THE IMPACT OF A HIGH COMPLEX CARBOHYDRATE DIET AND A VERY LOW CARBOHYDRATE DIET ON VISCERAL ADIPOSE TISSUE MASS, VOLUME AND AREA AMONG HEALTHY OVERWEIGHT AND OBESE ADULTS

By

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CERTIFICATE OF APPROVAL

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LIST OF ABBREVIATIONS

AgRP	Agouti-Related Peptide
ANOVA	Analysis of Variance
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CT	Computed Tomography
DASH	Dietary Approaches to Stop Hypertension
DEXA	Dual Energy X-ray Absorptiometry
GCRC	General Clinical Research Center
HCCD	High Complex Carbohydrate Diet
hs-CRP	High Sensitivity C-Reactive Protein
IRB	Institutional Review Board
JAK/STAT	Janus Kinase/ Signal Transducer Activator of Transcription
m-HCCD	Energy Matched High Complex Carbohydrate Diet
MRI	Magnetic Resonance Imaging
NPY	Neuropeptide Y
OHSU	Oregon Health & Science University
PVAT-TBM	Visceral Adipose Tissue as a Percent of Total Body Mass
PVAT-TFM	Visceral Adipose Tissue as a Percent of Total Fat Mass
ROI	Region of Interest
SAT	Subcutaneous Adipose Tissue
WHO	World Health Organization
VAT	Visceral Adipose Tissue
VLCD	Very Low Carbohydrate Diet

VLCKD	Very Low Calorie-Ketogenic Diet
αMSH	α Melanocyte Stimulating Hormone

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ABSTRACT

It is unclear if different dietary weight loss strategies affect visceral adipose tissue (VAT) mass to different extents among individuals who are overweight and obese. We evaluated whether consuming a high complex-carbohydrate diet (HCCD) or a very low carbohydrate diet (VLCD) for six weeks under controlled feeding study conditions resulted in similar reductions in abdominal VAT mass. We also evaluated the relationship between change in fasting serum leptin and plasma hs-CRP concentrations and change in VAT mass during these dietary interventions.

Thirty-five overweight and obese but otherwise heathy adults were randomly assigned to consume an *ad libitum* very low carbohydrate diet (VLCD), an *ad libitum* high complex carbohydrate diet (HCCD) or an energy matched high complex carbohydrate diet (m-HCCD) for 6 weeks. The *ad libitum* intervention groups were provided 120% of their energy needs and the m-HCCD intervention group was given approximately 66% of their energy needs. Body composition, including VAT mass, was quantified by Dual Energy X-ray Absorptiometry, and blood samples, including fasting serum leptin and plasma hs-CRP concentrations, were measured pre- and post- dietary intervention.

Overall, mean weight loss was -3.51 ± 0.40 kg ($p<0.001$), mean reduction in BMI was -1.25 ± 0.14 kg/m^2 ($p<0.0001$), and mean total fat mass loss was -2.12 ± 0.24 kg ($p<0.0001$). Mean fasting serum leptin concentration was -1.8 ±0.33 ng/ml lower after the dietary intervention ($p<0.0001$) but change in fasting plasma hs-CRP concentration was not significantly altered post-intervention (-0.69 ± 0.46 mg/l, $p=0.14$). On average, participants lost an insignificant amount of VAT mass (-0.03 ± 0.02 kg; CI: -0.08, 0.02, $p=0.217$). There were no significant differences in mean change of any of the variables between dietary intervention groups.

These results suggest that energy restriction, but not dietary macronutrient composition, influences change in weight, body mass, BMI, total fat mass, total percent fat mass, and fasting serum leptin concentrations. Our findings also suggest that neither energy restriction nor differences in macronutrient composition during our 6-week intervention significantly altered abdominal VAT mass or fasting plasma hs-CRP concentrations. Finally, our results suggest that changes in VAT mass are not correlated with changes in total body mass, total fat mass or fasting serum leptin and plasma hs-CRP concentrations.

Chapter 1

Introduction and Specific Aims

According to the Centers for Disease Control and Prevention (CDC), more than one third of adults in the United States (US) have obesity [1] which is associated with increased medical costs, comorbidities and mortality rates. Dietary behaviors and patterns are important contributors to obesity and although dietary recommendations are established, the CDC has reported an increase in obesity prevalence among adults from 34.9% in 2011-2013 to 37.7% in 2013-2014 [2].

The estimated annual medical cost of individuals with obesity is $1,429 higher than those without obesity[1]. And, as of 2008, the annual medical cost in the US of individuals with obesity was $147 billion dollars[1]. There is an additional annual cost to the nation due to obesity and obesity-related absenteeism which ranges between $3.38 to $6.38 billion dollars in lost productivity [1]. Obesity is a serious concern that is associated with the leading causes of death in the US [1]. According to the World Health Organization (WHO), overweight and obesity are linked to more deaths than underweight and contributes to major health consequences including heart disease, diabetes, stroke, musculoskeletal disorders, and some cancers such as breast, ovarian, kidney, prostate, colon, liver and gallbladder [3].

Obesity is characterized by excess adipose tissue that accumulates within both visceral and subcutaneous sites. Visceral adipose tissue (VAT) is stored deep within the abdominal cavity and surrounds internal organs such as the liver, intestines and kidneys, while subcutaneous adipose tissue (SAT) is stored underneath the skin. Excessive deposition of VAT is associated with increased risk of developing insulin resistance, cardiovascular disease and metabolic syndrome which includes the constellation of higher waist circumference, blood pressure,

fasting blood glucose and triglyceride concentrations, and lower HDL cholesterol concentrations, while excessive deposition of SAT is not [4].

Technologies to assess VAT and weight loss strategies to reduce excessive VAT are important to reduce the rate of obesity and its comorbid conditions. Currently, it is unclear if different dietary weight loss strategies affect VAT mass to different extents among individuals with obesity. To answer this question, we evaluated whether consuming a high complex-carbohydrate diet (HCCD) or a very low carbohydrate diet (VLCD) for six weeks under controlled feeding study conditions resulted in similar reductions in abdominal VAT. We also evaluated the relationship between change in fasting serum leptin and plasma hs-CRP concentrations and change in VAT mass during these dietary interventions.

Specific Aims

Our first aim of this study was to describe the change in abdominal VAT mass within the abdominal Region of Interest (ROI) measured by Dual-Energy X-ray Absorptiometry (DEXA) after consuming an *ad libitum* high complex carbohydrate diet (HCCD), an *ad libitum* very low carbohydrate diet (VLCD) or an energy matched high complex carbohydrate diet (m-HCCD) for 6 weeks as part of a controlled feeding study. To address this aim we tested the following hypotheses:

1. Mean abdominal VAT mass within the abdominal ROI, expressed as both absolute VAT mass loss in kilograms and percent of total fat mass loss, is lower after dietary intervention than before in each study group.
2. Loss of abdominal VAT mass, in kilograms, within the abdominal ROI is correlated with loss of total fat mass and loss of total body mass among participants in each dietary intervention group.

3. The amount of VAT mass loss, expressed as absolute VAT mass loss (kg), percent of total mass loss, and percent of total fat mass loss is similar among groups.

Our second aim was to describe the relationships between change from baseline in VAT mass within the abdominal ROI and change in fasting serum leptin and high sensitivity C-reactive protein (hs-CRP) concentrations after consuming an *ad libitum* HCCD, an *ad libitum* VLCD or an energy matched HCCD for 6 weeks as part of a controlled feeding study. To address this aim we tested the following hypotheses:

1. Changes in fasting serum leptin (indexed to total fat mass) and hs-CRP concentrations are similar among dietary intervention groups.
2. Change in VAT mass, in kilograms, is directly related to change in fasting serum leptin (indexed to total fat mass) and hs-CRP concentrations among participants in each dietary intervention group.

Chapter 2

Background

Overview of Visceral Adipose Tissue and Subcutaneous Adipose Tissue

Adipose tissue and body fat are often used synonymously but these terms are different. Body fat is a subgroup of compounds, also referred to as lipids, that are hydrophobic and stored as triglycerides within adipocytes [5]. Adipose tissue is the connective tissue that is comprised of adipocytes and is distributed throughout the body [5]. Adipose tissue is divided into two main compartments, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). While these two types of adipose tissue are similar in the way they offer the body cushion and insulation, they are different in their metabolic functions and the regions in which they reside [6].

Subcutaneous Adipose Tissue

Subcutaneous adipose tissue is the layer of fat found between the skin and the aponeuroses and fasciae of muscle [7]. SAT is comprised of superficial adipose tissue and deep subcutaneous adipose tissue. The superficial adipose layer is found directly underneath the skin layer, while deep subcutaneous adipose tissue is located underneath the superficial layer. A layer of connective tissue separates the two layers of SAT [7]. Unlike VAT, SAT has been shown to be the least metabolically harmful storage site of body fat [8,9]. For example, the Dallas Heart Study demonstrated this by looking at the incidence of diabetes and its association to VAT and SAT through measurement by DEXA and magnetic resonance imaging (MRI). Higher VAT was associated with developing diabetes while SAT was not [8].

Visceral Adipose Tissue

The classification of visceral adipose tissue is illustrated in Figure 1. Visceral adipose tissue is dispersed into three cavities of the body: intrathoracic, intraabdominal and intrapelvic. Intraabdominal VAT has been investigated the most although it is not known if VAT deposited within the three body cavities has different metabolic features [5]. The intraabdominal and the intrapelvic regions are often grouped together within the intrabdominopelvic region. Within the abdominal cavity there is further classification of VAT. The two main regions of the intraabdominal cavity are the intraperitoneal and extraperitoneal regions. The intraperitoneal region includes omental adipose tissue, the layer surrounding the organs, and mesenteric adipose tissue, the layer attaching the organs together. The extraperitoneal region includes the preperitoneal and retroperitoneal areas [5]. Within the retroperitoneal region is the adipose tissue that surrounds the kidneys, aortic valve and pancreas.

