height were measured, and physical activity was assessed using Monica Optional Study of Physical Activity (MOSPA) patient-reported questionnaire which has been validated to measure physical activity over moderate-to-long term follow-up.[98] Body composition was assessed by whole-body DEXA and abdomen CT scans. Resting energy expenditure and substrate oxidation was analyzed by indirect calorimetry.

5.3 Weight stabilization period

During this weight stabilization period, participants were told to conduct daily activities without any intention of gaining or losing weight. Participants were weighed twice weekly during this period and following this stabilization period, participants were invited to attend the

pre-intervention study visit if <2% weight fluctuation was observed. Participants were excluded if ≥2% weight gain or loss was observed.

5.4 Nutritional/weight loss intervention

Throughout the weight-loss period, participants consumed a high-protein ketogenic diet that provided a 20% caloric deficit with a targeted macronutrient distribution of 35% protein, 10% carbohydrate, and 55% fat.[37] Participants were instructed to prepare and consume two high-protein shakes per day, and one ketogenic meal. Each shake provided approximately 500 kcal and comprised of either pea/lupine mix (pea protein: ~45%, lupine protein: ~55%), or whey protein powder (Nutritional Fundamentals of Health [NFH], Quebec, Canada). Regardless of protein source, each shake also contained medium-chain triglyceride (MCT) oil to promote ketosis. Participants were given written and verbal explanations of the individualized meal plan, and a registered dietitian adjusted the meal plans and provided dietary counselling as needed to ensure participant adherence. Participants were instructed to maintain their routine daily physical activity throughout the study duration. Compliance was assessed through biweekly weigh-ins and weekly assessment of ketone body levels through urinary ketone analysis strips (Urinox-10, Diagnox, United States) and self-reporting upon final study visit. Participants were considered compliant if they reported >80% daily dietary compliance, trended towards 10% weight loss throughout the study, and had high levels (8.0-16.0 mmol/L) of ketones in the urine by Week 4. Additional dietary support was provided by the dietitian as needed.

5.5 Body composition

For body composition measurements, participants wore minimal clothing and were asked to remove all items that could impact the measurements (glasses, jewelry, underwear containing wire, etc.). In the rare event there were items that were permanent or unremovable such as piercings, or rings, these were included at each time point. Body composition was obtained while fasted using dual-energy x-ray absorptiometry (DEXA, GE Lunar Prodigy Advance, GE Healthcare, United States).[99] Prior to each scan, the machine was calibrated using a standard calibration block supplied by the manufacturer. For the measurement, participants laid supine.[100] Participants also underwent a single slice CT scan to analyze intrabdominal adipose tissue (GE Discovery PET/CT 690, GE HealthCare, United States).[21] While lying supine on the CT table, sagittal and coronal images were used to landmark Lumbar 2-Lumbar 3 (L2-L3) vertebrae prior to taking a transverse image at L2-L3 vertebrae. This vertebral location is a reliable measurement location to represent abdominal adiposity in people with obesity.[21,101,102] CT images were segmented using Slice-O-Matic 6.0 software (TomoVision, Quebec, Canada) (Figure 3). Inter- and intra-rater reliability was controlled using standard operating procedures. Briefly, <5% coefficient of variation (CV) was achieved between raters and within the same rater across three different sample slices. Subcutaneous and visceral adipose tissue, and skeletal muscle surface area were segmented using a previously published protocol.[103,104] Segmentation was completed through an automated region growing mode according to standard Hounsfield unit (HU) ranges for the respective tissue (adipose tissue: -190 to -30 HU, skeletal muscle: -29 to 150 HU)[105] and following the region growing process, these values were adjusted if necessary following visual verification. Visceral adipose tissue (VAT) was calculated using the following formula[21]:

$$VAT\ (kg) = \frac{Visceral\ Adipose\ Tissue\ (cm^2)}{Total\ Adipose\ Tissue\ (cm^2)} \times Android\ FM\ (kg)$$

Upper body fat mass was calculated using the following formula:

$$Upper\ Body\ FM\ (kg) = Total\ Fat\ Mass\ (kg) - Leg\ Fat\ Mass\ (kg) - \ VAT\ (kg)$$

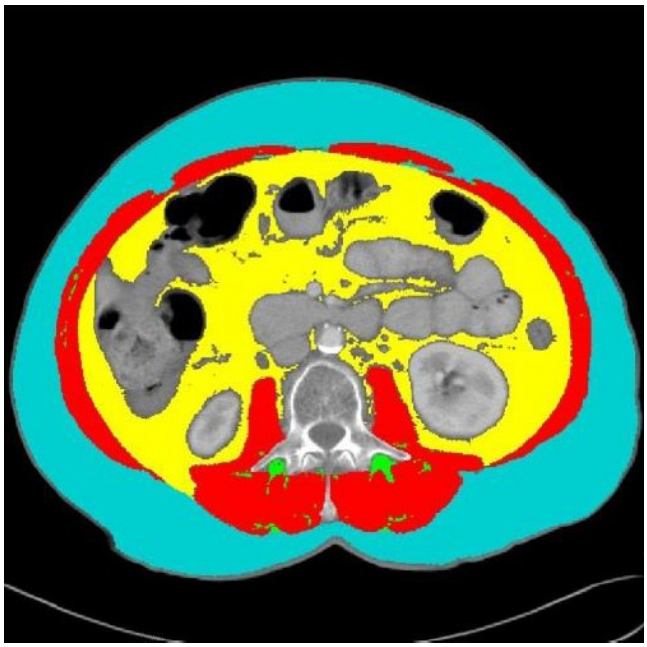

Figure 3: Example of tissue segmentation using SliceOmatic

5.6 Resting energy expenditure and substrate oxidation

Resting energy expenditure and respiratory exchange ratio were measured using the Sable Systems Field Metabolic System (Nevada, USA). Participants rested in a supine position for at least 60-minutes prior to analysis.[106] Indirect calorimetry was used to measure the volume of oxygen uptake (VO_2) and carbon dioxide production (VCO_2) simultaneously with a plastic hood canopy in the supine position. Participants were instructed to remain awake and still and breathe normally for the duration of the 30-minute measurement. The system was set to record the fractional amount of O_2 and CO_2, mixing chamber temperature, water vapour pressure, barometric pressure, subsample flow rate, and mass flow rate in a negative pressure design. The

indirect calorimetry system was calibrated prior to each study visit according to manufacturer protocol (Sable Systems Field Metabolic System, United States). To account for an acclimatization period, the initial 10-minutes of sampling were removed from result calculations.

Carbohydrate oxidation (CHO_{ox}) and lipid oxidation (FAT_{ox}) were assessed using the volume of carbon dioxide and volume of oxygen measured during the indirect calorimetry assessment and calculated using the following formulas[107]:

$$CHO_{ox}(g/min) = 4.59V_{CO_2}(l/min) - 3.2V_{O_2}(l/min)$$

$$FAT_{ox}(g/min) = -1.70V_{CO_2}(l/min) + 1.70V_{O_2}(l/min)$$

5.7 Statistical analysis

Using the Shapiro-Wilk test, normality of all data was confirmed. Independent t-tests were used to assess differences in baseline characteristics and mixed model ANOVA was used to assess differences between groups across timepoints. When data were not comparable at baseline, the baseline value was added as a covariate ofa mixed model ANOVA. When a significant event was observed, t-test post-hoc analysis was completed. Exploratory analysis was completed to analyze the differences between groups when separating the dataset by sex and mixed model ANOVA was used to assess differences between groups across timepoints. All data are expressed as mean ± standard deviation (SD) and results were considered statistically significant when $p<0.05$. All statistical analyses were completed using IBM SPSS software version 28.0.0 (IBM Corp, Armonk, NY, USA).

6. Results

6.1 Participant characteristics

The CONSORT diagram to describe the flow of participants is presented in Figure 4. A total of 60 participants were recruited and 35 participants finished the study and were included in the analyses, 19 in the plant-based group (11 F) and 16 in the animal-based group (8 F). No participants were excluded during the weight stabilization period due to lack of weight loss or gain during this period and all participants reported >6-months of weight stability. Based on self-report, participants who completed the study reported ≥80% dietary compliance throughout the study, defined as the percentage of days during the study participants followed the diet without fluctuating. Further compliance was assessed by measurement of urinary ketone bodies. Of the 34 participants who completed the study, 24 (76.5%) participants had high concentration of ketone bodies (8.0-16.0 mmol/L) in the urine by Week 2, and all 34 participants had high levels of ketone bodies by Week 3. Of the participants who dropped out of the study, ten discontinued the intervention due to dietary inconvenience (n=5 in both groups), 1 from the plant group due to suspected allergy, and 1 from the plant group due to personal reasons. None of the participants in either group who finished the intervention self-reported any serious adverse effects. There were 2 participants in the plant-based and 4 participants in the animal-based group that reported minor gastrointestinal issue that resolved within two weeks of the original complaint.