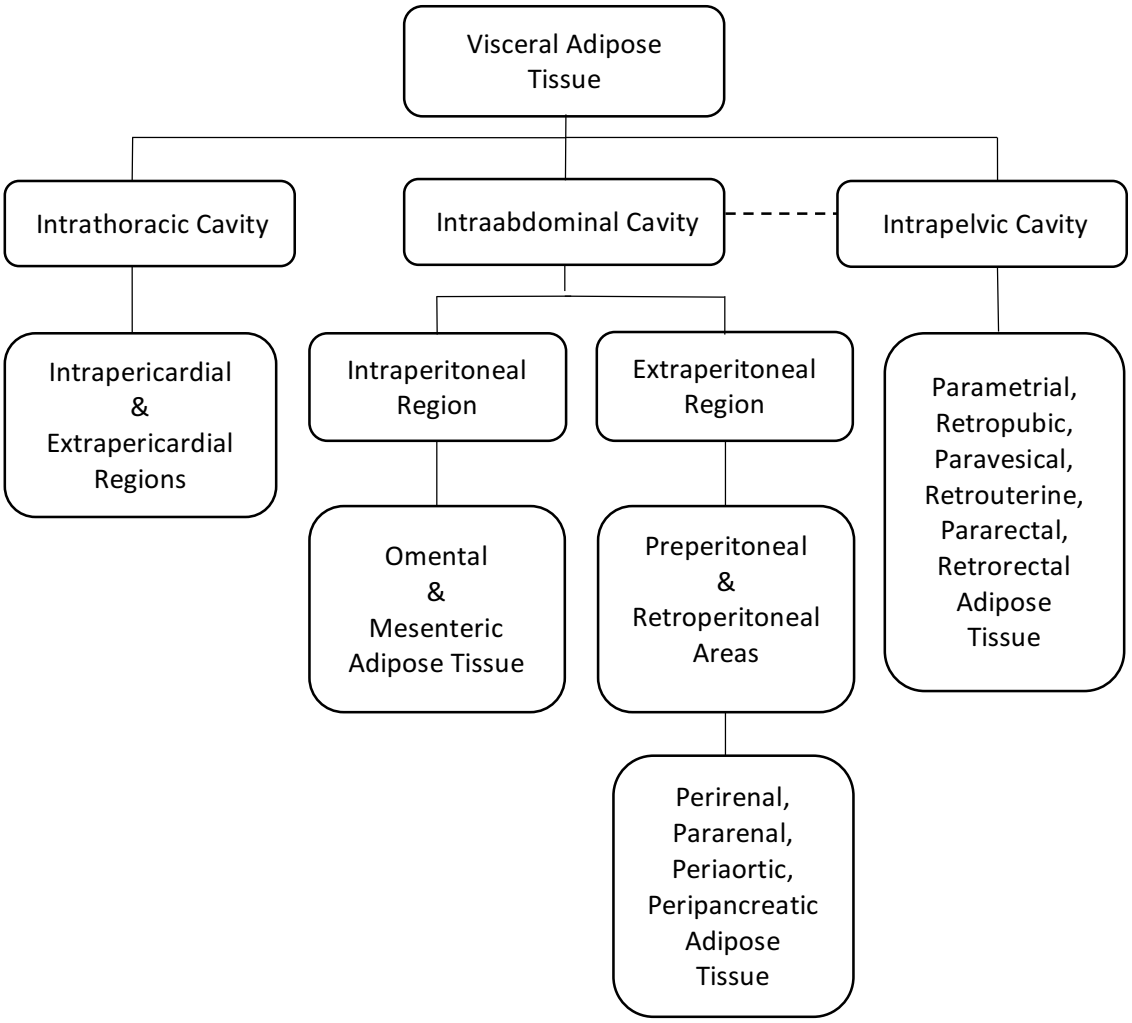


Figure 1: Classification of Visceral Adipose Tissue (Modified from Shen et al, 2003[5])

Gender, Age and Adiposity

Adult men and women differ in how fat is distributed within their bodies. In males, adipose tissue is more likely to be stored in the trunk and abdominal region. This fat deposition pattern is called the android shape pattern of fat distribution. In women, adipose tissue is more likely to be stored in the hips and thighs, which is called the gynoid shape pattern of fat distribution [7]. Total VAT volumes are positively associated with age in both males and females [10- 12].

A sub study of the Framingham Heart Study Third Generation investigated SAT and VAT using 8-slice multidetector computed tomography (CT) and risk factors such as waist circumference, Body Mass Index (BMI), blood pressure, and HDL cholesterol, total cholesterol, and blood glucose concentrations. In both men and women, VAT and SAT volumes were significantly associated with increased odds of having hypertension, impaired fasting glucose concentrations, diabetes and metabolic syndrome ($p<0.01$) [12]. VAT was positively correlated with age in both men and women ($p<0.001$) while SAT was positively correlated with age in women only [12].

The relationship between age and adipose tissue volume has continued to be investigated particularly in women. Three longitudinal studies from the Lifespan Health Research Center in Dayton, OH of 728 apparently healthy, overweight or obese African American and European American women aged 18-80 years investigated total body fat, SAT and VAT mass using DEXA and abdominal Magnetic Resonance Imaging (MRI) axial images [13]. Similar to the Framingham Heart Study results, in women, age and abdominal VAT mass were positively associated but age and abdominal SAT mass were negatively associated [13]. Other studies examined waist-hip-ratio as a determinant of abdominal adipose tissue and determined that in both men and women

there is a strong correlation between increased age and increased waist-to-hip ratio [7]. These studies indicate that aging in both men and women is associated with increased adipose tissue.

Adiposity and Disease

Since the 1980's, regional distribution of adipose tissue has become a topic of interest because of its association with disease risk and its implications on public health. It was discovered that having obesity, in general, may not be the primary cause of a person developing obesity-related co-morbidities, such as heart disease and metabolic syndrome, but rather the distribution of the adipose tissue within the body [7]. Reduction of chronic disease should therefore be more thoroughly investigated by examining the reduction in adipose tissue in various regions of the body.

Metabolic risk factors, such as hypertension, higher waist circumference, increased BMI, higher total- cholesterol, higher LDL-cholesterol, elevated blood glucose concentrations, and lower HDL cholesterol concentrations, in addition to insulin resistance have been studied in association to SAT and VAT [12,14]. The Framingham Heart Study Third Generation demonstrated through two large cross sectional studies that VAT and SAT are significantly associated with metabolic syndrome [12,14]. In both studies, CT images were used to measure SAT and VAT and examine the relationship between SAT and VAT and metabolic risk. The first study demonstrated that in both men and women, SAT and VAT were directly associated with blood pressure, and fasting plasma glucose, triglycerides, and HDL cholesterol concentrations and the higher VAT and SAT volumes were associated with increased odds of having hypertension, impaired fasting glucose, diabetes, and metabolic syndrome ($p<0.01$). In normal weight, overweight and obese individuals, the prevalence of metabolic syndrome increased significantly ($p<0.001$) across quartiles of VAT [12]. A follow-up study found similar results but in addition, quantified abdominal

VAT for a period over 6.2 years. The authors observed that for each additional 500 cm^3 of abdominal VAT at baseline, there was a 2.34 mg/dL increase in fasting glucose concentration and a 1.62 mg/dL decrease in HDL-cholesterol concentration from baseline to follow-up ($P<0.0001$ for both). For each additional 500 cm^3 of abdominal VAT at baseline, the odds of having metabolic syndrome were 2.58 times higher ($P<0.0001$) [14].

Other studies found significant correlations between VAT and disease but not SAT and disease. In a cross sectional study where VAT and SAT thickness were measured by ultrasonography, VAT thickness was significantly higher in individuals with gallstone disease (62.56 ± 23.00 mm vs. 57.27 ± 22.61 mm, $p = 0.001$), while subjects with or without gallstone disease did not exhibit significant differences in mean SAT thickness ($p = 0.07$) [15]. Endothelial function was examined in 35 individuals who gained approximately 4 kg in 8 weeks with VAT and SAT measured using DEXA and CT scans. An increase of VAT (between 8 and >16 cm^2), but not SAT, was significantly correlated ($p=.004$) with a reduction of flow mediated dilatation, a measure of arterial dysfunction [16].

Three scenarios help to explain the relationship between VAT and disease risk. First, the abundance of fat in omental adipose tissue interferes with the action of insulin causing insulin resistance [17]. Insulin resistance exposes the liver to high amounts of free fatty acids that alter many hepatic metabolic processes such as lipid, glucose, lipoprotein and fatty acid metabolism. These altered processes can cause hyperinsulinemia, glucose intolerance and hypertriglyceridemia [17]. Second, adipose tissue has metabolically active regions that secrete substances such as adipocytokines, including leptin and adiponectin, and inflammatory substances that can contribute to insulin resistance and the proinflammatory state of obesity [11-13,17]. Third, SAT has a limited capacity to store excessive fat in the abdominal region. When there is an impaired uptake of circulating fat by subcutaneous adipose tissue, fat will likely start to be

stored around vital organs such as the liver, heart and pancreas [17]. One, or a combination, of these scenarios likely leads to obesity related diseases.

Overview of Techniques used to Measure Adipose Tissue

Imaging Technology

Various types of imaging technology are used to measure VAT including computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography, and Dual-Energy X-Ray Absorptiometry (DEXA). All of these techniques require a skilled technician and a high initial capital investment which makes it less accessible for field use [18]. These techniques generate images of the body, specifically the abdominal region, and allow VAT and SAT to be distinguished, quantified and linked to health outcomes. CT is considered the gold standard method to measure VAT and SAT because CT technology uses higher doses of radiation (3,100 μSv) and separates the images into slices that produce a higher resolution and more discreet ability to distinguish between different types of adipose tissue [19]. MRI is similar to CT in that the images are separated into slices at specific levels of the body and show detailed images. Also, MRI does not use radiation so it is fitting for longitudinal studies that require repeated scans to assess change over time. However, MRI technology takes longer to acquire the images, and is often more expensive [19].

Dual-Energy X-Ray Absorptiometry (DEXA)

DEXA uses X-ray photons that absorb at two energies to differentiate fat mass from fat-free mass [19]. The lower intensity energy beam is absorbed by soft tissue (muscle and fat) while the higher intensity beam is absorbed by bone and soft tissue (e.g. muscle). DEXA is frequently used over other imaging techniques because of its convenience, lower cost, shorter scan duration (~15 minutes) and lower dose of radiation (~1mSv or 1000 μSv) [19]. This method is

limited by the size of the scanning area, the weight capacity of the scanning bed and the limited ability to differentiate between types of fat, until recently. Now, application of a new algorithm, available in some DEXA software, estimates VAT mass and volume within the android region of interest by subtracting SAT from total android fat [20].

Several studies have examined the efficacy of DEXA scans to estimate SAT and VAT compared to other body composition measurement techniques [18,20-22]. In a study that compared DEXA to CT in individuals with a wide range of BMI values (18.5-40 kg/m^2), VAT volume was measured in men and women with a mean difference of 56 cm^3 (95% CI: -339, 472 cm^3) between the two techniques [21]. In comparison to CT, which has been previously suggested as the gold standard for measuring VAT, DEXA was shown to accurately measure VAT and to be useful in research because of its short scanning time and low dose of radiation.

Very Low Carbohydrate Diet (VLCD)

The Atkins diet is a very low carbohydrate-high protein diet that allows unlimited fat and protein consumption while strictly limiting carbohydrate intake. VLCD have been used for weight loss over time but regained popularity after the release of the "Dr. Atkins' Diet Revolution" book in 1981. Typical VLCD limit consumption of carbohydrate to 20-90 grams per day. In comparison, the World Health Organization recommends consuming about 275 grams of carbohydrate per day in a 2000 kcal diet [23]. The goal of a VLCD is to induce a state of ketosis during which fat, instead of carbohydrate, is metabolized to generate energy. The Atkin's diet typically includes four phases in which the first phase limits consumption of net carbohydrate (total carbohydrate – fiber) to 20-25 grams each day followed by a gradual increase in carbohydrate intake through the phases to consumption of a daily net carbohydrate intake of 80-100 grams per day in phase four [24].

Very Low Carbohydrate Diet & Weight Loss

Research on VLCD has often compared low carbohydrate diets to low fat diets. Although greater weight loss while on VLCD was typically demonstrated in the short term, there was often no significant difference in weight loss between intervention groups in the long term. The duration of the weight loss intervention within each study yielded different results. In one study, after 3 months of low to moderate carbohydrate consumption, (41-42% of daily energy intake) compared to baseline, both men and women demonstrated significant weight loss (70 $\pm$ 13.3 kg to 69.1 $\pm$ 13.1 kg and 57.2 $\pm$ 12.3 kg to 55.7 $\pm$ 12.1 kg, respectively; $p<0.001$ for both)[25]. In another 3-month intervention, 63 obese but otherwise healthy men and women were instructed by a dietitian to consume either a low carbohydrate diet of ~20 g/day, or a conventional diet composed of ~60% carbohydrate [26]. The low carbohydrate diet group lost significantly more weight (-6.8 $\pm$ 5.0 % body weight) compared to the conventional diet group (-2.7 $\pm$ 3.7 % body weight) by 3 months ($p=0.001$).

When investigating a longer-term intervention of 6 months, results are conflicting. Some studies found significant differences in weight loss, while others did not. Lack of consistency between studies may be attributed to differences in carbohydrate intakes, differences in adherence to the dietary intervention and differences in study design. One randomized controlled trial instructed participants to consume either a low carbohydrate diet (~20 g/day) or a conventional diet (~60% carbohydrate/day). Those consuming the low carbohydrate diet lost significantly more weight (-9.7 $\pm$ 5.7 kg, $p=0.03$) compared to those consuming the conventional diet (-5.3 $\pm$ 6.4 kg) [26]. Comparably, a cohort study of five trials compared a low carbohydrate diet (~20 g/day) to a low-fat diet and concluded that those consuming the low carbohydrate diet lost significantly more weight than those consuming the low-fat diet (mean difference, –3.3 kg;

95% [CI], −5.3 to −1.4 kg) [28]. In both studies, differences in weight loss between groups was no longer significant at 12 months [26,28]. The Pounds Lost trial reported contrasting results when examining weight loss among various "at home" diet interventions. There was no significant weight loss from baseline to 6 months among participants assigned to either a ~65% carbohydrate diet or a ~35% carbohydrate diet nor was there a significant difference in weight loss between diet groups [29]. Overall, it is unclear if VLCD result in greater weight loss after 6 months than conventional diets.