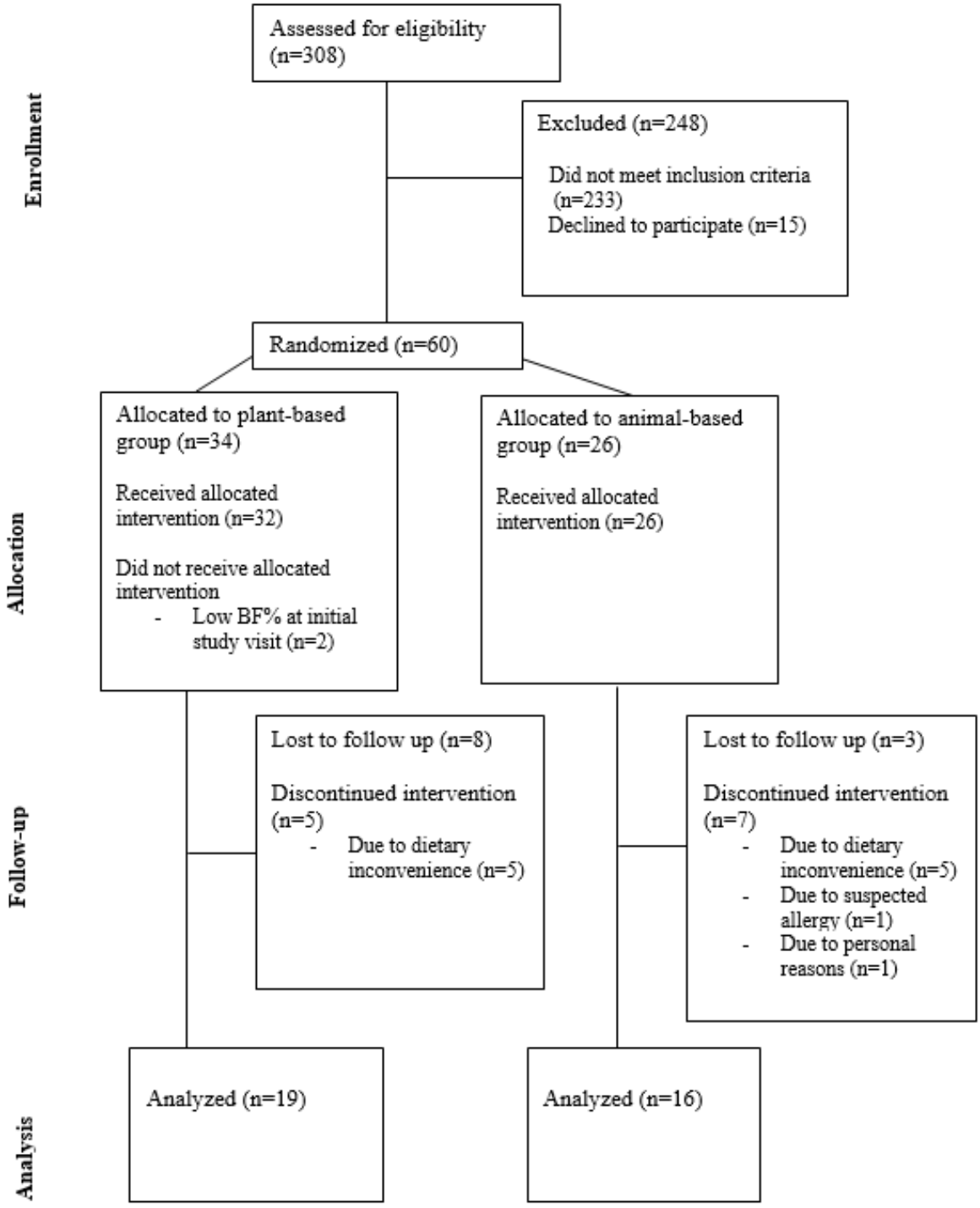

Figure 4. CONSORT participant flow diagram. BF%: body-fat percentage.

Participant characteristics are shown in Table 2. The groups were comparable at baseline for all variables other than respiratory exchange ratio (p=0.025) and lipid oxidation (p=0.05). Participants in the plant-based and animal-based groups were comparable at baseline for physical activity levels (p=0.878).

Table 2: Baseline participant characteristics.

Variables	Plant-based (n=19, 11F)	Animal-based (n=16, 8F)	P-value
Age, years	36.9 (10.2)	41.2 (10.8)	0.235
Height, cm	171.7 (8.6)	169.3 (8.7)	0.402
Weight, kg	107.2 (16.0)	107.9 (18.2)	0.906
BMI, kg/m^2	36.2 (3.8)	37.4 (4.0)	0.368
FM, %	46.3 (6.2)	43.8 (4.7)	0.199
FM, kg	48.1 (10.6)	46.2 (10.3)	0.611
Lean mass, kg	55.8 (9.2)	58.4 (9.8)	0.426
FFM, kg	58.7 (9.6)	61.4 (10.1)	0.420
Upper body FM, kg	31.1 (8.4)	30.8 (7.9)	0.968
Lower body FM, kg	16.2 (3.8)	14.7 (3.1)	0.220
VAT mass†, kg	1.34 (0.99)	1.52 (0.92)	0.477
REE‡, kcal/day	1923 (376)	1968 (293)	0.516
RER‡	0.86 (0.05)	0.83 (0.05)	0.025
CHO_{ox}‡, mg/min	197.5 (60)	161.5 (57)	0.107
FAT_{ox}‡, mg/min	62.3 (25.8)	82.2 (27)	0.050

Note: All values reported are mean (SD). †n=17 for the plant group and n=15 for animal group. ‡n=17 for the plant group and n=13 for the animal group. BMI: body mass index, CHO_{ox}: carbohydrate oxidation; FAT_{ox}: lipid oxidation; FFM: fat-free mass, FM: fat mass; SD: standard deviation; VAT: visceral adipose tissue.

6.2 Overall weight loss and anthropometric measurements

Overall, participants lost an average of 10.0 ± 3.4 kg or 9.5 ± 3.2% of their baseline weight following a 12-week high-protein ketogenic diet and both groups had a significant reduction in weight ($p<0.001$ in both groups). Participants in the plant-based group lost 9.8 ± 3.3 kg or 9.1 ± 2.8% of total body weight ($p<0.001$) (Figure 5). Participants in the animal-based group lost 10.3 ± 3.5 kg or 9.8 ± 3.6% of total body weight ($p<0.001$) (Figure 5). There were no significant differences in total weight loss ($p=0.646$) and percent weight loss between groups ($p=0.517$). No changes (plant-based group: $p=0.187$, animal-based group: $p=0.676$) in physical activity were reported (Appendix 4).

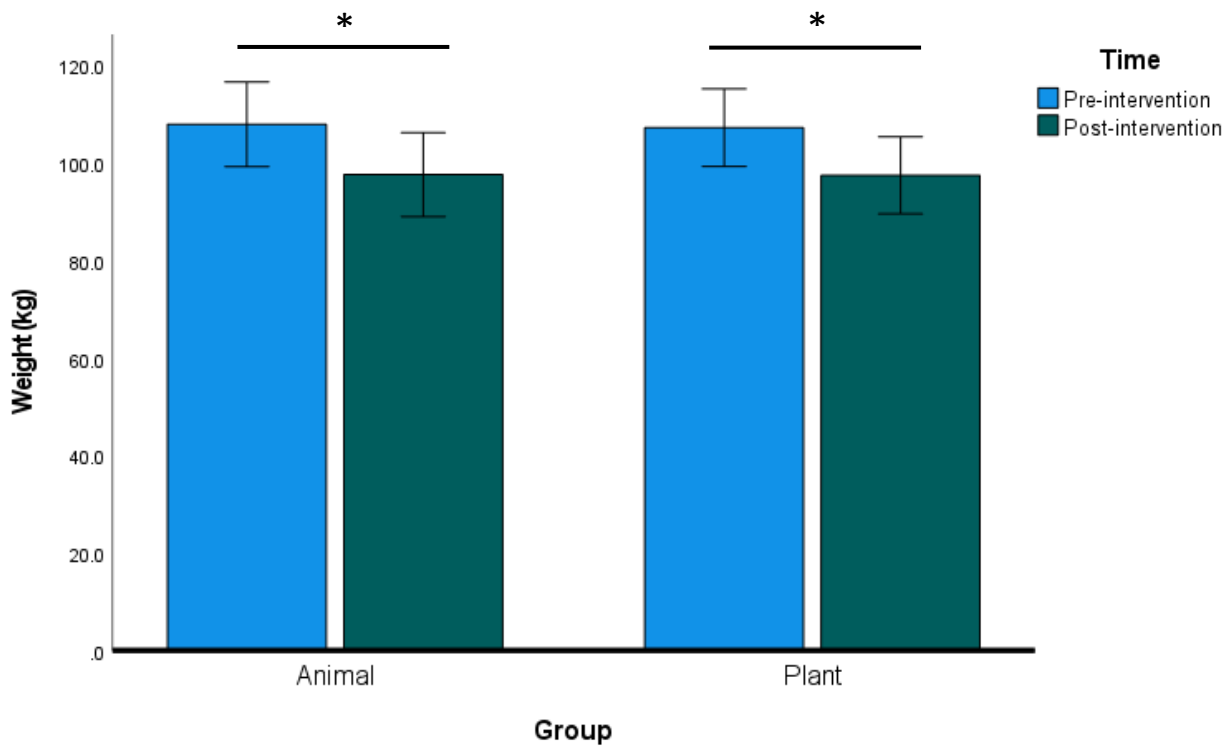

Figure 5: Weight (kg) before and after 12-week dietary weight loss intervention. *p<0.001.

6.3 Body composition

The body composition data are presented in Table 3. After 12 weeks, there was a reduction in percent fat mass, fat mass, lean mass, fat-free mass, upper fat body mass, and lower body fat mass in both groups ($p<0.001$ for all). A reduction (plant-based group: $p=0.024$, animal-based group: 0.031) in visceral adipose tissue mass was also observed. There were no significant differences between groups across time.

Table 3: Body composition variables separated by intervention group.

Variables	Plant-based (n=19, 11F)		Animal-based (n=16, 8F)		P-value$_{time*group}$
	Pre	Post	Pre	Post	
FM, %	46.3 (6.2)	43.2 (7.3)	43.8 (4.7)	40.5 (6.2)	0.691
FM, kg	48.1 (10.6)	40.8 (10.2)	46.2 (10.3)	38.8 (11.5)	0.805
Lean mass, kg	55.8 (9.2)	53.5 (9.4)	58.4 (9.8)	55.8 (9.7)	0.692
FFM, kg	58.7 (9.6)	56.5 (9.7)	61.4 (10.1)	58.8 (10.1)	0.546
Upper body FM†, kg	31.1 (8.4)	26.5 (8.0)	30.8 (7.9)	25.6 (8.9)	0.434
Lower body FM†, kg	16.2 (3.8)	14.2 (3.8)	14.7 (3.1)	12.7 (3.2)	0.879
VAT mass†, kg	1.34 (0.99)	0.94 (0.51)	1.52 (0.92)	1.16 (0.67)	0.863

Note: All values reported are mean (SD) and were recorded through DEXA unless otherwise stated. †n=17 for the plant group and n=15 for animal group. CT: computed topography; DEXA: dual energy x-ray absorptiometry; FFM: fat-free mass; FM: fat mass; SD: standard deviation; VAT: visceral adipose tissue.

6.4 Energy usage and substrate oxidation

Metabolism data separated by group are presented in Table 4. Data was not collected for five participants (n=2 in the plant group; n=3 in the animal group) due to equipment failure at the follow-up visit. There was a significant decrease in respiratory exchange ratio in both groups (plant-based group: $p<0.001$, animal-based group: 0.017) and a trend to suggest a group*time interaction ($p=0.057$). Carbohydrate oxidation (mg/min) was significantly reduced following the intervention in the plant-based group ($p<0.001$) and in the animal-based group though the reduction did not reach statistical significance ($p=0.091$). There was a group*time interaction observed for carbohydrate oxidation ($p=0.037$) indicating the plant-based group showed a greater reduction in carbohydrate oxidation, or a greater shift towards lipid oxidation, compared to the animal-based group. Lipid oxidation (mg/min) was increased ($p<0.001$) but was unchanged ($p=0.271$) in the animal-based group. There was a group*time trend observed ($p=0.057$) for lipid oxidation but did not reach statistical significance. However, when accounting for baseline differences, there were no differences between groups for respiratory exchange ratio, carbohydrate oxidation, and lipid oxidation (group*time $p=0.350$, $p=0.189$, $p=0.287$, respectively). There were no differences observed in resting energy expenditure following the intervention in either group (plant-based group: $p=0.393$, animal-based group: 0.155) nor differences between groups over time ($p=0.607$).

Table 4: Metabolic parameters separated by intervention group.

Variables	Plant-based (n=17, 9F)		Animal-based (n=13, 7F)		P-value$_{time*group}$
	Pre	Post	Pre	Post	
REE, kcal/day	1923 (376)	1873 (328)	1968 (293)	1874 (304)	0.607
RER†	0.86 (0.05)	0.79 (0.04)	0.83 (0.05)	0.79 (0.04)	0.057†
CHO_{ox}‡, mg/min	197.5 (60.0)	106.1 (47.5)	161.5 (57.0)	125.3 (54.2)	0.037‡
FAT_{ox}¶, mg/min	62.3 (25.8)	96.5 (30.4)	82.2 (27.0)	92.2 (31.1)	0.054¶

Note: All values are reported as mean (SD). †p-value between groups at baseline: 0.025 and p-value$_{group*time}$=0.350 when baseline values were accounted for during analysis. ‡p-value$_{group*time}$=0.189 when baseline values were accounted for during analysis. ¶p-value between groups at baseline: 0.05 and p-value$_{group*time}$=0.287. CHO_{ox}: carbohydrate oxidation; FAT_{ox}: lipid oxidation; REE: resting energy expenditure; RER: respiratory exchange ration; SD: standard deviation.

6. 5 Exploratory analyses: Sex differences

We completed exploratory analyses to determine if males and females exposed to different protein sources during a 12-week high-protein ketogenic diet exhibited different results in weight loss, body composition, and substrate oxidation. When separating the dataset by sex, females and males in both groups were comparable at baseline (Appendix 5 and Appendix 6). Females lost 8.7 ± 2.3% of total body weight in the plant-based group and 9.9 ± 3.2% total body weight in the animal-based group. Males lost 9.8 ± 3.5% of total body weight in the plant-based group and 9.8 ± 4.2% of total body weight in the animal-based group. There were no differences in percent weight loss between protein source following the intervention in females (p=0.347) nor males (p=0.98).

There was a significant effect of time for percent fat mass, fat mass, lean mass, fat-free mass, upper body fat mass, and lower body fat mass with post-hoc tests indicating a significant reduction following the intervention in females in both groups. Visceral adipose tissue mass was significantly reduced in females in the animal-based group ($p<0.001$) but was unchanged in the plant-based group ($p=0.287$). No group*time interactions were observed ($p=0.181$).

There was significant effect of time for percent fat mass, fat mass, lean mass, fat-free mass, upper body fat mass, and lower body fat mass with post-hoc test indicating a significant reduction following the intervention in males in both groups. Unlike in females, visceral adipose tissue mass of males in the animal-based group did not change ($p=0.217$). In contract, visceral adipose tissue mass was reduced ($p=0.041$) in males in the plant-based group. No group*time interactions were observed ($p=0.446$).

After the 12-week intervention in females, there was a group*time interaction observed ($p=0.027$) in resting energy expenditure indicating there was a different effect on resting energy expenditure demonstration between groups. Closer examination showed a decrease in respiratory exchange ratio in the plant-based group ($p<0.001$) with no changes observed in the animal-based group ($p=0.086$). Females in the both groups saw a decrease in carbohydrate oxidation (plant-based group: $p=0.002$, animal-based group: 0.044) after the intervention. No differences between groups were observed ($p=0.174$). There was an increase in lipid oxidation in females in the plant-based group ($p=0.003$). There were no differences observed in the animal-based group ($p=0.236$). There were no differences in resting energy expenditure between groups (plant-based group: $p=0.862$, animal-based group: 0.287) nor group*time effects ($p=0.181$) in females (Appendix 7).

Much like in the female dataset, there was a significant decrease in respiratory exchange ratio in the plant-based group ($p<0.001$) in males and a group* time trend observed between groups ($p=0.084$) as there was no significant change observed in the animal-based group ($p=0.126$). Males in the plant-based group saw a significant decrease in carbohydrate oxidation ($p=0.01$) at the end of the study; however, there were no significant differences in the animal-based group ($p=0.62$) nor between groups for carbohydrate oxidation following the intervention ($p=0.137$). There were no significant differences in lipid oxidation in either group following the intervention (plant-based group: $p=0.087$, animal-based group: $p=0.624$). Much like in females, there were no significant differences in resting energy expenditure between groups (plant-based group: $p=0.066$, animal-based group: 0.699) nor between groups over time ($p=0.457$) in males (Appendix 8).

7. Discussion

This study is the first to examine the effect of plant- and animal-based protein supplementation in a ketogenic diet on weight loss, body composition, and substrate oxidation. We found that regardless of protein source supplementation, people following a high-protein ketogenic diet lost ~10% weight loss after 12-weeks. Our results also indicate for the first time that regardless of protein source, people with obesity lost weight and yet maintained resting energy expenditure. Interestingly, we also determined that people who consume plant-based protein supplement may have a larger reduction in respiratory exchange ratio, increase in lipid oxidation, and reduction in carbohydrate oxidation compared to people who consume animal-based protein.

The weight loss obtained by both groups in this study is reflective of other 12-week dietary weight loss interventions.[108] Similar weight loss is likely given similar caloric composition of the two diets. There is only one other study we know of that has examined the effects of protein source in the context of a ketogenic diet. Basciani *et al*[109] compared ketogenic diets consisting of whey protein or vegetable protein (derived from soya, green peas, or cereals) in older adults (males and females, 50-70 y) with obesity over 45 days. In contrast to our findings, this study observed a decrease in body weight in those who consumed the whey protein-based ketogenic diet while those who consumed the vegetable protein did not lose weight. The contradictory results between the current study and Basciani *et al*[109] could be explained by the age of the patient population studied. The current study focused on adults between the ages of 18 and 60 while Basciani *et al*[109] studied older adults and sarcopenia, the decline of skeletal muscle tissue with age,[81] or physical activity levels of the participants could impact the weight loss observed. The ages of the groups studied were not reported and therefore, we are unable to know if these groups were comparable at baseline. Additionally, though daily dietary data were collected for the Basciani study, this was not reported and therefore, the compliance to daily caloric intake could not be assessed.

Interestingly, there were no difference in the changes in body composition between groups. Both the plant-based and animal-based ketogenic diets resulted in decreased fat and fat-free mass. People following a ketogenic diet tend to lose water weight at the beginning of the diet as glycogen stores become depleted due to the low carbohydrate intake. The significant reductions in fat-free mass may be partially reflected in this loss in water weight since muscle is the major site of glycogen storage, and the water content in lean body mass is approximately 73% while the water content in adipose tissue is approximate 10%.[110]

A previous study of adults with obesity following a very-low-carbohydrate ketogenic diet for 4 months saw weight loss mainly attributable to fat mass, and more specifically, visceral fat mass.[17] Our results show that weight loss due to a ketogenic diet comprises of fat-free mass loss, as well as, fat mass. The contradictory results demonstrated between the current study and the previous may be due to method of measurement as the current study used DEXA in conjunction with a single CT slice while the previous study assessed body composition through DEXA, multifrequency bioelectrical impedance analysis, and air displacement plethysmography which may lack accuracy. We also saw a more statistically significant loss in upper body fat mass, lower body fat mass, and fat-free mass than visceral fat mass which may also be due to method of assessment or to the differences in macronutrient composition of the diets. There were also no differences observed between groups for changes in body composition in the current study. As discussed earlier, most research related to protein source manipulation and body composition has been completed in patient populations other than people with obesity and has focused on muscle growth and function rather than fat-free mass preservation. In those studies, animal-based proteins have demonstrated greater fat-free mass growth and muscle strength,[91,109] likely due to the hypothesis that animal-based proteins support lean muscle mass preservation by producing a protein-sparing effect[111,112] but our results suggest there is no fat-free mass preservation component when comparing protein source.

Given the lack of difference in weight loss and body composition changes between the groups, it is not surprising that resting energy expenditure was not different between groups. However, it is surprising that given ~10% weight loss, resting energy expenditure remained constant. A previous weight loss study evaluating total energy expenditure changes by Johannnsen *et al*[113] reported that after >10% weight loss, resting energy expenditure was reduced

by ~15% (baseline: ~2614kcal/d, week 6: ~2258kcal/d).[26] These results may differ from the current study due to the difference in dietary intervention and the rapid weight loss seen in this study which may affect metabolic efficacy. Additionally, a recent review suggested that resting energy expenditure may be reduced following short-term ketogenic diets due to the reduction of gluconeogenesis and increase in lipid oxidation.[114] The previous study's conclusions do not align with our results; however, this may be due to differences in study design, patient population, and intervention details.[114] In contrast to the aforementioned studies, there have been a few studies to support our findings that resting energy expenditure remains unchanged following a ketogenic diet.[70,115] Rubini *et al*[70] and Brehm *et al*[115] both demonstrated ketogenic diets had no effect on resting energy expenditure in females with overweight and obesity when compared to Mediterranean or low-fat diets, respectively. It is important to note that Rubini *et al*[70] had a shorter study duration of 20 days than the current study and therefore out results suggest there are metabolic benefits to ketogenic diets of >3 weeks. Interestingly, Brehm *et al*[115] allowed participants to increase dietary carbohydrate at various time intervals within their 4-month study, dependent on urinary ketone strip analyses to assess ketosis. Thus, the macronutrient composition of Brehm's dietary intervention varied between participants and muddies the ability to draw conclusions. Our results confirm the metabolic effects of a ketogenic diet over a longer duration (12 weeks), in both males and females

When baseline differences were taken into consideration, neither the plant-based nor the animal-based group significantly impacted carbohydrate oxidation or lipid oxidation of participants following the 12-week intervention. Substrate oxidation is influenced by multiple factors such as exercise, sex, and dietary intake.[116] Our study controlled for physical activity within the study period and both groups were well-balanced for sex. We also controlled for

dietary intake through the provision of shakes within the weight-loss period. However, given that the dietary intervention started after baseline measurements, the carbohydrate oxidation was lower and lipid oxidation was higher in the animal- compared to the plant-based group. Unfortunately, dietary data were not collected at baseline and thus, we cannot be sure whether difference in macronutrient composition of the diet was the reason for the difference in substrate oxidation between groups at baseline. However, given the baseline level of carbohydrate and lipid oxidation in the animal-based group was potentially reflective of a diet that was lower in carbohydrates and higher in fat, the implementation of a ketogenic diet that was similar in composition was unlikely to have an effect in further changing macronutrient oxidation in this group.