When investigating interventions lasting at least one year, studies found various differences in weight loss from baseline such as -5.1 $\pm$ 8.7 kg ($p = 0.003$)[30], -7.3 $\pm$ 7.3 kg ($p < 0.05$) [26] and -3.9 kg ($p=0.009$) [31]. However, in each of these studies there was no significant difference in weight loss when the low carbohydrate diet was compared to the other dietary intervention groups. These findings suggest long term weight loss is not due to a particular distribution of macronutrients within the dietary intervention.

Very Low Carbohydrate Diet & Total Fat Mass Loss

The impact of diet composition on loss of fat mass has also been explored. In a randomized control trial of 46 men with abdominal obesity, differences in fat mass loss, measured by CT, were compared across dietary interventions [32]. The participants were instructed on how to consume either a very high-fat, low-carbohydrate diet or a low-fat, high-carbohydrate diet for 12 weeks. The diet interventions were equal in energy and protein content and compliance to the dietary intervention was promoted through individual counseling sessions. There was a significant reduction in mean fat mass of -10.3 kg (95% CI: - 11.5, - 9.16 kg, $p < 0.001$) from baseline to 12 weeks in the pooled data. However, there was no significant difference in fat mass loss between the diet intervention groups. This study indicates that for a

period of 12 weeks, macronutrient composition did not differentially influence fat mass loss.

Along with dietary carbohydrate composition, researchers have also investigated how energy intake affects fat mass loss. In a randomized clinical trial, 120 obese participants referred to a Specialist Obesity Clinic were recruited to consume a low fat, energy restricted diet for 3 months. Seventy-two participants who were not able to lose 5% of their body weight after 3 months were assigned to either a low carbohydrate diet (<40 g/day) with an energy intake of 800-1500 kcal/day or a very low calorie diet (550 kcal/day, 36% carbohydrate) for 9 months. The very low calorie diet involved two stages. In the first stage, participants consumed soups, shakes and bars in place of conventional foods for 3-6 months to promote weight loss. In the second stage, participants were reintroduced to solid foods to promote weight maintenance. From baseline to 3 months those consuming the very low-calorie diet lost significantly more fat mass (59.5 ± 15.1 kg to 50.3 ± 15.1 kg), compared to those consuming the low carbohydrate diet (54.1 ± 10.2 kg to 52.0 ± 11.3 kg; $p<0.001$). The difference in fat mass loss remained significant through 9 months where those consuming the very low calorie diet continued to lose an average of ~4 kg fat mass while those consuming the low carbohydrate diet regained ~1 kg of fat mass ($p<0.001$) [27]. This study suggests that energy intake had a greater impact on fat mass loss than the dietary carbohydrate composition.

A similarly designed trial randomized obese participants to either a low-calorie diet (800-1500 kcal/day; representing 10% below basal metabolic rate) or a very low calorie-ketogenic diet (600-800 kcal/day with <50g of carbohydrate/day). Those assigned to the very low calorie-ketogenic diet lost significantly more fat mass from baseline to 6 months (an average of 19.1 kg fat mass) than those consuming the low-calorie diet lost (an average of 6.1 kg fat mass) [33]. However, we cannot differentiate whether reduction in energy, reduction in carbohydrate or a

combination of both factors caused the greater decrease in fat mass in the very low calorie-ketogenic diet group.

One way to control diet in a clinical trial is to provide the food in an inpatient setting. In a metabolic balance study, 19 obese men and women were admitted for a pair of two-week inpatient periods separated by a 2-4-week washout period. For the first 5 days, each participant consumed a eucaloric baseline diet. Then, each participant was assigned to either a carbohydrate-restricted diet or a fat-restricted diet with 30% fewer calories than their baseline diet. Individuals in the carbohydrate-restricted and fat-restricted dietary intervention groups lost significant amounts of fat mass compared to baseline (- 0.529 $\pm$ 0.13 kg, p = 0.0015 vs. – 0.588 $\pm$ 0.14 kg, p = 0.001). However, there was no significant difference in fat mass loss between dietary intervention groups (p = 0.78) [34]. This study suggests that when energy restriction is the same, dietary macronutrient distribution does not affect degree of fat mass loss over a short period in a controlled setting.

Very Low Carbohydrate Diet & Visceral Adipose Tissue Loss

Recent research has investigated the impact of dietary intervention on fat mass more thoroughly by measuring changes in the distribution of fat, such as VAT. The Pounds Lost trial measured VAT by CT in 424 overweight or obese men and women who were randomized into diet intervention groups with carbohydrate intake distributed between 35% - 65%. There was no significant change in mean VAT mass from baseline to 6 months in either the 35% carbohydrate or the 65% carbohydrate diet intervention groups [29]. Several other studies used CT to measure VAT while investigating carbohydrate intake using food records [25,32]. In a 3-month study of diabetic patients, change in carbohydrate intake was significantly associated with change in mean VAT in men ($p<0.025$) but not in women($p>0.025$) [25]. Self-reported food records were also

used to investigate carbohydrate intake in a randomized control trial involving 46 obese men consuming diets of ~10% or ~53% carbohydrate for 12 weeks [32]. Although no significant difference in VAT mass loss was found between intervention groups, when data was pooled there was a significant reduction in the mean visceral fat area (-57.2 cm^2 95% CI: -65.2, -49.1, $p<0.001$).

VAT was also measured while investigating a low-calorie diet that consisted of 800-1500 kcal/day (10% below their basal metabolic rate) or a very low calorie-ketogenic diet that provided 600-800 kcal/day and <50 g of carbohydrate/day for the first 45-60 days to obese men and women. VAT mass was significantly different ($p<0.001$) among diet groups during the duration of the study (2-24 months). The average reduction of VAT mass was greater in the very low-calorie ketogenic diet group (706 cm^3) than it was in the low-calorie diet group (212 cm^3) at 24 months ($p<0.001$) [33]. Although calorie intake contributed heavily to the outcome of this study, the low carbohydrate diet with energy restriction resulted in greater VAT mass loss compared to energy restriction on its own.

High Complex Carbohydrate Diet (HCCD)

The Dietary Approaches to Stop Hypertension (DASH) diet pattern has been shown to lower blood pressure. This dietary pattern is rich in fruits and vegetables, fat-free or low fat milk products, fish, poultry, beans, seeds and nuts. This diet emphasizes the consumption of high complex carbohydrates (6-8 servings/day) to improve cardiovascular health and can result in weight loss as a secondary outcome. It is also low in sodium, sweets, fats and red meat [35].

High Complex Carbohydrate Diets & Weight Loss

The DASH diet is recommended by health professionals as a general healthy diet that may also provide the added beneficial effect of weight loss. Several studies have investigated the effect of the DASH dietary pattern on weight loss with interventions as short as 6 days to as long as 1 year. In many studies, the diets are self-reported and assessed through dietary food records, interviews or food frequency questionnaires which makes it difficult to accurately assess carbohydrate intake. In an intervention study, the DASH diet was maintained through group education and individual counseling [36]. After completion of the 2-month education program, 77% of the participants on the DASH diet lost an average of 3.6 pounds and had a significant reduction in BMI ($p<0.01$). Another study followed participants for 1 year who participated in a healthy-eating/ exercise program patterned after the DASH diet [37]. DASH diet quality scores and physical activity scores were calculated and compared to body composition measurements. After 1 year, average weight was significantly lower compared to baseline ($p<0.001$). On average, the participants with high physical activity and high diet quality scores lost more weight than those with lower dietary and physical activity adherence scores; however, differences between groups were not significant. Similarly, a cross sectional study used a food frequency questionnaire to evaluate the association between whole grain intake and body composition. Mean BMI was significantly higher in the quartile with the lowest whole grain intake (27.4 $kg/m^{2,}$ 95% CI: 27.0, 27.8) compared to the quartile with the highest whole grain intake (26.3 kg/m^2, 95% CI: 25.9, 26.7, $p<0.001$) [38]. Although there are significant associations in these studies, we cannot determine if carbohydrate intake affected weight loss independent of overall lifestyle changes.

Several randomized control trials have been completed to study the effects of the Mediterranean diet pattern on weight loss. This dietary pattern is similar to the DASH diet in that it emphasizes consumption of whole grains, fruit, vegetables, fish, oils and de-emphasizes consumption of red meat and saturated fats. A meta-analysis was performed to explore the associations between this dietary pattern and weight loss in 6 randomized control trials. Compared to those assigned to the control diets, those assigned to the Mediterranean diet lost slightly more weight (0.29 kg, CI: -0.55, to 0.04; p=0.924) which resulted in a slightly greater reduction in BMI compared to control diets (mean difference, - 0.29 kg/m^2 CI: -0.46 to -0.12; p=0.976) [39] .

High Complex Carbohydrate Diet & Total Fat Mass Loss

HCCD have also been shown to be beneficial for fat mass loss. In several studies that reported weight loss as part of the outcomes, total fat mass was not reported. Studies that did describe changes in fat mass were often more complex. For example, in a year-long study that assessed diet quality using the DASH derived diet quality score in a cohort of 93 men, those with higher diet quality scores had significantly lower fat mass as a percent of total body weight (23.4 $\pm$ 5%) than men with lower diet quality scores (28.4 $\pm$ 6.5%) [37].

High Complex Carbohydrate Diet & Visceral Adipose Tissue Loss

While there is evidence of the beneficial effects of the DASH diet on weight loss and fat mass loss, there is limited research on whether this diet has the same effect on VAT mass. As mentioned above, one study used the DASH derived diet quality score and related these scores to adipose tissue reduction 1 year after following a healthy eating/physical activity program.

This study concluded that higher DASH derived quality scores and higher physical activity, was associated with significantly greater change in VAT from baseline to 1 year (1880 $\pm$ 459 cm^3 to 1230 $\pm$ 416 cm^3; $p<0.0001$)[37]. The authors emphasized a synergistic effect between a higher diet quality score and physical activity score, but when looked at independently, there was a significant loss of VAT at 1 year among those with a higher DASH derived diet quality score.

Another study investigated consumption of whole grains versus refined grains and the effect on adipose tissue [38]. Although this is not specifically the DASH diet approach, whole grains are strongly emphasized as part of the DASH diet. Investigating how whole grains impact VAT mass can provide information on the role of complex carbohydrates in the DASH diet approach. This study concluded that the mean volume of VAT was significantly higher (1864 cm^3 CI: 1805, 1923) in the quartile of participants who consumed the lowest amount of whole grains, compared to the quartile of participants who consumed the highest amount of whole grain (1676 cm^3 CI: 1614, 1739; $p<0.001$). The authors also concluded that there is a relationship between whole grain and refined grain consumption. In participants who consumed whole grains and more than 4 servings of refined grains the effect of whole grains on VAT volume was reduced. The higher consumption of whole grains along with a reduction in refined grains within the DASH diet may be associated with the reduction of VAT volume.

In both studies discussed above, reductions in VAT and SAT were significant. Although neither study strictly implemented the DASH diet, these results suggest that VAT is significantly lower among those with a higher DASH diet quality score, increased whole grain intake and decreased refined grain intake.

Neuroendocrine Regulation of Food Intake

The central nervous system plays a large role in the regulation of food intake. Since 1953,

when the "lipostat theory" was proposed by Gordon Kennedy, it was suggested that body fat is preserved by hormones secreted by adipose tissue that send signals to the brain [40]. This physiologic regulation of food intake helps to tightly maintain the balance between energy intake and energy expenditure. The hypothalamus within the brain is the main control response center for adipose tissue derived hormones, such as leptin, to interact with orexigenic and anorexigenic neurons that either stimulate or suppress appetite.