This study had many strengths and limitations. This study was a randomized, single-blinded trial where participants were not informed of which protein source they received. This results in reduced participant-related bias within the study. Generalizability to certain populations is limited since we only included healthy participants based on inclusion and exclusion criteria including only females that were premenopausal. However, the reasons we set the criteria for participant inclusions as we did was because variables such as menopausal status act as a potential confounder and would limit the interpretation of our findings. Our study did, however, include a wide age range of both males and females, which allows the results to be largely generalizable. Physical activity can have a large impact on weight loss and body composition.[117] By measuring physical activity through a self-reported questionnaire, we were able to assess whether this variable would impact the weight loss seen. As physical activity remained unchanged, this variable could reasonably be removed as a confounding variable. We did not use daily dietary tracking for this study and therefore, we cannot confirm the daily

dietary composition for all participants. However, participants self-reported a high level of dietary compliance, which we were further able to confirm that all participants who completed the study were in a ketogenic state. The gold standard measurement to assess ketone bodies is mass spectrometry, but this method is expensive and requires technical expertise that we did not presently have.[118] Ketone bodies can also be measured through the blood which is also costly and burdens participants further due to having to undergo weekly needle sticks. Given that we did not need a precise quantification of ketone concentrations and only wanted to determine that our participants were in ketosis, we found urinary strips to be an inexpensive and viable option. Though urinary analysis may provide less precise results of overall ketone levels within the body, they can still be used to verify ketosis and measure adherence.[43] Additionally, by providing participants with individualized meal plans, we were able to ensure all participants were prescribed the appropriate caloric deficit depending on their physical activity levels and dietary preferences; however, participants did not conduct daily dietary tracking. Notably, by having our research team educate participants about macronutrients, the mechanism of ketosis, and how to read nutritional information labels, we were able to provide participants with autonomy to make better informed decisions about their intake after the study which is a strength that may not be scientifically analyzed but is important to mention when the goal of research is to better inform people impacted by obesity. Finally, despite DEXA being the gold standard to measure body composition, weight and body composition can fluctuate based on the female menstrual cycle[119] and we did not take this into account during our study.

8. Conclusion/Significance

Our results indicate that regardless of protein source, people who followed a 12-week high-protein ketogenic diet saw significant weight loss, fat loss, and fat-free mass loss. The findings of this study begin to shed light on the impacts of protein source on weight loss, body composition, and substrate oxidation following a ketogenic diet and this may be interesting in the context of obesity management options. Future research is needed to determine whether sex effects following a high-protein ketogenic diet may be linked to protein source. This study serves as a base for future studies over longer durations, in different sample populations, and within larger sample sizes to continue to examine how protein source may impact people with obesity.

9. Funding

Funding for this study was provided by NFH, Mitacs Accelerate Grant, and a PERFORM Centre Multidisciplinary Grant.

10. Appendix

Appendix 1: Ethic approved study protocol

Titre du projet de recherche - Protocol title.

Ketogenic Diets: Does Protein Source Affect Lipid Metabolism and Dietary Safety?

Numéro de protocole, si applicable - Protocol identifying number, if applicable.

Date - Date.

October 5, 2023

Chercheur responsable du projet de recherche - Principal investigator.

Sylvia Santosa and Dajana Vuckovic

Co-chercheur - Co-investigator.

Jose Morais

Organisme subventionnaire - Funding agency.

PERFORM Multidisciplinary Grant and MITACS

Commanditaire - Sponsor.

Nutritional Fundamentals for Health

1. Résumé - Background information.

Weight loss is the primary strategy to decrease the risks associated with obesity. Though there are several ways weight loss can be achieved, ketogenic diets have experienced consistent popularity. Ketogenic diets can have different macronutrient compositions but the one thing they all have in common is that they are low carbohydrate, high fat, and low-high protein. In consuming such a diet, the body increases fat breakdown resulting in the production of ketone bodies. Indeed, in the short term, such diets have been shown to result in effective weight loss. However, the long-term safety of these diets is contentious as some authors showed that low carbohydrate-high protein diets were associated with increasing incidence of CVD, whereas others showed high protein intake to be associated with decreased risk. The variability in these findings may stem from the type of protein source consumed. Animal protein consumption is associated with higher saturated fat. More recently, higher total, animal protein, and animal:plant protein intake has been associated with increased mortality. Indeed, plant-based (vegan) diets have been consistently gaining in popularity with searches for the word “vegan” consistently showing peak popularity in global Google trend data since 2017. Common health beliefs surrounding plant-based diets include better weight control, lower risk of CVD, and prevention of diabetes. However, evidence supporting the advantages of plant-based diets is lacking and more research is needed to determine the validity of these claims. Similarly, the influence of low

carbohydrate, high protein diets on lipid profiles also showed contradictory results. For example, circulatory total triglycerides (TGs) decreased, did not change, or increased, possibly indicating the presence of confounding factors such as protein source. Furthermore, total lipid measurements are insufficient to monitor and understand health effects of a given weight loss diet.

2. Objectifs et but de l'essai - Trial objectives and purpose.

The objective of this study is to compare how a ketogenic weight loss diet, supplemented with either animal- or plant-based protein affects: (i) metabolic markers (adipokine and cytokine) in adipose tissue and blood, (ii) adipose tissue immune cell profiles, and (iii) adipocyte characteristics and their interaction with other cells (e.g. immune cells and muscle cells). To better determine how tissue characteristics affect the whole body level, the relationships between adipose tissue characteristics and blood lipid and inflammatory markers will also be examined.

Hypothèses - Hypotheses.

We hypothesize that there will be more favourable outcomes (ie more weight loss, better lipid and inflammatory profiles, etc) after weight loss with the plant-based than with the animal-based ketogenic protein diet.

Conception de l'essai - Trial design.

Participants: Participants will be randomized to two weight loss groups. One group will receive a hypocaloric high protein, ketogenic diet containing either a plant-based (lupine/pea) protein supplement (PL), or an animal-based (whey) protein supplement (AN).

Protocol: All participants will undergo two study visits, before and after a 12-week weight loss period. Prior to each study visit, participants will undergo a 2-week weight stabilization period after which 24 h urine will be collected for measurement of protein oxidation. For each study visit, participants will arrive in the morning fasted and will undergo a baseline indirect calorimetry measurement, a needle- aspirated biopsy of the abdominal and thigh subcutaneous adipose tissue, and blood draw. Whole body dual x-ray absorptiometry (DXA) scan will be conducted for measurement of regional body composition and a CT scan of the abdominal area (L2-L3) will be conducted for quantification of visceral fat.

Description de la population à étudier - Description of the population to be studied.

1) Taille de l'échantillon - Sample size.

30 participants will be recruited per group for a total of 60 participants

2) Sélection des participants - Participants selection criteria.

Participants will be recruited from the Montreal and surrounding area.

3) Critère d'inclusion - Inclusion criteria.

18 - 60 yo with obesity (BMI>30 kg/m2). All participants will be generally healthy with no history of chronic disease.

4) Critère d'exclusion - Exclusion criteria

Any chronic metabolic conditions (e.g. CVD), renal impairment (creatinine clearance <60 mL/min), uncontrolled hypothyroidism, past (<6 year) or present use of nicotine products, or use of any other medications that may affect study outcomes (e.g. anti-depressants). Participants who have had several CT scans in the course of the year will also be excluded.

Procédure et lieux de recrutement - Recrutement procedure.

Participants will be recruited from the Montreal area via advertising (flyers, social media, etc) and word of mouth. Past participants that have consented to be contacted for future studies will also be phoned.

5) Retrait des participants - Participants withdrawal criteria.

Participants will be withdrawn from the study if they do not follow the study protocol or unable to perform the study visits.

Procédure de l'essai - Study procedure.

Methods:

Weight Stabilization Period: All participants will be weight stable for 2 weeks prior to the measurement. During this period, participants will be weighed twice weekly.

Weight loss protocol: In both groups, weight loss will be accomplished with a ketogenic diet consisting of a 20% caloric deficit with a macronutrient distribution of at least 1.8 g/kg/d protein based on fat free mass, 10% carbohydrate, and the remaining fat (~55% based on a 2000 kcal diet). This level of protein was chosen as previous research by Dr. Morais has shown this to be a level sufficient to maintain muscle mass. The PL and AN groups will receive a plant- or animal-based protein shake (or a recipe on how to make one depending on the circumstances), respectively, twice daily. A dietitian will instruct participants on what to eat for the remaining meals using an exchange system.

Interview and Health Assessment. Participants will be asked to arrive after an overnight fast (no food for at least 8 hours before visit; water only). Participants will be interviewed about their medical history, and diet. **Participants will also be asked to provide pictures from childhood (around the age of puberty) to confirm obesity onset.** The health assessment will include a measurement of blood pressure, heart rate, weight and height.

The following will take place before and after the weight loss protocol, towards the end of the stabilization periods.

Questionnaires. Participants will be asked to complete questionnaires about their health, eating behaviour, and quality of life.

Blood draws: Blood will be drawn at the PERFORM Centre by a qualified individual (e.g. nurse). A finger prick blood draw will also be collected (optional) to determine whether measurements can be accurately done with a finger prick vs. a venous blood draw.

Body Composition Assessment by Whole body DEXA scan. Participants will be asked to remove all jewellery and to lie supine on the instrument table as the arm of the machine passes over them. The scan should take less than 15 min.

Body Composition Assessment by Single-slice CT scan. Female participants will have a urine pregnancy test prior to the scan to ensure they are not pregnant. Participants will be asked to change into scrubs. They will be asked to lie supine on the CT table that will take them into the machine. Sagittal and coronal images will be taken for the purpose of landmarking L2-3. A transverse image will then be taken at L2-3. The procedure should take approximately 5 – 10 minutes to complete.

Circumference Measurements. The circumferences of waist and hip will be measured with a measuring tape according to WHO standards.

Energy Expenditure Assessment by Indirect Calorimetry. After an overnight fast of at least 8 hours, participants will rest comfortably for 2 hours before the test. Participants will breathe normally under a clear, plastic canopy for around 30 minutes while lying down to measure energy expenditure.