The Role of Leptin in Energy Balance and Body Composition

Overview

Leptin is a peptide hormone comprised of 167 amino acids that form a four-helix bundle structure typical of the class-I family of cytokines [41]. Leptin is mainly secreted by white adipose tissue in a rhythmic fashion with higher circulating concentrations at night reaching peak concentrations around 2:00 AM [42,43] . This adipocyte derived hormone is either bound to a leptin receptor in the hypothalamus or circulates in free form [44]. The circulating concentration of leptin is positively correlated with body fat mass; and, it is produced in proportion to body fat stores which communicates the amount of stored energy reserve to the central nervous system [41]. With the discovery of leptin deficient ob/ob mice and leptin receptor deficient db/db mice, it was revealed that lack of leptin or leptin receptors results in significantly increased food intake and reduced energy expenditure [41]. Both of these genetic mutations result in hyperphagia, obesity, diabetes, neuroendocrine abnormalities and infertility in humans [43].

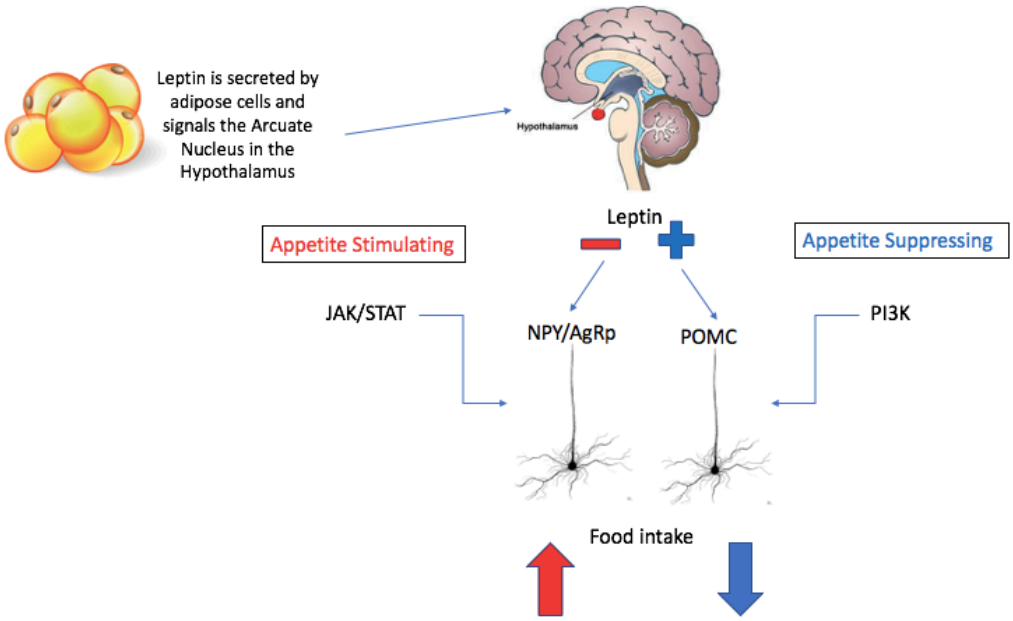


Figure 2. Leptin Action on Appetite Control. Figure modified from Kelesidis et al.[48] JAK/STAT: Janus kinase/signal transducer activator of transcription, NPY: neuropeptide Y, AgRp: agouti-related peptide, POMC: proopiomelanocortin, PI3K: phosphoinositide 3 kinase

As presented in Figure 2, leptin is secreted by white adipose tissue into the circulation. In the arcuate nucleus of the hypothalamus, leptin binds to leptin receptors to mediate its actions. Leptin stimulates two different types of neurons that increase or decrease appetite. The janus kinase/signal transducer activator of transcription (JAK/STAT) pathway stimulates appetite by synthesis of the orexigenic peptides, agouti-related peptide (AgRP) and neuropeptide Y (NPY)[41]. The phosphoinositide 3 kinase (PI3K) pathway suppresses appetite by synthesis of proopiomelanocortin (POMC)[41]. The synthesis of POMC produces α melanocyte stimulating hormone (α – MSH) signaling a decrease in appetite. When leptin signaling works correctly, leptin acts via the leptin receptor to activate the PI3K pathway stimulating the synthesis of POMC and simultaneously suppressing the JAK/STAT pathway limiting the production of NPY/AgRp [45].

Leptin & VAT

Obese individuals have been shown to have higher circulating leptin concentrations and may be resistant to leptin's effects[43,46]. The compartment in which the adipose tissue resides may have different effects on leptin secretion. In a cross-sectional study of 782 middle aged to elderly, normal weight Japanese participants, the combined effect of visceral obesity and leptin concentrations was explored [47]. In men with visceral obesity, mean visceral adipose area (154.2 $\pm$ 2.3 cm^2) was significantly higher than in men with normal weight (52.8 $\pm$ 39.8 cm^2, $p<0.001$). Leptin concentrations were also significantly higher in men with visceral obesity (4.3 $\pm$ 2.3 ng/ml) compared to those with normal weight (1.9 $\pm$ 2.3 ng/ml, $p<0.001$) [47]. Similar differences were reported for the women, but data was not provided. In another cross-sectional study of 452 obese individuals with sleep apnea, abdominal visceral adipose tissue was positively correlated with circulating leptin concentrations [48]. Mean leptin concentrations were significantly different between groups and were higher among those with higher BMI. For those with a BMI <30 kg/m^2 the mean leptin concentration was 6.7 ng/ml (CI: 6.0, 7.3), for those with a BMI of 30-35 kg/m^2 the mean leptin concentration was 10.8 ng/ml (CI: 9.8, 11.8), and for those with a BMI >35 kg/m^2 the mean leptin concentration was 17.9 ng/ml (CI: 16.1, 19.9; $p<0.001$) [48]. Leptin concentrations have been shown to be significantly higher among individuals that possess more visceral adipose tissue than those with less [48].

Circulating Leptin Concentrations & Dietary Composition

Circulating leptin concentrations may be affected by dietary macronutrient intake [49]. Leptin concentrations have been shown to be slightly lower following consumption of a high fat, low carbohydrate meal than after a low fat, high carbohydrate meal in healthy postmenopausal non-obese women [50]. After 3 days of a low carbohydrate diet (35% carbohydrate, 50% fat and

15% protein), or a high carbohydrate diet (70% carbohydrate, 15% fat, and 15% protein), mean fasting leptin concentrations were 16.9 $\pm$ 2.1 ng/ml and 18.4 $\pm$ 2.6 ng/ml; (p=0.08), respectively.

Another well-controlled feeding study investigated dietary fat and its effects on leptin concentrations on 18 middle-aged, overweight but otherwise healthy adults [51]. For the first 2 weeks, subjects consumed a baseline moderate-fat diet (35% fat, 45% carbohydrate, and 20% protein) and for the next two weeks, subjects consumed an isocaloric low-fat diet (15% fat, 65% carbohydrate, and 20% protein). During the remaining 12 weeks, the diet was fixed at 15% fat, 65% carbohydrate, and 20% protein but the subjects were instructed to only eat as much as they wished (*ad libitum*). During the *ad libitum* portion of the study, daily energy intake decreased by 16%. Mean circulating fasting leptin concentrations were not significantly different after the moderate-fat diet (17.8 ng/ml $\pm$ 1.9) compared to the isocaloric low-fat diet (17.2 $\pm$ 2.0 ng/ml). However, while consuming the low-fat *ad libitum* diet, mean fasting leptin concentrations were significantly lower than both the moderate-fat and the isocaloric low-fat diets (12.2 $\pm$ 1.8 ng/ml; p<0.001) [51]. The *ad libitum* low-fat diet led to reduced body weight with a significant reduction in mean circulating leptin concentrations. This reduction in leptin concentrations may not be due to the macronutrient content of the meals but rather to weight loss that occurred during the *ad libitum* conditions. After the *ad libitum* low-fat phase, participants had a mean weight of 70.8 $\pm$ 2.7 kg, which was significantly lower compared to the weight maintenance phases (mean weight for 15% fat: 74.6 $\pm$ 2.4 kg; for 35% fat: 74.9 $\pm$ 2.4 kg; p<0.001).

The Role of C-Reactive Protein (CRP) in Energy Balance and Body Composition

Overview of C-Reactive Protein

CRP is a pentraxin protein made up of five identical subunits arranged around a central core [52-54]. Each subunit has a phosphocholine binding site consisting of a hydrophobic pocket and two calcium ions [53,54]. CRP binds to phosphocholine molecules found in some bacterial species and the surface of dead or dying cells. Along with phosphocholine, CRP binds to other ligands that induce phagocytic cells to respond and protect the body [53].

Increased circulating CRP concentrations is part of the acute phase inflammatory response and is a sensitive marker of inflammation, infection and tissue damage. CRP is mainly synthesized in the liver with a normal circulating concentration of 0.8-10 mg/L in healthy individuals [54]. During the acute phase inflammatory response, CRP concentrations can increase up to 10,000-fold. As such, CRP is a useful marker of inflammation and infection and also a marker of response to treatment due to its rapid synthesis and rapid degradation [54].

BMI and circulating CRP concentrations are positively correlated, as are weight loss and lower CRP concentrations. This association may not be caused by the acute phase response but rather chronic inflammation associated with obesity and implication of the altered metabolic state [54]. CRP may also be useful for monitoring low-grade inflammation within obese individuals and response to treatment of metabolic syndrome caused by obesity.

CRP and VAT

A positive association between obesity and circulating CRP concentrations has been established [55,56]. Investigators have also examined whether this association changes when measuring circulating CRP concentration in relation to fat deposition. In the SMART study, a large ongoing cohort study of patients with vascular disease, abdominal VAT was measured by ultrasound imaging [55]. Subjects were ranked into quartiles according to their intra-abdominal fat

thickness (cm). In subjects with the highest intra-abdominal fat thickness (9.0-19.8 cm), median CRP concentration was 2.4 mg/L (95% CI: 1.3-4.4 mg/L) compared to subjects in the lowest intra-abdominal quartile (2.7-7.8 cm), in which median CRP concentrations were 1.1 mg/L (95% CI: 0.5-2.6 mg/L). VAT, waist circumference and BMI were positively associated with circulating CRP concentrations in both men ($p<0.01$) and women ($p<0.01$). In a cross-sectional study of generally healthy sedentary men and women, CRP and abdominal VAT, measured by dual-energy X-ray absorptiometry and abdominal computed tomography, were investigated [56]. In men, there was significant association between plasma CRP concentrations and abdominal VAT and SAT and total fat mass and abdominal fat area. In women, there was only a significant association between plasma CRP concentrations and total fat mass. This gender difference in the relationship may be caused by men having a significantly larger VAT area and significantly larger waist circumferences than the women in this study.