Biopsies. Participants will be asked to arrive after an overnight fast. They will be asked to not consume caffeine or alcohol, or to engage in any strenuous exercise for at least 24 hours prior to this visit.

o Fat. Fat biopsies will be conducted in the femoral and abdominal areas. Approximately 2-3 g of subcutaneous fat will be collected at each site by needle aspiration technique (like a mini liposuction). On average the procedure takes approximately 30 minutes. Surgical sterile technique is implemented during this procedure, including the use of scrubs, a mask, eye protection and sterile gloves. At each site a fan-like area will be numbed with a 1:2 lidocaine: lactated ringer's solution. A small punch incision will be made with a scalpel of about 0.5 cm long. A 13-gauge fat biopsy needle connected to a syringe will then be inserted through the incision subcutaneously. The fat will then be aspirated by suction. Once the procedure is complete, the area will be cleaned and treated with benzoine. The incision will be closed with steri-strips and a bandage. Ice will be applied for 20 minutes, and participants will be provided with post-biopsy care instructions.

Instruments de mesures, tâches - Data acquisition.

Urine: Urine collected over 24 h will be analyzed for nitrogen content.

Blood and tissue analyses: We will measure insulin, inflammatory cytokines (e.g. IL6, TNFα), and adipokines (e.g. leptin, adiponectin) in blood via Luminex or ELISA. Untargeted lipidomics will be performed which will include in-depth coverage of glycerolipids, phospholipids and sphingolipids. This analysis will be further supplemented by oxylipin analysis of lipid mediators with known pro- and anti-inflammatory roles. Fat cell size will be determined after adipose tissue collagenase digestion. Flow cytometry will be used to identify and quantify the species of immune cells present in the SVF and blood. Adipose tissue and muscle cellular characteristics (e.g adipogenesis and myogenesis) will also be examined.

Mesure de sécurité - Safety considerations.

The following risks are included in the consent form and will also be discussed with the participants:

- Blood draws: Risk of discomfort resulting from the insertion of the needle, as well as a risk of pain, bruising or infection (rare). The total blood drawn could be as much as 150 ml. Blood should not be donated for 8 weeks following the end of the last visit.

- Fat biopsies: The most common risks of fat biopsies include pain, a small dent or bump and bruising at the site where the fat sample is taken. Bruising may last one to two weeks. Less common risks of fat biopsies may include bleeding, infection, a small scar, and numbness of the skin around the site of the biopsy. The chance of these risks is less than 1% (1 in 100). There is also a chance of an allergic reaction to the lidocaine used for local anesthesia. Care will be taken to reduce the chance of these risks. Participants should not take aspirin 3 days before or after the study. Participants will be advised not to participate in any vigorous activities for three days before and after the biopsies. Participants should avoid exposure to water for prolonged periods, i.e. hot tubs or swimming, for 5 days after the study. Normal daily activities will not be affected.

Radiation risk: Participants will be exposed to a small amount of radiation from the DEXA and single-slice CT scan. The amount of radiation has a low risk of harmful effects. Radiation exposure from the DEXA scan is like the amount one would receive from sun exposure on a sunny day (1/10th that of a chest x-ray). The amount of radiation exposure from the CT scan is less than that of one return transatlantic plane flight (about 2-3 chest x-rays). The radiation does not remain in the body after the scan.

Indirect Calorimetry. There is a slight risk of discomfort and hyperventilation from claustrophobia when under the clear, plastic hood. Staff will be present, and the hood is easily removable.

We do not foresee any major risk or long-term effects from any of the procedures to be performed as these procedures have been performed safely in humans for over 30 years. Members of the research team will be certified in First Aid and CPR level C+ and a registered nurse will be on hand during the blood draws and biopsies. Our team will help the participant by either contacting or directing the participant to proper medical help should there be adverse side effects from any procedure.

Blood draws: A certified person will be on hand to perform the procedure and proper aseptic technique will be employed. The blood draws will take place where proper facilities are in place should untoward reactions (fainting, etc) occur. Should volunteers experience unusual pain or discomfort from the venipuncture site, they will be encouraged to contact study staff or a health care professional.

Fat biopsies: The risk of pain from lidocaine administration and bruising from adipose tissue biopsies is high, and the volunteers are given this information. The biopsies will be performed by a MD with assistance from an RN using sterile technique. Each volunteer is informed of the signs and symptoms of infection and is told to call the study staff or health care professional immediately should any of them occur. A record is kept of all adverse reactions to biopsies. If an increase in the number of adverse reactions relative to historical controls is observed during a particular study a careful assessment of technique and approaches is made. The protocol might then be modified or additional instruction or supervision of the individual performing the biopsies could be undertaken. The subsequent results are then audited to determine how effective the intervention has been. As aspirin reduces coagulation, participants will be asked not to take aspirin 3 days before or after the study. However, if they choose to continue to take aspirin, they will not be excluded from the study. For administration of anesthetics, care is taken to administer the minimum amount of lidocaine necessary to achieve adequate anesthesia. We are careful to limit the amount administered to much less than that which could cause cardiac arrhythmias. To minimize the risk of infection, participants will be advised not to participate in any vigorous activities for three days before and after the study, to avoid exposure to water for prolonged periods, i.e. hot tubs or swimming, for 5 days after study. Proper care of the incision site and bandage will be explained to the subject, and written instructions will be given to the subject in the form of a pamphlet. Normal daily activities of the subjects will not be affected.

Analyses statistiques - Data management and statistical analysis.

All data will be tested for normality using a Shapiro-Wilks test. Data that are not normal will be transformed. Difference between timepoints will be measured via a mixed model. Relationships between variables will be examined using a Pearson or Spearman correlation, depending on data distribution. Mass spectrometry data will be processed using LipidSearch software to identify the lipids followed by multivariate statistical analysis using Umetrics Simca software and pathway analysis using LipidMaps Biopan.

Description des mesures pour réduire ou éviter les biais - Description of the measures taken to minimize or avoid bias.

Randomization into treatment diets will minimize bias. Also, all data collection will be performed according to standard operating procedures to minimize or avoid bias.

Contrôle et assurance de la qualité - Quality control and quality assurance procedures.

As all the techniques used here have been used before, Drs. Santosa and Vuckovic have data quality benchmarks against which to compare the collected data. Calibration of equipment ensures consistent measures accross different study days and participants. Use of reference standards where applicable and duplicate measures helps ensure data quality.

Éthique - Ethics.

Ethics will be obtained from the CCER.

Traitement des données et tenue des dossiers - Data handling and record keeping.

All non-coded data will be kept in a secure location in Drs. Vukovic's or Santosa's lab and only the principal investigators and the students involved directly in the project will be able to access it. All data collected will be entered onto spreadsheets and stored on a computer by participant code which will be backed up regularly. Short- and long-term storage will be on office and laboratory computers in the Science Pavilion and the PERFORM Centre of Concordia University. The offices and laboratory will be locked when unattended. The principal investigator will have control over who has access to these areas. A password will be required to access the computer directly.

With the permission of the participant, the data and samples will become part of the data and tissue bank for the metabolism, obesity, nutrition lab, which has previously been approved. Data will be processed using computing resources at Concordia and on computation ervices such as Calcul Quebec and Compute Canada.

All data will be kept for a period of 50 years after the end of the project.

Financement et assurance - Financing and insurance.

This study will be financed through an internal PERFORM Centre Multidisciplinary grant obtained by the co-PIs. The project is also funded by a MITACS Accelerate grant in partnership with Nutritional Fundamentals for Health, Inc. (NFH). NFH will also provide in-kind protein and MCT supplements for the entire project.

Règles en matière de publication - Publication policy.

Manuscripts steming from this research will be submitted to peer-reviewed journals.

Retombées - Anticipated results.

Obesity has been associated with a host of adverse health conditions, such as insulin resistance, systemic inflammation and is a major cardiovascular disease risk factor. In 2015, Public Health Agency of Canada stated that 64% of Canadians over 18 are overweight or obese. With the prominent role that obesity plays in origins/progression of metabolic disease, effective and safe means of weight loss are increasingly more important. Ketogenic diets that are rich in protein and low in carbohydrates have earned attention due to their proven potential for weight reduction. However, despite their apparent success and popularity for weight loss, multiple animal studies and recent epidemiological evidence in large-scale human studies have shown possible correlation between these weight loss diets and higher mortality and CVD, with animal-based protein sources found to increase the health risks. However, the underlying biochemical pathways and possible biomarkers of these processes are not currently known. Our study will address this important knowledge gap by determining how circulatory and tissue lipid profiles and inflammation may be impacted by plant-derived versus animal-derived protein dietary intake during weight loss intervention. In addition, this study will provide us with first evidence as to whether our newly discovered lipid biomarkers are dysregulated in individuals with obesity, both before and after diet intervention.

Échéancier - Timeline.

We expect study recruitment and visits to take place over 1 y and data acquisition to take place over 2 y. Knowledge translation will take place over 1 y. There are currently no plans to follow participants beyond their participation in the protocol.

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Appendix 2: Ethics approved informed consent form

Research project title:	**Ketogenic Diets: Does Protein Source Affect Lipid Metabolism and Dietary Safety?**
Principal investigators of the project:	Sylvia Santosa, Ph. D. Dajana Vuckovic, Ph. D.
Co-investigator:	Jose Morais, M.D.
Funding agency:	PERFORM Multidisciplinary Grant, Nutritional Fundamentals for Health, Inc., MITACS

1. Introduction.

We are inviting you to participate in a research project. However, before you agree to participate in this project and sign this information and consent form, please take the time to read, understand and carefully consider the following information.

This form may contain words that you do not understand. Please ask the principal investigator of this project or a member of his research staff all the questions you consider useful and ask them to explain to you any word or information that is not clear.

2. Nature and objectives of the research project.

Weight loss is the primary strategy to decrease the risks associated with obesity. A popular way that people lose weight is through a low carbohydrate - high protein and fat (ketogenic) diets. Usually, ketogenic diets include animal-based protein. Recently, plant-based proteins sources have increased in popularity.

A ketogenic diet is one where we don't eat enough sugars from carbohydrates (sugars) resulting in our bodies relying more on fat for energy. This type of diet has been popular for example, in weight loss, treatment of cancer, and seizures.

The aim of this study is to determine whether the type of protein, animal-based vs plant-based, consumed in a ketogenic diet affects how much weight is lost, dietary safety and how we use fat in our bodies.

This research project also aims to contribute to the MON lab sample and tissue bank, to encourage research, innovation, cohesion, and the visibility of interest in obesity nutrition and metabolic disease. By agreeing to participate in this project, you agree that your research data will be added to the MON lab blood and tissue bank.

To carry out this research project, we intend to recruit 60 participants, men and women, aged 18 to 60 years old.

3. How the research project will proceed.

Please note that enrolling in this study is a major commitment and will require much of your time and energy.

3.1 Location of the research project, duration, and number of visits.

This research project will be held at Concordia University's PERFORM Center and your participation in this research project will last 4 months and will consist of several study visits described below.

3.2 Distribution of groups

In participating in this study, you will be assigned in one of the following 2 groups:

a. Animal-based ketogenic protein diet
b. Plant-based ketogenic protein diet

Note that the possibility to be assigned to either one of these diets is 50/50 (i.e., like a flip of a coin). You will not be able to choose which dietary group you will be in.

3.3 Nature of your participation

3.3.1 Stage one: Pre-intervention

3.3.1.1 Screening and Information Visit:

The screening process will be done over the phone and will last approximately 30 minutes.

The screening process will determine whether you are eligible for the study and will provide information for designing your weight loss protocol.

There might also be a group information session on zoom or in person at Concordia's PERFORM Centre, which you can choose to attend. The offering of this session would depend on interest and need. Should this information session be offered, it would take place over 1 hour. During this session, you will meet the members of the research staff to discuss the study and have your questions answered.

Four your participation, you will be asked to provide pictures from childhood, around the age of puberty to confirm obesity onset.

3.3.1.2 Interview and Health Assessment Visit:

This visit will last approximately 1.5 hours and will take place at the PERFORM Centre.

You will be asked to arrive after an overnight fast (no food for at least 8 hours before visit; water only). You will be interviewed about your medical history, weight history and diet. The health assessment will include a fasted blood draw (approximately 60CC), urine collection, and measurement of blood pressure, heartrate, weight, and height.

If there are any subclinical markers, based on clinical blood markers for example high blood pressure that may result in study exclusion, you will be informed.

3.3.1.3 Pre-Weight Loss Stabilization Periods (~4 visits over 2 Weeks)

The aim of the pre-weight loss stabilization period is to ensure your body weight is stable for 2 weeks, to prepare you for the weight loss period, and to take baseline measurements prior to commencing weight loss.

During this stabilization period, there will be:

- ~3 Weight-In Visits that will last 5-10 minutes to take body weight.
- One Nutrition Visit lasting ~1h.
- One ½ day Assessment Visit.

You will be instructed to follow your usual diet to maintain a stable weight for 2 weeks both before and after the weight loss protocol. You will also record your food intake for 3 days (2 weekdays and 1 weekend day).

- **3 Weight-In Visits (3 Visits x ~5-10 min)**

To ensure that you are weight stable, you will be weighed after fasting for at least 8 hours at least twice a week.

- **One Nutrition Visits (~1h)**

During this visit, you will meet with a member of the study team/dietician who will do a preliminary

evaluation and discuss the ketogenic diet, which will require you to prepare and consume a plant or animal-based protein powder smoothie for breakfast and lunch. You will be instructed on how to choose dinner options.

Prior to the weight loss intervention, you will be provided with the protein powder and some other smoothie ingredients that are not as readily found in the grocery store (e.g., medium chain triglyceride (MCT) oil, stevia, flavourings, etc.).

You will be required to purchase or have other ingredients on hand that are more readily found at the grocery store depending on the flavour of the smoothie that you prefer (e.g., instant coffee, cocoa powder, frozen berries, etc.).

- **One ½ day Assessment Visit (~1/2 day)**

The following assessments will be conducted during this visit:

- ✓ **Questionnaires.**
  You will be asked to complete questionnaires about your health, eating behaviour, and quality of life.

- ✓ **Body Composition Assessment by Dual Energy X-ray Absorptiometry (DEXA).**
  DEXA is a radiographic technique using very low doses of radiation to measure the tissue density of the human body. This includes bone mineral density calculations or comparisons of soft versus lean tissue percentages.
  To perform a DEXA scan, you will be lying on the bed and will have to remain still. A moving arm will then traverse the region of interest (i.e. lower back or entire body) without ever touching it. The longest protocol is about 15 minutes for a whole-body scan depending on body habitus and height. The device is relatively silent, and you will not experience any more discomfort than you normally would lying flat on your back. There is usually no special preparation although some projects may add fasting and hydration for increased precision.

- ✓ **Body Composition Assessment by Computed Tomography (CT scan).**
  During this test, you will lie on a table that will be passed through a large, open circular tube. The CT scan machine will take pictures of your abdomen. The scan takes approximately 5-10 minutes to complete.

- ✓ **Circumference Measurements.**
  The circumferences of different parts of your body (e.g. waist, hip, chest, arm, thigh) will be measured with a measuring tape.

- ✓ **Energy Expenditure Assessment by Indirect Calorimetry.**
  You will arrive after an overnight fast of at least 8 hours and will rest comfortably for 2 hours before the test. You will breathe normally under a clear, plastic canopy for around 30 minutes while lying down. This will allow us to measure the rate at which your body burns calories (your energy expenditure). Information from this assessment will help us determine how many calories you need to maintain weight or to lose weight at a certain rate.

- ✓ **Blood Draw.**
  Your blood will be drawn after an overnight fast of at least 8 hours. We ask that you avoid taking any pain medication (e.g. tylenol, motrin, aspirin, etc) in the 24 h prior to the blood draw.

  In addition to a venous blood draw, a finger prick blood draw will also be taken. The finger prick blood draw is optional.

  The blood drawn will help us determine whether a finger prick can be used to measure some of our outcomes instead of a venous blood draw.

- ✓ **Biopsies.**
  The fat biopsies will be done by a physician.

You will be asked to arrive after an overnight fast (water only after midnight). You will also be asked to not consume caffeine or alcohol for at least 24 hours prior to this visit. Strenuous exercise should also be avoided for at least 24 hours before and at least 5 days after the procedures.

- **Fat.** A sample will be taken from the fat in your stomach and thigh region. The procedure involves cleaning the skin to remove any germs, numbing the skin by injecting a local anesthesia (to freeze the area) with a thin needle, making a small nick incision and then removing the fat just below the skin.
  To remove the fat, a small hollow tube attached to a syringe will be used to suction out a small amount of fat tissue underneath your skin. The procedure will not require stitches, as the incisions are small; the physician will simply place sterile tape to close the incision.

  After the biopsies are done, post-biopsy care will be explained, and you will be provided with written instructions.

3.3.2 Stage two: Intervention

Immediately after the Pre-Weight Loss Stabilization Period, you will start the weight loss intervention.

During this period, you will lose weight by diet. You will be instructed on how to decrease the number of calories you eat in your diet by 20% through the ketogenic diet (high protein/fat, low carbohydrate).

You will be provided with plant-based or animal-based protein sources, as well as a source of fat (medium chain triglyceride) to be incorporated as part of your diet.

You will also be asked to keep a paper or electronic-based record of your diet throughout the weight loss period.

Monthly educational or support group sessions may also be conducted.

- **Nutrition Follow-Ups (~2 Visits of ~30min each)**

Depending on your progress and level of comfort with the intervention, you will have up to 2 Nutrition Follow-Ups. These follow-ups may help to address any questions or concerns you may have with the ketogenic diet, and/or to evaluate the incorporation or compliance to the ketogenic diet.

- **Weekly Check-in Visits (1x/week over 12 weeks for 15 min each, ~12 visits)**

Weekly check-ins will take place at Concordia's PERFORM centre and will last approximately 15 minutes.

Your weight will be measured, and your weight loss progress will be monitored on a weekly basis.

3.3.3 Stage three: Post-intervention

The purpose of these visits is to assess the changes that occurred as a result of the weight loss via the plant-/animal-based ketogenic diets.

You will be required to stop losing weight and remain weight stable for 2 weeks during this period.

With the exception of the Nutrition Visit, all of the activities performed during the Pre-Weight Stabilization Period will be repeated during this 2-week Post-Weight Loss Stabilization Period, including the Weigh-In Visits and all of the one ½ day assessment visit.

4. Ownership of Research Data.

All of your research data will be placed in the MON lab Data Bank, which has been established for research purposes to encourage research, innovation, cohesion and visibility of research on obesity nutrition and metabolic disease.

Please note that your data will remain your own at all times. Under no circumstances will your data be sold. Your data will only be used for research purposes.

Please also note that the researcher responsible for this project will act as a trustee for the data set, which will be placed in the MON lab Data Bank established for research purposes. That is, in accordance with the Data Bank Management Framework, the lead researcher will be responsible for the development, maintenance, custody, security, access and transfer of the data.

5. Incidental finding.

Although they do not undergo a formal medical evaluation, the results of all tests, tasks and procedures that you have to do during your participation in this project can highlight problems previously unknown, called incidental findings. Therefore, in the presence of a particular feature, the researcher responsible for the project will call you to do a follow-up.

6. Advantages associated with the research project.

You may benefit personally from participating in this research project, but we cannot guarantee it. However, the results that are obtained will contribute to the advancement of scientific knowledge in this field of research.

7. Risks associated with the research project.

7.1 Blood Draws.

There is a risk of discomfort, pain, fainting, bruising or infection (rare) from the blood draw. The amount of blood drawn at each time point will vary. Total blood drawn throughout the study will not exceed ~150 mL. It is recommended that you avoid taking aspirin 24 hours before and after the blood draws, and that you don't donate blood for up to 8 weeks following your participation in the study.

7.2 Fat Biopsies

Please note that the fat biopsies will be done by a physician.

The most common risks of fat biopsies include pain, a small dent or bump and bruising at the site where the sample was taken. The bruising may last one to two weeks. Less common risks of biopsies include bleeding, infection, a small scar, and numbness of the skin around the biopsy site. The chance of these risks is less than 1% (1 in 100). There is also a chance of an allergic reaction to the lidocaine used for local anaesthesia. Care will be taken to reduce the chances of these risks. Aspirin should be avoided 3 days before and after the biopsies. It is not advised to participate in any vigorous activities for 3 days before and after the biopsies. Exposure to water for prolonged periods should be avoided (e.g. bathtubs, hot tubs or swimming) for 5 days after the biopsies. Showering is permitted, however band-aids must be changed afterwards. Normal daily activities will not be affected.

7.3 Indirect Calorimetry.

There is a slight risk of discomfort and hyperventilation from claustrophobia when under the clear, plastic hood. Staff will be present, and the hood is easily removable.

7.4 DEXA Scan.

This technique uses a very small amount of x-rays of the same nature as those used to obtain conventional radiographs. These x-rays come from the arm described above and is captured by a second arm within the bed of the machine. The dose of radiation received is much lower than that of standard radiological studies. Depending on the protocol used you will receive a dose to the order of 0.0002 millisievert (mSv) compared to doses of the order 0.06 mSv from a typical pulmonary x-ray. Based on current knowledge there is likely no significant risk associated with exposure at this level.