CRP & Dietary Consumption

Investigators have also considered the relationship between dietary composition and CRP concentrations. A well-controlled feeding study of 22 healthy post-menopausal women with a mean BMI of 29 kg/m^2 and age of 61 years investigated a low fat, high carbohydrate diet and circulating CRP concentrations. The first 4 months consisted of a eucaloric phase where all food was provided. The participants then went through 3 more phases to eventually consume a diet consisting of 14% fat and 67% carbohydrate at a stabilized weight. This was followed by 8 months of an *ad libitum* 15% fat diet in free living conditions. There was no significant weight loss from baseline to the end of the eucaloric diet. However, after the *ad libitum* low fat diet phase, mean weight was significantly lower (73.6 ± 3.4 kg vs 69.0 ± 3.3 kg; $p<0.001$), mean energy intake was significantly lower (2238 ± 68 kcal vs 1251 ± 66 kcal; $p=0.004$) and mean circulating hs-CRP concentrations were significantly lower (4.3 ± 0.6 mg/L vs 2.5 ± 0.5 mg/L;

p=0.001) [57]. Another study examined circulating CRP concentrations in 100 obese women randomly assigned to either a high carbohydrate (64% carbohydrate) diet or a high protein (46% carbohydrate) diet for 12 weeks [58]. Mean weight loss of the high protein diet group was not significantly different from the high carbohydrate group (7.6$\pm$ 0.4 kg vs 6.9 $\pm$ 0.5 kg; $p = 0.29$). There was no significant difference in circulating CRP concentrations between diets, but overall, compared to baseline, mean CRP concentrations were significantly lower in participants consuming the high protein and high carbohydrate diets (6.6 $\pm$ 0.7 mg/L vs 4.9 $\pm$ 0.6 mg/L and 4.8 $\pm$ 0.5 mg/L vs 4.0 $\pm$ 0.4 mg/L; p<0.001). These results suggest that weight loss may have a more significant effect on circulating CRP concentrations than macronutrient composition of a diet.

Chapter 3

Methods

General Design

This is a secondary analysis of data collected as part of the "Energy Balance Study", a randomized, controlled, feeding study, reviewed and approved by the OHSU Institutional Review Board (IRB), and directed by Dr. Diane Stadler. This study explored the mechanisms through which a very low-carbohydrate diet and a high complex carbohydrate diet impact weight regulation and energy balance. Participants who completed the study included 35 overweight and obese but otherwise healthy adults who met the inclusion criteria described in Table 1. All participants lived in and were recruited from the Portland, OR community.

Table 1: Inclusion and Exclusion Criteria for Participant Recruitment

Inclusion Criteria

1. BMI: 30-50 kg/m^2
2. Age: 21-65 years
3. Willing to eat either a low or high carbohydrate diet
4. Willing to stop taking vitamins, mineral, or other dietary supplements for duration of study
5. Permission from primary care provider
6. Weight stable for 6 months (no gain or loss of 10 lbs. or 3-5% of body weight)

Exclusion Criteria

1. Major mental or physical illness that would interfere with participation
2. Renal or hepatic disease, diabetes
3. History of stroke or heart disease of any kind
4. Current/history of swallowing disorders, esophageal or bowel strictures, fistulas, or gastrointestinal obstructions
5. History of gallbladder disease/removal
6. Hyper-or hypothyroidism
7. Poorly controlled hypertension
8. Use of lipid lowering medication
9. Chronic use of prescription pain medication
10. Food allergies to eggs, nuts or wheat or lactose intolerance to cheese
11. Pregnancy or lactation
12. Current moderate or excessive use of alcohol
13. Current/recent chronic use of tobacco or recreational drugs
14. Plans to leave area in next year
15. Iron deficiency anemia

Participant Recruitment and Screening

Recruitment was done through posted advertisements on the Oregon Health & Science University (OHSU) campus, research participation website and the local newspaper. The initial screening was done by telephone interview where the inclusion and exclusion criteria were described and the participant was invited to additional screenings if he/she thought they qualified. Three more screenings took place where the procedures and protocols were explained, informed consent was obtained, medical history was evaluated, biochemical and physical measurements were taken and analyzed. If the participant qualified for participation they were scheduled for a Meal Trial Week in which they were asked to come to the General Clinical Research Center (GCRC) every morning to complete routine measurements (weight, blood pressure and urine ketones) and eat breakfast. This allowed the participant to assess the time requirement and expectations of the study. Eligible and interested participants were enrolled into the study and written consent to participate in the full study was obtained.

Study Intervention

The design of the Energy Balance Study is illustrated in Figure 3. During the first 14 days of the study, participants ate a standardized diet individualized to meet each participant's energy requirements to maintain weight. On day 15 each participant spent about 26 hours in the General Clinical Research Center (GCRC) at OHSU during which time baseline measurements and timed blood sample collections were performed. For the next 5 days, each participant returned to the standardized diet and was randomized to one of three intervention groups: an *ad libitum* low-carbohydrate diet, an *ad libitum* high-complex carbohydrate diet, and an energy-matched high complex carbohydrate diet. On day 21, each participant was re-admitted to the GCRC for about 14 hours to complete additional measurements, timed blood sample collections and to initiate either the low-carbohydrate or high carbohydrate diet. Each participant

continued to consume their assigned research diet for the next 6 weeks after which they were readmitted to the GCRC to complete a third set of measurements and timed blood sample collections.

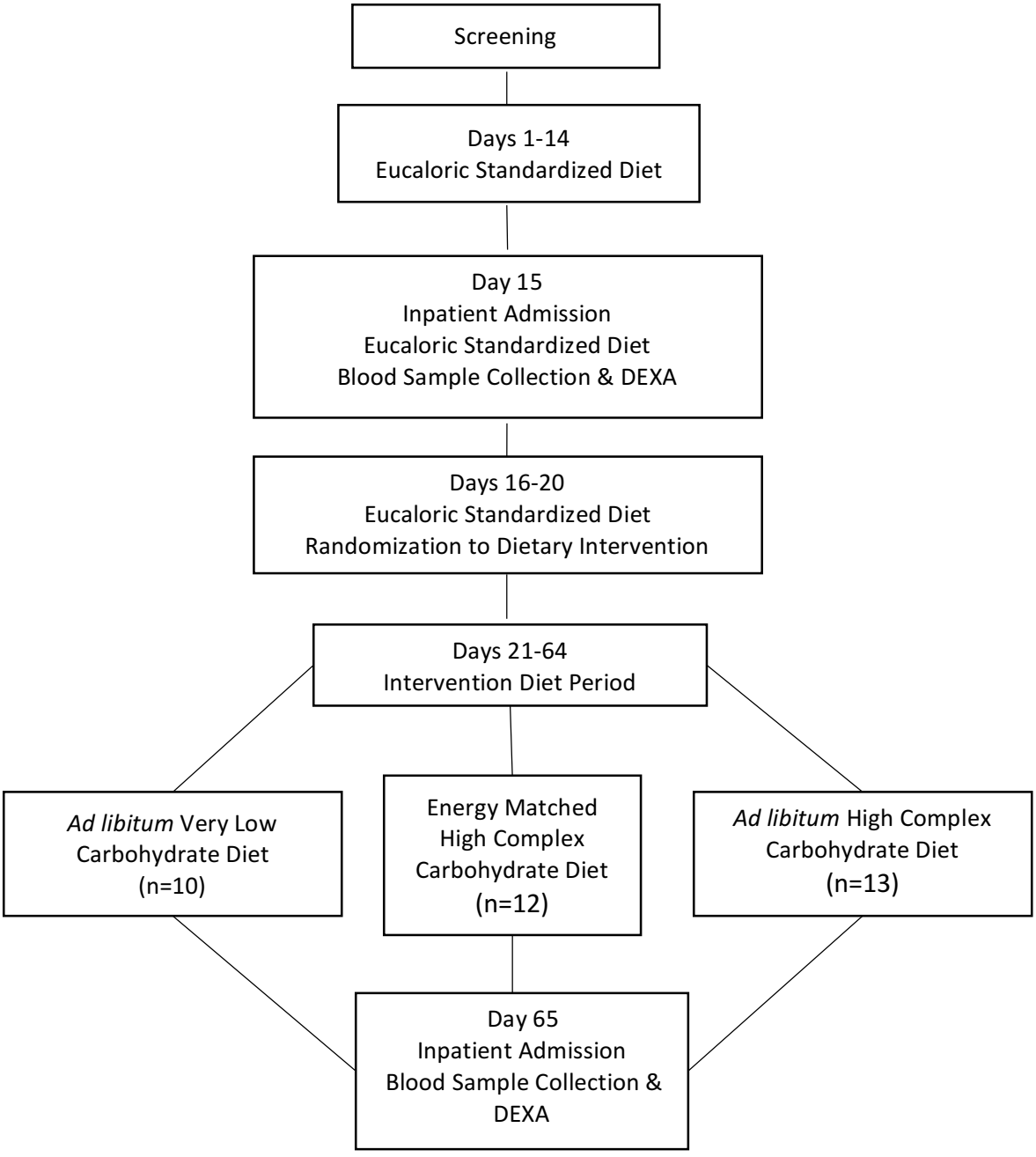


Figure 3. Modified Study Design of the Original Energy Balance Study
DEXA: Dual-Energy X-ray Absorptiometry

Description of Study Diets

Standard Eucaloric Diet

The standardized eucaloric diet consisted of 3 meals and 1 snack each day that provided 100% of the participant's estimated energy needs for weight maintenance. This diet was comprised of 15% protein, 35% fat and 50% carbohydrate. Breakfast was eaten at the GCRC on Monday, Wednesday and Friday and all other meals were provided for participants to eat off-site.

Very Low Carbohydrate Diet

The very low carbohydrate diet was limited to 28 g of carbohydrate per day and was comprised of approximately 66% fat, 30% protein and 4% carbohydrate to meet the established carbohydrate restriction. Patterned after the traditional "Atkins Diet", foods such as fruits, starchy vegetables and fluid milk were provided in very limited amounts and foods such as eggs, meat, fish, poultry, cheese, cream, oils and butter were included.

High Complex Carbohydrate Diet

The high complex carbohydrate diet consisted of approximately 58% carbohydrate, 18% protein and 27% fat. Patterned after the DASH diet, foods emphasized were fruits, vegetables, low-fat dairy products, whole grains, poultry, fish, nuts. Foods that were restricted included fats, sweets, red meat, and sugar sweetened beverages.

Estimation of Energy Requirement for Weight Maintenance

Each participant's estimated energy intake was calculated using the Boothby and Berkson Food Nomogram that takes into consideration the participant's sex, age, weight, height and physical activity level [59] and the Harris Benedict equation that includes the participant's height, weight and age[60]. Height and weight measurements taken during screening visits along

with the expertise of the Bionutritionists at the GCRC were used to determine the participant's estimated energy requirement.

Nutrient Analysis

The diets were formulated into a six-day menu cycle and generated using the ProNutra nutrient analyses system (Viocare Technologies, Inc., Princeton, NJ). This system matched the food items and recipes given to the participants to the USDA Standard 17 database and analyzed the energy and nutrient content of each participant's daily diet before and after consumption. Food items and nutrients not included in the USDA database were added to ProNutra using similar foods in another nutrient analysis database, Nutrition Data System for Research (NDSR 2008, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) [61].

Energy Intake

The participants were provided a standardized diet meeting 100% of their energy needs to maintain weight during the first 3 weeks of the study. During weeks 4-9, individuals in the *ad libitum* intervention groups were given diets with specific foods and beverages to provide 120% of their energy needs and were told to eat as much of the food as needed to satisfy their appetite. Individuals in the m-HCCD intervention group were given food and beverages that offered approximately 66% of their energy needs and were instructed to consume all of the food provided. This degree of energy restriction was based on prior studies conducted at OHSU in which it was observed that participants consuming an *ad libitum* VLCD consumed, on average, 66% of their energy needs.

Breakfast was eaten at the GCRC on Monday, Wednesday and Friday and all other meals were provided to eat off-site. Foods that were not consumed were returned to the GCRC and weighed. The weight of the returned food was entered into the ProNutra program and

subtracted from the weight of the food provided to calculate each individual's daily intake of energy, macro- and micronutrients.

Dual-Energy X-ray Absorptiometry (DEXA) Analysis

Body composition was measured by DEXA (Hologic, Inc., QDR Discovery A, Bedford, MA) on days 15 and 65 of the study. Participants changed into hospital gowns and removed any metallic objects. As illustrated in Figure 4, participants reclined on the scanning table in the supine position with arms and hands at their sides. The participant was positioned so all components of the body were in the scanning area. The participants head was positioned at the upper scan limit line with the spine aligned along the midline of the table. Whole-body DEXA scans were initially analyzed for total and regional lean mass (fat free mass plus bone mass) and fat mass composition and distribution. The same DEXA scans were reanalyzed using Hologic's Horizon DEXA system software to measure VAT within an established region of interest (ROI). The established ROI is the anatomical region located directly above the pelvis, establishing the lower limit to the upper limit defined as one-fifth the distance from the pelvis to the neck, as shown in Figure 4. The software calculated VAT area (cm^2), volume (cm^3) and mass (g) by subtracting SAT from total abdominal fat within the ROI. The DEXA scan output included total body mass, fat mass, lean mass, % fat mass, skeletal mass, and estimated VAT mass within the ROI.