7.5 CT Scan.

The CT scan will also expose you for a short time to a dose of radiation. The main risk associated with

radiation exposure of the type and level of those used in the CT studies is to develop cancer in the future that would not have developed if you had not received the radiation dose associated with the study. Although radiation is responsible for some cancers, this risk has never been formally established at the doses provided in this study. This risk is very low and may even be zero.

Having said that, before participating in our project, it is important that you tell us if you have participated in other research protocols involving radiation exposure in the past year. In addition, if you participate in other research projects in the future involving radiation exposure, you should inform the researchers of your involvement in this study.

8. Risks associated to pregnancy.

Participation in this research project may involve risks, known or not, for pregnant women, unborn children or breast-fed infants. This is why pregnant and lactating women cannot participate in this project.

Women who may become pregnant should have a pregnancy test before performing a DEXA and a CT scan and may participate in this project only if the pregnancy test result is negative.

9. Voluntary participation and possibility of withdrawal.

Your participation in this research project is voluntary, and you are free to refuse to participate. You can also withdraw from the project at any time, without giving reasons, by telling the research team.

The researcher in charge of the research project and the Comité central d'éthique de la recherche du ministre de la Santé et des Services sociaux may end your participation, without your consent. This may happen if new findings or information indicate that your participation in the project is no longer in your interest, if you do not follow the research project instructions or else if there are administrative reasons for abandoning the project.

If you withdraw or are withdrawn from the project, the information and material already collected in the context of this project will nonetheless be stored, analyzed or used to ensure the integrity of the project.

Any new knowledge acquired during the course of the project that could have an impact on your decision to continue participating in this project will be communicated to you quickly.

10. Contribution, retention, access to data, and confidentiality.

During your participation in this research project, the researcher in charge of this project and members of her research staff will collect, in a research file, information about you that is necessary to meet the scientific objectives of this research project.

This information may include information about your past and present medical history, your lifestyle as well as the results of all tests, examinations and procedures that will be performed, including your biological material. Your file may also include other information such as your name, gender, date of birth and ethnicity.

All of your information, including your biological materials, collected as research data will be stored securely in the Data Bank and Biological Materials in accordance with the Research Data Bank Management Framework.

Your research data will be kept confidential to the extent permitted by law. To preserve your identity and confidentiality, you will be identified only by a code number. The code key linking your name to your data set will be kept by the researcher responsible for this research project.

We remind you that the MON lab data and biological material bank will provide researchers with a platform to carry out research projects of interest on obesity nutrition and metabolic disease.

Thus, your research data will be used by researchers to conduct research projects on obesity nutrition

and metabolic disease. All research projects will be reviewed and approved by Concordia University's Research Ethics Board before they are conducted. The Research Ethics Board will also monitor the research.

The research data in the data bank and biological material will be shared with different researchers. This transfer of information implies that your research data could be transmitted to countries other than Canada. However, the researcher in charge of this research project will respect the rules of confidentiality in effect in Quebec and in Canada, in all countries.

Your research data will be kept for as long as it is useful for the advancement of scientific knowledge. When it is no longer useful, your research data will be destroyed. Please note that at any time, you may request that your research data not be used by contacting the researcher in charge of the research project. In such an event, your research data already obtained in the context of this project will nevertheless be retained, analyzed, or used to ensure the integrity of the project and to comply with regulatory requirements, but no new project will be conducted with your research data.

For purposes of surveillance, monitoring, protection and security, your research file may be consulted by a person mandated by regulatory bodies as well as by representatives of the institution, the Concordia University Research Ethics Board or the Comité central d'éthique de la recherche du ministre de la Santé et des Services sociaux . These individuals and organizations adhere to a confidentiality policy.

You have the right to consult your research file to verify the information collected and have it corrected if necessary.

11. Participating in future studies.

Do you agree that the principal investigators of this research project or a member of his research staff may contact you again to suggest that you participate in other research projects? Of course, during this call, you will be free to agree or refuse to participate in the research projects suggested. **Yes No**

12. Possibility of marketing.

The research results stemming from your participation could lead to the creation of commercial products. However, you will not be able to receive any financial benefit from this.

13. Funding for the research project.

The principal investigators received funding from the granting agency to carry out this research project.

14. Compensation.

As compensation for the expenses incurred because of your participation in the research project, you will receive an amount of $700 upon completion of the study. If you withdraw from the project or if your participation is ended before it is completed, compensation will be proportional to your participation.

15. In the event of injury.

Should you suffer any injury whatsoever due to your participation in the research project, you will receive all of the care and services required by your health condition.

By agreeing to participate in this research project, you do not waive any of your rights and you do not release the principal investigator of this research project, the funding agency and the University from their civil and professional liability.

16. Medical emergency procedures.

In the event of a medical condition requiring immediate care, first aid will be provided by the staff and arrangements will be made to transfer you, if necessary, to the emergency room of a nearby hospital.

17. Identification of contact persons.

If you have any questions or have any problems related to the research project or if you wish to withdraw from it, you can contact the principal investigator of this research project or someone on the research team, at the following number, (514) 848-2424, extension 5841.

For any question concerning your rights as a participant in this research project or if you have any complaints or comments to make, you can contact the ombudsman office at Concordia University at: (514) 848-2424, ext. 8658 or by email at: ombuds@concordia.ca.

18. Monitoring the ethical aspects of the research project.

The Comité central d'éthique de la recherche du ministre de la Santé et des Services sociaux has approved this research project and continues to monitor it. For further information, please contact Ms. Johane de Champlain, Vice President, at (514) 873-2114.

Consent.

1. **Participant's consent.**

I have read and understood the information presented to me on this consent form. The research project and the information on this consent form have been explained to me; my questions have been answered and I have been given enough time to make a decision. After thinking it over, I consent to participate in this research project under the conditions set out in this form.

Participant's name and signature Date

2. Signature of the person who obtained consent, if different from the principal investigator of the research project.

I have explained the research project and this information and consent form to the participant and I have answered all of the questions that were asked.

Name and signature of the person obtaining consent Date

3. **Signature and commitment of the principal investigator of this research project.**

I certify that this information and consent form have been explained to the participant and that their questions have been answered to their satisfaction.

Together with the research team, I agree to comply with that which has been agreed to in the information and consent form and to give a signed and dated copy of it to the participant.

Name and signature of the principal investigator of this research project Date

Appendix 3: MOSPA questionnaire

MOSPA Questionnaire

Please indicate your answer in the space provided. To do this, enter the number corresponding to your answer in the box to the right.

OCCUPATIONAL PHYSICAL ACTIVITY

1. Please indicate the category that best describes your current situation.
(select only ONE. If two or more apply, select the best one.)

1 Student → go to question 2.
2 Homemaker → go to question 2.
3 Retired → go to question 2.
4 Disabled → go to question 2.
5 Unemployed → skip to question 10.
6 Employed → skip to question 3.
7 Other → go to question 2.

2. Are you employed ?
1 Yes → go to question 3.
2 No → skip to question 10.

3. What is your occupation ?
(Choose ONE from the following list.)

1 Professional and technical workers
2 Managers, officials and proprietors
3 Clerical workers
4 Sales workers
5 Craftsman
6 Machine and equipment operators.
7 Non-farm labourers
8 Private household workers
9 Service workers except private household
10 Farmers and farm managers
11 Farm labourers and foreman

4. How many hours do you work during a typical week ?

hours

5. How many days do you work during a typical week ?

days

6. On a typical day at work, how much time do you spend sitting or standing ?
(Do not include time spent going to and from work.
Do not include time spent walking, lifting or carrying moderately heavy objects or very heavy objects.)

h min

7. On a typical day at work, how much time do you spend walking ? ***(Do not include time spent going to and from work.*** ***Do not include time spent lifting or carrying moderately heavy objects or very heavy objects.)***	☐ h ☐ min
8. On typical day at work, approximately how much time do you spend actually lifting or carrying moderately heavy objects (about 5-10 kg) or doing activities of a similar effort.	☐ h ☐ min
9. On a typical day at work, approximately how much time do you spend actually lifting or carrying very heavy objects (more than 10 kg) or doing activities of a similar effort.	☐ h ☐ min

TRANSPORTATION TO AND FROM WORK, SCHOOL, AND SHOPPING

10. Going to and from work or school, or shopping, how much time do you spend walking each day ? ***(Do not include hiking or walking for sport, exercise, or pleasure.)***	☐ h ☐ min
11. Going to and from work or school, or shopping, how much time do you spend bicycling each day? ***(Do not include bicycling for sport, exercise, or pleasure.)***	☐ h ☐ min

WALKING DURING LEISURE-TIME FOR PLEASURE OR EXERCISE

12. During an average week, how many hours do you spend walking? ***(Do not include time spent at work, or going to and from work or school, or shopping.)***	☐ hours
13. When you are walking what usually happens to the rate or depth of your breathing ? **1** No change **2** Small increase **3** Moderate increase **4** Large increase	☐

HOUSEWORK

14. On the average, how much time do you spend every day doing moderately vigorous or very vigorous chores at home such as sweeping, vacuuming, washing clothes, scrubbing floors, etc.?	☐ h ☐ min

15. When you do these chores, what usually happens to the rate or depth of your breathing ?

1 No change
2 Small increase
3 Moderate increase
4 Large increase

LEISURE-TIME SPORT, SPORT TRAINING, OR EXERCISE

16. During the past 12 months, did you play any sports or do any exercise such as running, skiing, soccer, table tennis, gardening, aerobics, cycling, etc. for exercise or pleasure at least 12 times?

1 Yes → go to question 17.
2 No → skip to question 28.

17. What sport or exercise did you do most frequently ?
(Choose ONE from the following list.)

1 Aerobic exercise or dancing
2 Baseball or softball
3 Basketball, european handball or Australian netball
4 Bowling
5 Callisthenics or gymnastics
6 Cricket
7 Cycling or biking
8 Dancing
9 Gardening
10 Golf
11 Handball (American) Racquetball, squash
12 Hiking with pack or in mountains
13 Jogging, running
14 Martial arts (judo, karate, Tai Chi)
15 Orienteering
16 Rowing
17 Rugby, football (American, Australian, Canadian)
18 Ice-skating, ice-hockey
19 Skiing-cross country
20 Skiing-downhill
21 Soccer (European football)
22 Swimming (not bathing)
23 Table tennis
24 Tennis
25 Volleyball
26 Weight lifting (or body building)
27 Other : _____

18. During the past year, in how many months did you do this sport or exercise ?

months

19. In the months when you did this sport or exercise, how many times per week did you usually do it?

1 Less than one time per week
2 One time per week
3 Two times per week
4 Three times per week
5 Four times per week
6 Five times per week
7 Six times per week
8 Seven or more times per week

20. When you did this sport or exercise, how much time did you usually

spend for each session? h min

21. When you did this sport or exercise, what usually happens to the rate or deqth of your breathing ?

1 No change
2 Small increase
3 Moderate increase
4 Large increase

22. During the past 12months, did you play any other sport or do any other exercise at least 12times?

1 Yes → go to question 23.
2 No → skip to question 31.

23. What sport or exercise was it?
(Choose ONE from the following list.)

1 Aerobic exercise or dancing
2 Baseball or softball
3 Basketball, european handball or Australian netball
4 Bowling
5 Callisthenics or gymnastics
6 Cricket
7 Cycling or biking
8 Dancing
9 Gardening
10 Golf
11 Handball (American) Racquetball, squash
12 Hiking with pack or in mountains
13 Jogging, running
28 Martial arts (judo, karate, Tai Chi)
29 Orienteering
30 Rowing
31 Rugby, football (American, Australian, Canadian)
32 Ice-skating, ice-hockey
33 Skiing-cross country
34 Skiing-downhill
35 Soccer (European football)
36 Swimming (not bathing)
37 Table tennis
38 Tennis
39 Volleyball
40 Weight lifting (or body building)
41 Other : _____

24. During the past year, in how many months did you do this sport or exercise ?

months

25. In the months when you did this sport, or exercise how many times per week Did you usually do it ?

0 Less than one time per week
1 One time per week
2 Two times per week
3 Three times per week
4 Four times per week
5 Five times per week
6 Six times per week
7 Seven or more times per week

26. When you did this sport or exercise, how much time did you usually spend for each session ?

h min

27. When you did this sport or exercise, what usually happened to the rate Or depth of your breathing ?

1 No change → skip to question 31.
2 Small increase → skip to question 31.
3 Moderate increase → skip to question 31.
4 Large increase → skip to question 31.

☐

28. Prior to the past 12 months, did you play any sports or do any other exercise such as running, skiing, soccer, table tennis, gardening, aerobics, or cycling for exercise or pleasure at least 12 times in any one year ?

1 yes → go to question 29.
2 No → skip to question 31.

☐

29. what was the most recent sport that you did on a regular basis prior to this past year ?
(Choose ONE from the following list.)

☐

1 Aerobic exercise or dancing
2 Baseball or softball
3 Basketball, european handball or
4 Australian netball
5 Bowling
6 Callisthenics or gymnastics
7 Cricket
8 Cycling or biking
9 Dancing
10 Gardening
11 Golf
12 Handball (American) Racquetball, squash
13 Hiking with pack or in mountains
14 Jogging, running
15 Martial arts (judo, karate, Tai Chi)
16 Orienteering
17 Rowing
18 Rugby, football (American, Australian, Canadian)
19 Ice-skating, ice-hockey
20 Skiing-cross country
21 Skiing-downhill
22 Soccer (European football)
23 Swimming (not bathing)
24 Table tennis
25 Tennis
26 Volleyball
27 Weight lifting (or body building)
28 Other : _____

30. How many years ago did you stop doing this sport ?

☐ yrs

SUMMARY QUESTION ABOUT VIGOROUS PHYSICAL ACTIVITY

31. Which of the following four activity classes best describes your present activity outside of your job? Please consider transportation to and from work, sporting activity and other physical activity effort during your leisure time, like gardening or dancing. **1** No physical activity weekly. **2** Only light physical activity in most weeks. **3** Vigorous physical activity at least 20minutes once or twice a week, (Vigorous activity causes shortness of breath, a rapid heart rate, and sweating). **4** Vigorous physical activity for at least 20 minutes three or more times Per week.	☐

Appendix 4: Patient-reported physical activity

	Plant-based (n=18, 11F)		**Animal-based (n=16, 8F)**		**P-value-time†**	**P-value$_{time}$‡**	**P-value$_{time*group}$**
	Pre	**Post**	**Pre**	**Post**			
MOSPA score, met-minutes/week	7229 (3262)	6436 (2276)	7075 (2533)	6726 (2314)	0.187	0.676	0.655

Note: All values reported are mean (SD). Data from one participant in the plant-based group was excluded due to missing data in the patient-completed questionnaire. †p-value reported corresponds to the plant-based group. ‡p-value reported corresponds to the animal-based group. MOSPA: Monica Optional Study of Physical Activity; SD: standard deviation.

Appendix 5: Baseline characteristics separated by sex: Female dataset

Variables	Plant-based (n=11)	Animal-based (n=8)	P-value
Age, y	36.5 (9.6)	36.4 (10.1)	0.986
Height, cm	167.7 (8.1)	166.0 (7.5)	0.650
Weight, kg	102.4 (15.1)	100.4 (14.3)	0.767
BMI, kg/m^2	36.2 (3.3)	36.1 (3.3)	0.960
FM, %	50.0 (3.0)	46.3 (3.3)	0.021
FM, kg	49.5 (9.3)	45.5 (9.3)	0.366
Lean mass, kg	49.8 (6.9)	51.8 (5.0)	0.496
FFM, kg	52.4 (7.1)	54.7 (5.3)	0.449
Upper body FM, kg	31.0 (7.8)	28.6 (6.7)	0.590
Lower body FM, kg	18.5 (2.7)	15.9 (2.9)	0.077
VAT mass†, kg	0.85 (0.35)	1.03 (0.40)	0.218
REE‡, kcal/day	1686 (332)	1866 (201)	0.306
RER‡	0.88 (0.06)	0.85 (0.04)	0.204
CHO_{ox}‡, mg/min	188.4 (77.2)	172.8 (52.3)	0.655
FAT_{ox}‡, mg/min	46.3 (21.9)	70.0 (19.9)	0.042

Note: All values reported are mean (SD). †n=10 for the plant group. ‡n=9 for the plant group and n=7 for the animal group. BMI: body mass index, CHO_{ox}: carbohydrate oxidation; FAT_{ox}: lipid oxidation; FFM: fat-free mass, FM: fat mass; SD: standard deviation; VAT: visceral adipose tissue.

Appendix 6: Baseline characteristics separate by sex: Male dataset

Variables	Plant-based (n=8)	Animal-based (n=8)	P-value
Age, y	37.5 (11.6)	46 (9.7)	0.134
Height, cm	177.3 (6.0)	172.5 (8.9)	0.230
Weight, kg	113.7 (15.7)	115.4 (104.6)	0.853
BMI, kg/m^2	36.2 (4.6)	38.7 (4.5)	0.292
FM, %	41.4 (5.8)	41.4 (4.8)	0.927
FM, kg	46.1 (12.5)	47.0 (11.9)	0.882
Lean mass, kg	64.0 (4.0)	64.9 (9.0)	0.795
FFM, kg	67.4 (4.1)	68.1 (9.4)	0.832
Upper body FM, kg	31.2 (9.8)	33.4 (9.8)	0.665
Lower body FM, kg	13.0 (2.9)	13.4 (3.0)	0.818
VAT mass†, kg	2.0 (1.2)	2.1 (1.0)	0.931
REE‡, kcal/day	2190 (209)	2087 (354)	0.984
RER‡	0.847 (0.02)	0.813 (0.04)	0.048
CHO_{ox}‡, mg/min	207.8 (34.2)	148.3 (64.4)	0.078
FAT_{ox}‡, mg/min	80.3 (16.6)	96.5 (28.7)	0.204

Note: All values reported are mean (SD). †n=7 for the plant group and n=7 for animal group. ‡n=6 for the animal group. BMI: body mass index, CHO_{ox}: carbohydrate oxidation; FAT_{ox}: lipid oxidation; FFM: fat-free mass, FM: fat mass; SD: standard deviation; VAT: visceral adipose tissue.

Appendix 7: Metabolic parameters separated by sex: Female dataset

Variables	Plant-based (n=9)		Animal-based (n=7)		P-value$_{time*group}$
	Pre	Post	Pre	Post	
REE, kcal/day	1686 (332)	1703 (281)	1866 (201)	1726 (239)	0.237
RER	0.877 (0.044)	0.785 (0.042)	0.847 (0.057)	0.815 (0.033)	0.027
CHO_{ox}, mg/min	188.4 (77.2)	94.5 (40.3)	172.8 (52.3)	122.1 (27.9)	0.174
FAT_{ox}†, mg/min	46.3 (21.9)	87.9 (23.1)	70.0 (19.9)	80.8 (25.2)	0.041

Note: All values are reported as mean (SD). †p-value between groups: 0.042. CHO_{ox}: carbohydrate oxidation; FAT_{ox}: lipid oxidation; REE: resting energy expenditure; RER: respiratory exchange ration; SD: standard deviation.

Appendix 8: Metabolic parameters separated by sex: Male dataset

Variables	Plant-based (n=8)		Animal-based (n=6)		P-value$_{time*group}$
	Pre	Post	Pre	Post	
REE, kcal/day	2190 (209)	2065 (274)	2087 (354)	2046 (296)	0.457
RER	0.847 (0.022)	0.797 (0.045)	0.813 (0.041)	0.772 (0.035)	0.765
CHOox, mg/min	207.8 (34.2)†	119.1 (54.0)	148.3 (64.4)†	128.8 (78.1)	0.137
FATox, mg/min	80.3 (16.6)	106.4 (36.0)	96.5 (28.7)	105.5 (34.2)	0.436

Note: All values are reported as mean (SD). †p-value between groups: 0.078. CHO_{ox}: carbohydrate oxidation; FAT_{ox}: lipid oxidation; REE: resting energy expenditure; RER: respiratory exchange ration; SD: standard deviation.

Appendix 9: Amino acid composition of the protein supplementation in each group as described by Nutritional Fundamentals of Health nutritional labels.

Amino acid	**Pea/lupine (g per 100g)**	**Whey (g per 100g)**
Alanine	3.3	4.1
Arginine	7.4	2.1
Aspartic acid	8.9	8.7
Cystine	0.8	1.9
Glutamic acid	14.2	13.9
Glycine	3.1	1.5
Histidine*	1.9	1.5
Isoleucine*	3.7	4.9
Leucine*	6.4	8.6
Lysine*	5.7	7.2
Methionine*	0.8	1.6
Phenylalanine*	4.2	2.6
Proline	3.4	4.7
Serine	3.9	4.2
Threonine*	2.8	5.7
Tryptophan*	0.7	1.5
Tyrosine	3.1	2.8
Valine*	4.0	4.6

*Essential amino acids.

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