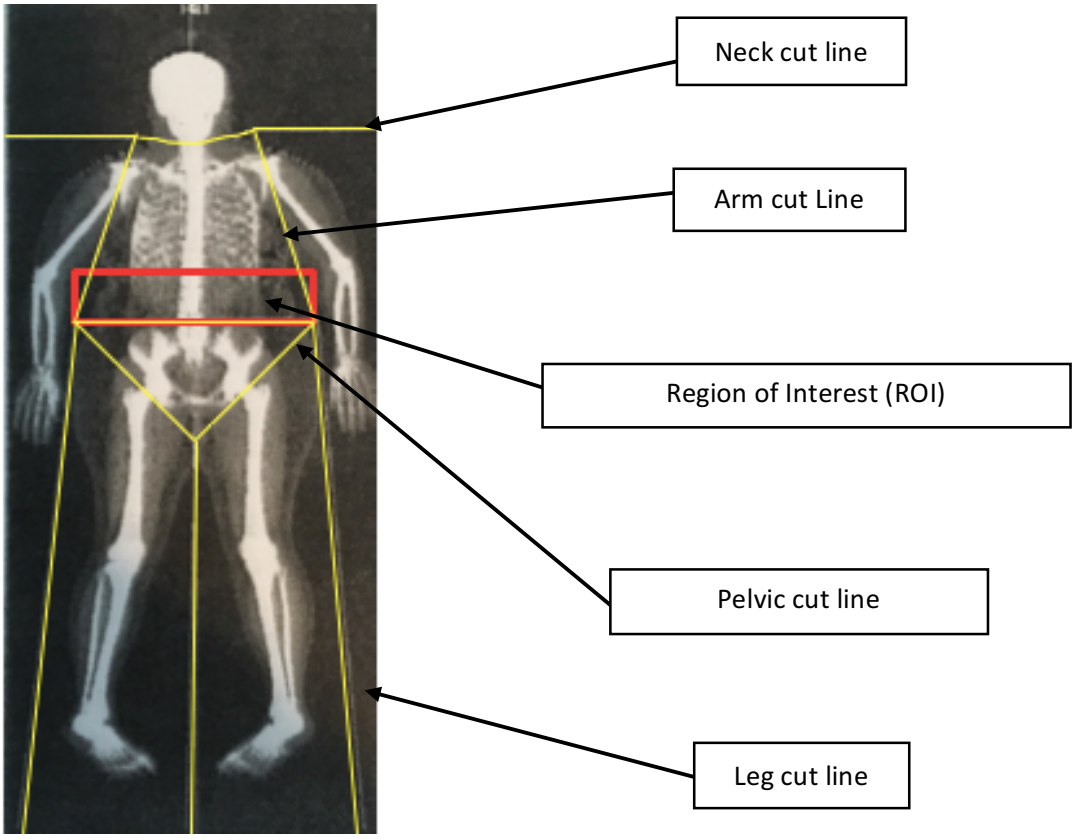


Figure 4. Dual-Energy X-Ray Absorptiometry Image
Region of Interest defined as one-fifth the distance from the pelvic cut line to the neck cut line.

Blood Sample Collection

For this sub analysis**,** fasting blood samples were collected on days 15 and 65. Tubes containing heparinized blood samples were processed immediately and tubes containing blood without anticoagulants were allowed to sit at room temperature for 20 minutes before processing. All tubes were centrifuged for 12 minutes at 2600 rpm under 4°C refrigerated conditions. Serum samples for leptin analysis and heparin plasma samples for hs-CRP analysis were collected and divided into polypropylene aliquot tubes. All tubes were frozen at -20°C and then transferred within 24 hours to -80°C freezers and stored frozen until analysis.

Analytical Methods

Leptin and hs-CRP concentrations were measured by research staff of the core biochemistry lab at the GCRC. Serum leptin concentrations were measured in duplicate using an immunoradiometric assay (IRMA) (Diagnostic Systems Laboratories Inc., Webster, TX). The lowest concentration of leptin able to be detected by the assay was 0.5 ng/ml. Plasma high sensitivity CRP (hs-CRP) was measured using a high sensitivity latex-enhanced turbidimetric *in vitro* immunoassay (Catalog # LKCR1, Immulite/Immulite 1000 (Siemens), Deerfield, IL) [62]. The minimum limit of detection of CRP was at 0.1 mg/L; the interassay %CV was between 7.0-7.6%.

Statistical Analysis

Data from the DEXA analyses and blood sample analyses were recorded and stored in an Excel spreadsheet, Microsoft Office 2017 version 15.33. All statistical analyses were performed using Stata/IC 15.0 for Mac. Demographic data including sex, age and race were recorded and frequencies and proportions were calculated for each categorical variable. Anthropometric data including height, weight, BMI, total body mass, total fat mass, percent fat mass, abdominal VAT mass, VAT volume, VAT area, android fat mass, android total mass, gynoid fat mass, and gynoid total mass derived from the participant's DEXA scans were recorded for days 15 and 65. Fasting serum leptin and hs-CRP concentrations were recorded for days 15 and 65. Summary statistics including means, minimum, maximum and standard deviations and 95% confidence intervals were calculated for each continuous variable.

Analysis of variance (ANOVA) was used to test whether baseline, post-intervention or differences between post-intervention and baseline means were different among the three dietary intervention groups (HCCD, VLCD, and m-HCCD). T-tests (based off the ANOVA) were used to determine if any two groups were significantly different from one another. In particular, we examined the mean VLCD response compared to the HCCD and m-HCCD response and also

the mean HCCD response was compared to the m-HCCD response for each variable, as presented in Figure 5. Bland-Altman plots were used to illustrate how changes over time potentially differed among the three treatment groups. Spearman tests were used to determine the correlation between the average of post-intervention and baseline values and the difference between post-intervention and baseline values. Pearson's correlations were used to determine if any change in VAT mass was correlated with the change in total body mass, total fat mass, and fasting serum leptin and plasma hs-CRP concentrations. Spearman's tests were also used to determine if an observed correlation was driven by outlier data.

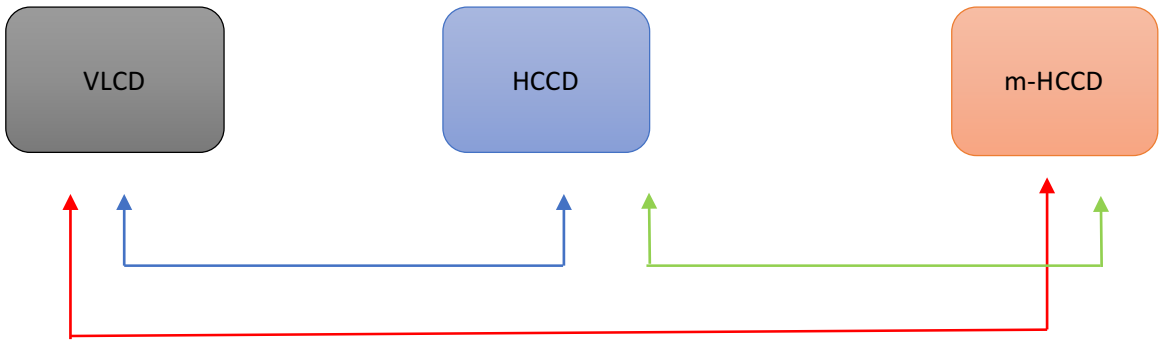


Figure 5. Comparison Between Treatment Groups
VLCD: Very Low Carbohydrate Diet, HCCD: High Complex Carbohydrate Diet, m-HCCD: Energy-Matched High Complex Carbohydrate Diet

Chapter 4

Results

This secondary analysis, conducted using data collected as part of a randomized, controlled feeding study, was completed to determine the impact of very low-carbohydrate and high complex-carbohydrate diets on changes in abdominal visceral adipose tissue and circulating concentrations of leptin and hs-CRP in overweight and obese participants. Data obtained for 35 participants were included in the analysis: 10 in the VLCD group, 13 in the HCCD group and 12 in the m-HCCD group.

Baseline Characteristics

Demographic Characteristics

As designed, and as presented in Table 2, mean age, and sex and race distribution were similar between groups. Of the 35 participants analyzed, 30 were female and 5 were male; 27 were white and 7 were nonwhite; and the average age was 45.5 ± 9.8 years old.

Table 2. Participant Demographic Characteristics at Baseline

Baseline Characteristic	Diet Group			P-value*
	Very-low-Carbohydrate (n= 10)	**High-Complex-Carbohydrate** (n= 13)	**Matched-High-Complex-Carbohydrate** (n=12)	
Age (yrs.) **	45 ± 7.9 [33,58]	47 ± 10.7 [27,62]	44 ± 10.8 [25, 60]	0.78
Sex ***				0.59
Male	1 (10)	3 (23.1)	1 (8.3)	
Female	9 (90)	10 (76.9)	11 (91.7)	
Race ***				0.24
White	6 (60)	10 (83.3)	11 (91.7)	
Non-White	4 (40)	2 (16.6)	1 (8.3)	

*P-value of difference between groups
**Values represent Mean ± SD [Min, Max]
***Values represent Number (Percent)

Body Composition Characteristics at Baseline

At baseline, mean body composition measurements were similar between dietary intervention groups as presented in Table 3. Mean BMI in the VLCD, HCCD and m-HCCD groups was 36.6 ± 4.6 kg/m^2, 34.6 ± 4.7 kg/m^2 and 36.2 ± 4.5 kg/m^2, respectively; (p=0.56). Mean weight in the VLCD, HCCD and m-HCCD groups was 103.3 ± 13 kg, 99.3 ± 14.3 kg, and 96.9 ± 10.8kg, respectively; (p=0.48). Mean total fat mass in the VLCD, HCCD and m-HCCD groups was 46.1 ± 11.8 kg, 43.2 ± 8.1 kg, and 43.6 ± 7.5 kg, respectively; (p=0.80). Mean total percent fat mass in the VLCD, HCCD and m-HCCD was 44.3 ± 7.1%, 43.8 ± 4.8%, 44.9 ± 3.7%, respectively; (p=0.82). Overall, mean BMI was 35.7 ± 4.6 kg/m^2, mean weight was 99.6 ± 12.7 kg, mean total fat mass was 44.2 ± 8.9 kg, and mean percent body fat mass was 43.9 ± 5.2 %. On average, gynoid fat mass, 7.3 ± 1.6 kg, was higher than android fat mass, 3.9 ± 0.92 kg.

Table 3. Participant Body Composition Characteristics at Baseline

Baseline Characteristic	Diet Group			P-value*
	Very-low-Carbohydrate (n= 10)	**High-Complex-Carbohydrate** (n= 13)	**Matched-High-Complex-Carbohydrate** (n=12)	
BMI (kg/m^2) **	36.6 ± 4.6 [30.9, 46.0]	34.6± 4.7 [30.2, 47.0]	36.2 ± 4.5 [29.6, 43.3]	0.56
Weight (kg)	103.3 ± 13 [84.1, 126]	99.3 ± 14.3 [78.3, 123]	96.9 ± 10.8 [84.4, 117]	0.48
Total Mass (kg)	103 ± 13 [83.8, 125]	98.9 ± 14.1 [77.5, 120.7]	96.8 ± 10.7 [84.4, 117]	0.49
Total Fat Mass (kg)	46.1 ± 11.8 [24.3, 64.7]	43.2 ± 8.1 [34.6, 62.5]	43.6 ± 7.5 [30.9, 57.4]	0.80
Total % Fat Mass	44.3 ± 7.1 [26.1, 51.8]	43.8 ± 4.8 [35.5, 51.8]	44.9 ± 3.7 [34.9, 49.0]	0.82
Android Fat Mass (kg)	3.8 ± 0.98 [2.3, 5.8]	4.0 ± 1.0 [2.7, 6.7]	3.8 ± 0.8 [2.6, 5.5]	0.80
Android Total Mass (kg)	8.3 ± 1.6 [2.3, 5.8]	8.4 ± 1.7 [6.1, 11.5]	8.1 ± 1.4 [6.4, 10.9]	0.88
Gynoid Fat Mass (kg)	7.6 ± 1.94 [3.8, 9.6]	7.2 ± 1.6 [5.1, 9.6]	7.3 ± 1.3 [4.4, 9.5]	0.90
Gynoid Total Mass (kg)	16.9 ± 2.3 [13, 10.3]	16.2 ± 2.6 [11.8, 20.0]	16.1 ± 2.1 [13.3, 20.5]	0.71

*P-value of difference between groups

**All values are presented as Mean ± SD, [Min, Max]

BMI = Body Mass Index

Weight represents standing weight measured by electronic scale, total mass represents total body mass measured by DEXA.

Visceral Adipose Tissue Measurements at Baseline

Mean VAT measurements were similar among dietary intervention groups at baseline as shown in Table 4. For each dietary intervention group, mean VAT mass, mean VAT volume and mean VAT area within the android region of interest (ROI) are presented. Mean VAT mass in the VLCD, HCCD and m-HCCD groups was 0.73 ± 0.17 kg, 0.82 ± 0.17 kg, and 0.72 ± 0.18 kg, respectively; (p=0.33). Mean VAT volume in the VLCD, HCCD and m-HCCD groups was 791 ± 181.3 cm^3, 883 ± 187.9 cm^3, and 777 ± 197.9 cm^3, respectively; (p=0.33). Mean VAT area in the VLCD, HCCD and m-HCCD groups was 152 ± 34.8 cm^2, 170 ± 36 cm^2, and 149 ± 38 cm^2, respectively; (p=0.33). Overall, the mean VAT mass was 0.75 ± 0.17 kg. The amount of VAT mass relative to the amount of total body mass and total fat mass is shown as a percent of total body mass (PVAT-TBM) and percent of total fat mass (PVAT-TFM). On average, PVAT-TBM was 1.7 ± 0.45 % and PVAT-TFM was 0.76 ± 0.15 %.

Table 4. Visceral Adipose Tissue Mass, Volume and Area Within the Region of Interest at Baseline

Baseline Characteristic	**Diet Group**			**P-value***
	Very-low-Carbohydrate (n= 10)	**High-Complex-Carbohydrate** (n= 13)	**Matched-High-Complex-Carbohydrate** (n=12)	
VAT Mass (kg)**	0.73 ± 0.17 [0.46, 1]	0.82 ± 0.17 [0.57, 1.1]	0.72 ± 0.18 [0.42, 1.1]	0.33
PVAT-TBM (%)	0.71 ± 0.13 [0.52, 0.90]	0.83 ± 0.16 [0.61, 1.1]	0.73 ± 0.14 [0.48, 0.99]	0.15
PVAT-TFM (%)	1.6 ± 0.55 [1.1, 2.9]	1.9 ± 0.42 [1.4, 2.4]	1.6 ± 0.37 [1.1, 2.3]	0.24
VAT Volume (cm^3)	791 ± 181.3 [503,1109]	883 ± 187.9 [618, 1204]	777 ± 197.9 [462, 1207]	0.33
VAT Area (cm^2)	152 ± 34.8 [96.6, 213]	170 ± 36 [119, 231]	149 ± 38 [88.6, 232]	0.33

*P-value of difference between groups

** All values are presented as Mean ± SD [Min, Max].

PVAT-TBM = Percent VAT of Total Body Mass, PVAT-TFM = Percent VAT of Total Fat Mass

Fasting Serum Leptin and Plasma hs-CRP Concentrations at Baseline

Mean fasting leptin and hs-CRP concentrations were similar between intervention groups at baseline as shown in Table 5. Mean fasting serum leptin in the VLCD, HCCD and m-HCCD groups was 11.3 ± 6.35 ng/ml, 12.1 ± 6.4 ng/ml, and 11.7 ± 6.1 ng/ml, respectively; (p=0.96). Mean fasting plasma hs-CRP in the VLCD, HCCD and m-HCCD was 5.2 ± 3.75 mg/L, 4.9 ± 3.7 mg/L, and 4.0 ± 2.1 mg/L, respectively; (p=0.59). Overall, the average leptin concentration was 11.6 ± 6.1 ng/ml and the average hs-CRP concentration was 4.7 ± 3.2 mg/l. Since leptin is secreted from adipose tissue, leptin concentration was also analyzed indexed to total fat mass. Mean hs-CRP concentration was evaluated with and without one participant deemed to be an outlier with a post-intervention hs-CRP concentration that reflected recent injury.

Table 5. Fasting Serum Leptin and Plasma hs-CRP Concentrations at Baseline

Baseline Characteristic	**Diet Group**			**P-value***
	Very-low-Carbohydrate (n= 10)	**High-Complex-Carbohydrate** (n= 13)	**Matched-High-Complex-Carbohydrate** (n=12)	
Leptin (ng/ml) **	11.3 ± 6.35 [1.5, 23.5]	12.1 ± 6.4 [5.5, 27.5]	11.7 ± 6.1 [5.8, 29.2]	0.96
Leptin (ng/ml · kg) [a]	0.21 ± 0.17 [0.04, 0.67]	0.27 ± 0.15 [0.12, 0.67]	0.26 ± 0.13 [0.10, 0.66]	0.95
hs-CRP (mg/L)	5.2 ± 3.75 [0.74, 11.2)	4.9 ± 3.7 [0.8, 11.6]	4.0 ± 2.1 [1.1,7.8]	0.59
hs-CRP (mg/L) [b]	5.7 ± 3.6 [1.7, 11.2]	4.9 ± 3.7 [0.8, 11.6]	4.0 ± 2.2 [1.1, 7.8]	0.43

*P-value of difference between groups

** All values are presented as Mean ± SD [Min, Max].

[a] Leptin indexed to total fat mass

[b] Indicates analysis without the outlier.

Change in Body Composition Parameters from Baseline to 6 Weeks Within and Between Dietary Intervention Groups

Change in body composition variables from baseline to 6 weeks within and between groups are presented in Table 6. Mean weight loss in the VLCD, HCCD, and m-HCCD groups was -5.0 ± 0.91 kg, -2.8 ± 0.54 kg, and -3.08 ± 0.52, respectively; (p=0.14). Mean BMI reduction in the VLCD, HCCD, and m-HCCD groups was -1.8 ± 0.3 kg/m^2, -0.98 ± 0.18 kg/m^2 , and -1.1 ± 0.20 kg/m^2, respectively; (p=0.11). Mean total fat mass loss in the VLCD, HCCD, and m-HCCD groups was -2.8 ± 0.44 kg, -1.5 ± 0.38 kg, and -2.4 ± 0.36 kg, respectively; (p=0.08). Overall, with participants in all dietary intervention groups combined, there was significant weight loss (-3.51 ± 0.40 kg; p<0.001), BMI reduction (-1.25 ± 0.14; P<0.0001), and total fat mass loss (-2.12 ± 0.24 kg; p<0.0001). The mean difference between post-intervention and baseline for total percent fat mass was significantly lower at 6 weeks than baseline in the m-HCCD group (-1.22 ± 0.21; p<0.0001) and the VLCD group (-0.65 ± 0.30; p=0.036) but not in the HCCD group (-0.41 ± 0.26; P=0.132). There were no significant differences in mean body composition variables between diet intervention groups.

Table 6. Change in Body Composition Parameters from Baseline to 6 Weeks Within and Between Dietary Intervention Groups

	Baseline	Post-intervention	Difference from Baseline	P-value*	
	Day 15	Day 65	Δ week 6	Within Group	Between Groups
Weight (kg) **					
VLCD (n=10)	103.3 ± 4.1 (94.9, 111.6)	98.3 ± 3.9 (90.3, 106.3)	-5.0 ± 0.91 (-6.8, -3.1)	<0.001	
HCCD (n=13)	99.3 ± 4.0 (91.2, 107.5)	96.5 ± 3.9 (88.5, 104.5)	-2.8 ± 0.54 (-3.9, -1.7)	<0.0001	
m-HCCD (n=12)	96.9 ± 3.1 (90.6, 103.3)	93.9 ± 3.17 (87.4, 100.3)	-3.08 ± 0.52 (-4.2, -2.0)	<0.0001	
Overall (n=35)	99.6 ± 2.2 (95.3, 104.0)	96.1 ± 2.1 (91.9, 100.4)	-3.5 ± 0.4 (-4.3, -2.7)	<0.0001	0.14
Body Mass Index (kg/m^2)					
VLCD (n=10)	36.6 ± 1.5 (33.6, 39.5)	34.8 ± 1.4 (32, 37.6)	-1.8 ± 0.3 (-2.4, -1.1)	<0.0001	
HCCD (n=13)	34.6 ± 1.3 (31.9, 37.3)	33.6 ± 1.3 (31, 36.3)	-0.98 ± 0.18 (-1.3, -0.61)	<0.0001	
m-HCCD (n=12)	36.2 ± 1.3 (31.9, 37.3)	35.1 ± 1.3 (32.5, 37.7)	-1.1 ± 0.20 (-1.3, -0.61)	<0.0001	
Overall (n=35)	35.7 ± 0.78 (34.1, 37.3)	34.5 ± 0.75 (32.9, 36)	-1.3 ± 0.14 (-1.5, -0.97)	<0.0001	0.11

Table 6. Continued

	Baseline	Post-intervention	Difference from Baseline	P-value*	
	Day 15	Day 65	Δ week 6	Within Group	Between Groups
Total Fat Mass (kg)					
VLCD (n=10)	46.2 ± 3.7 (38.6, 53.68)	43.4 ± 3.59 (36, 50.7)	-2.8 ± 0.44 (-3.7, -1.9)	<0.0001	
HCCD (n=13)	43.3 ± 2.3 (38.7, 47.9)	41.8 ± 2.3 (37.1, 46.5)	-1.5 ± 0.38 (-2.3, -0.72)	<0.0001	
m-HCCD (n=12)	43.6 ± 2.2 (39.2, 48.1)	41.2 ± 2.1 (36.9, 45.5)	-2.4 ± 0.36 (-3.1, -1.7)	<0.0001	
Overall (n=35)	44.2 ± 1.5 (41.1, 47.3)	42 ± 1.5 (39, 45.1)	-2.1 ± 0.24 (-2.6, -1.6)	<0.0001	0.08
Total Percent Fat Mass					
VLCD (n=10)	44.3 ± 2.2 (39.7, 48.9)	43.6 ± 2.4 (38.8, 48.4)	-0.65 ± 0.30 (-1.3, -0.04)	0.036	
HCCD (n=13)	43.8 ± 1.32 (41.1, 46.5)	43.4 ± 1.37 (40.6, 46.2)	-0.41 ± 0.26 (-0.95, 0.13)	0.132	
m-HCCD (n=12)	44.9 ± 1.08 (42.67, 47.08)	43.65 ± 1.12 (41.38, 45.92)	-1.22 ± 0.21 (-1.65, -0.80)	<0.0001	
Overall (n=35)	44.3 ± 0.87 (42.5, 46.1)	43.6 ± 0.9 (41.7, 45.4)	-0.76 ± 0.16 (-1.1, -0.44)	<0.0001	0.05***

*P-value of difference within and between groups

**Values represent Mean ± SEM (95% Confidence Interval)

*** Follow up pairwise test indicates p-value non-significant after Bonferroni correction (α =0.016).

Change in Visceral Adipose Tissue from Baseline to 6 Weeks Within and Between Dietary Intervention Groups

Mean VAT mass, area, and volume and VAT mass as a percent of total fat mass (PVAT-TFM) and as a percent of total body mass (PVAT-TBM) at baseline and 6 weeks after dietary intervention are presented in Table 7 as are the mean changes in these parameters between the two-time points. There were no significant differences in mean VAT parameters at baseline or at 6 weeks within the dietary intervention groups, nor were there differences in the mean change

of VAT parameters from baseline to 6 weeks after dietary intervention between groups. Overall, the participants lost -0.03 ± 0.02 kg (CI: -0.08, 00.02) in VAT mass; -6.07 ± 4.74 cm^2 (CI: -15.71, 3.57) in VAT area; and -31.06 ± 24.72 $cm^{3,}$ (CI: -81.3, 19.18) in VAT volume.

Table 7. Change in Visceral Adipose Tissue from Baseline to 6 Weeks Within and Between Dietary Intervention Groups

	Baseline	Post-intervention	Difference from Baseline	P-value*	
	Day 15	Day 65	Δ week 6	Within Group	Between Groups
VAT Mass (kg)**					
VLCD (n=10)	0.73 ± 0.05 (0.62, 0.84)	0.69 ± 0.05 (0.59, 0.79)	-0.04 ± 0.03 (-0.09, 0.01)	0.12	
HCCD (n=13)	0.82 ± 0.05 (0.72, 0.92)	0.81 ± 0.08 (0.64, 0.99)	-0.00 ± 0.05 (-0.11, 0.11)	0.97	
m-HCCD (n=12)	0.72 ± 0.05 (0.61, 0.83)	0.67 ± 0.06 (0.56, 0.78)	-0.05 ± 0.03 (-0.11, 0.01)	0.12	
Overall (n=35)	0.76 ± 0.03 (0.70, 0.82)	0.73 ± 0.04 (0.65, 0.81)	-0.03 ± 0.02 (-0.08, 0.02)	0.22	0.76
VAT Area (cm^2)					
VLCD (n=10)	151.7 ± 10.9 (129.4, 173.9)	143.2 ± 10.6 (121.7, 164.7)	-8.5 ± 5.3 (-19.3, 2.3)	0.12	
HCCD (n=13)	169.5 ± 10.0 (149.1, 190)	168.9 ± 17.5 (133.3, 204.4)	-0.69 ± 11.0 (-23.2, 21.8)	0.95	
m-HCCD (n=12)	149.1 ± 11.0 (126.7, 171.5)	139.3 ± 11.5 (115.8, 162.7)	-9.9 ± 6.0 (-22.2, 2.4)	0.11	
Overall (n=35)	157.4 ± 6.2 (144.9, 170.0)	151.4 ± 8.3 (134.6, 168.1)	-6.1 ± 4.5 (-15.7, 3.6)	0.21	0.76

Table 7. Continued

	Baseline	Post-intervention	Difference from Baseline	P-value*	
	Day 15	Day 65	Δ week 6	Within Group	Between Groups
VAT Volume (cm^3)					
VLCD (n=10)	790.9 ± 56.9 (675.0, 906.8)	746.0 ± 55.1 (634.0, 858.5)	-44.7 ± 27.7 (-101.1, 11.7)	0.12	
HCCD (n=13)	883.2 ± 52.4 (776.5, 989.8)	880.9 ± 91.0 (695.5, 1066.2)	-2.3 ± 57.5 (-119.3, 114.7)	0.97	
m-HCCD (n-12)	776.9 ± 57.2 (660.4, 893	726.1 ± 60.0 (603.9, 848.3)	-50.8 ± 31.4 (-114.9, 13.2)	0.12	
PVAT-TFM (%)					
VLCD (n=10)	1.7 ± 0.17 (1.3, 2.0)	1.7 ± 0.16 (1.3, 2.0)	0.01 ± 0.05 (-0.10, 0.12)	0.87	
HCCD (n=13)	1.9 ± 0.12 (1.7, 2.2)	2.0 ± 0.14 (1.7, 2.2)	0.04 ± 0.09 (-0.15, 0.22)	0.70	
m-HCCD (n=12)	1.7 ± 0.11 (1.4, 1.9)	1.6 ± 0.10 (1.4, 1.8)	-0.03 ± 0.06 (-0.15, 0.09)	0.66	
Overall (n=35)	1.8 ± 0.08 (1.6, 1.9)	1.8 ± 0.08 (1.6, 1.9)	0.01 ± 0.04 (-0.08, 0.09)	0.87	0.83
PVAT-TBM (%)					
VLCD (n=10)	0.71 ± 0.04 (0.62, 0.08)	0.70 ± 0.04 (0.62, 0.79)	-0.01 ± 0.02 (-0.06, 0.04)	0.78	
HCCD (n=13)	0.83 ± 0.05 (0.74, 0.93)	0.85 ± 0.07 (0.70, 1.0)	0.02 ± 0.05 (-0.08, 0.11)	0.71	
m-HCCD (n=12)	0.74 ± 0.04 (0.65, 0.82)	0.71 ± 0.04 (0.62, 0.80)	-0.03 ± 0.03 (-0.08, 0.03)	0.29	
Overall (n=35)	0.76 ± 0.03 (0.71, 0.82)	0.76 ± 0.03 (0.69, 0.83)	-0.01 ± 0.02 (-0.05, 0.04)	0.80	0.66

* P-value of difference within and between groups

** Values represent Mean ± SEM (95% Confidence Interval)

PVAT-TBM: Percent VAT of Total Body Mass, PVAT-TFM: Percent VAT of Total Fat Mass,

Change in Fasting Leptin and hs-CRP Concentrations from Baseline to 6 Weeks Within and Between Dietary Intervention Groups

Mean fasting concentrations of serum leptin and plasma hs-CRP at baseline and 6 weeks after dietary intervention are presented in Table 8. Leptin is expressed as an absolute concentration (ng/ml) and indexed to total fat mass (ng/ml · kg). In each group, there was a significant reduction in mean fasting serum leptin concentration after the dietary intervention. The VLCD group had a reduction in mean fasting serum leptin of -2.92 ± 0.93 ng/ml ($p=0.004$), the HCCD group had a reduction in mean fasting leptin of -1.33± 0.46 ng/ml ($p=0.007$) and the m-HCCD had a mean reduction in mean fasting leptin of -1.40 ± 0.36 ng/ml ($p<0.0001$). When leptin was indexed to total fat mass, mean fasting leptin concentration was no longer lower after the intervention than before in the HCCD group -0.2 ± 0.01 ($p=0.136$). The reduction in mean serum leptin concentrations was not significantly different between groups ($p=0.2925$), whether expressed as an absolute value or indexed to total fat mass

Table 8 also shows the summary data of fasting plasma hs-CRP concentrations. Analyses were performed with and without data from one participant from the VLCD group who had a dramatic increase in circulating hs-CRP concentration at week 6 that was presumably associated with a recent injury. There were no significant differences in mean fasting plasma hs-CRP concentrations before or after the dietary intervention within, or between groups.

Table 8. Change in in Fasting Serum Leptin and Plasma hs-CRP Concentrations from Baseline to 6 weeks Within and Between Dietary Intervention Groups

	Baseline	Post intervention	Difference from Baseline	P-value*	
	Day 15	Day 65	Δ week 6	Within Group	Between Groups
Leptin (ng/ml) **					
VLCD (n=10)	11.3 ± 2.1 (7.0, 15.5)	8.3 ± 2.0 (4.2, 12.5)	-2.9 ± 0.93 (-4.8, -1.0)	0.004	
HCCD (n=13)	12.1 ± 1.8 (8.4, 15.7)	10.7 ± 1.8 (7.0, 14.5)	-1.3 ± 0.46 (-2.3, -0.39)	0.007	
m-HCCD (n=12)	11.8 ± 1.8 (8.2, 15.4)	10.4 ± 1.8 (6.7, 14.2)	-1.4 ± 0.36 (-2.1, -0.67)	<0.0001	
Overall (n=35)	11.6 ± 1.0 (9.5, 13.7)	9.8 ± 1.1 (7.7, 12.0)	-1.8 ± 0.33 (-2.5, -1.1)	<0.0001	0.29
Leptin Indexed to Fat Mass (ng/ml · kg)					
VLCD (n=10)	0.26 ± 0.06 (0.13, 0.39)	0.20 ± 0.06 (0.08, 0.33)	-0.06 ± 0.02 (-0.10, -0.02)	0.01	
HCCD (n=13)	0.28 ± 0.04 (0.20, 0.37)	0.26 ± 0.05 (0.17, 0.36)	-0.02 ± 0.01 (-0.05, 0.01)	0.14	
m-HCCD (n=12)	0.27 ± 0.04 (0.19, 0.34)	0.25 ± 0.04 (0.16, 0.33)	-0.02 ± 0.01 (-0.04, 0.00)	0.05	
Overall (n=35)	0.27 ± 0.03 (0.22, 0.32)	0.24 ± 0.03 (0.18, 0.29)	-0.03 ± 0.01 (-0.05, -0.01)	0.001	0.29

Table 8. Continued

	Baseline	Post intervention	Difference from Baseline	P-value*	
	Day 15	Day 65	Δ week 6	Within Group	Between Groups
Hs-CRP (mg/L)					
VLCD (n=10)	5.2 ± 1.2 (2.8, 7.6)	9.1 ± 4.7 (-0.55, 18.7)	3.9 ± 5.3 (-6.8, 14.6)	0.46	
HCCD (n=13)	4.9 ± 1.0 (2.8, 7.0)	4.1 ± 0.83 (2.4, 5.8)	-0.80 ± 0.63 (-2.1, 0.48)	0.21	
m-HCCD (n=12)	4.0 ± 0.63 (2.6, 5.3)	3.9 ± 0.85 (2.2, 5.6)	-0.11 ± 0.84 (-1.8, 1.6)	0.90	
Overall (n=35)	4.7 ± 0.54 (3.6, 5.8)	5.5 ± 1.4 (2.6, 8.4)	0.78 ± 1.5 (-2.4, 3.9)	0.62	0.57
Hs-CRP, (mg/L) ***					
VLCD (n=9)	5.7 ± 3.6 (3.3, 8.1)	4.4 ± 0.73 (2.9, 5.9)	-1.3 ± 1.0 (-3.4, 0.73)	0.20	
HCCD (n=13)	4.9 ± 3.7 (2.8, 7.1)	4.1 ± 0.83 (2.4, 5.8)	-0.80 ± 0.63 (-2.1, 0.48)	0.21	
m-HCCD (n=12)	4.0 ± 2.2 (2.7, 5.3)	3.9 ± 0.85 (2.2, 5.6)	-0.11 ± 0.84 (-1.8, 1.6)	0.90	
Overall (n=35)	4.8 ± 0.55 (3.7, 5.9)	4.12 ± 0.46 (3.2, 5.1)	-0.69 ± 0.46 (-1.6, 0.24)	0.14	0.64

* P-value of difference within and between groups

** Values represent Mean ± SEM (95% Confidence Interval)

***Indicates hs-CRP analysis without outlier

Correlation Between Change in VAT Mass and Change in Total Body Mass

The correlation between change in VAT mass and change in total body mass, measured by DEXA, is shown in Figure 6. There was no significant correlation between change in VAT mass and change in body mass in any of the dietary intervention groups individually or pooled. In the VLCD group (Panel A), nine of the ten participants lost body mass. The change in body mass ranged from -9.7 to 0.02 kg. Seven of the ten participants lost VAT mass, while three participants gained VAT mass. The change in VAT mass ranged from -0.18 to 0.05 kg. Two participants who lost body mass, gained VAT mass while one participant gained both body mass and VAT mass.

In the HCCD group (Panel B), 12 participants lost body mass while one participant gained body mass. The change in body mass ranged from (-5.6 to 0.99 kg). Of the 12 participants in the HCCD group who lost body mass, 8 participants also lost VAT mass while four participants gained VAT mass. One participant gained body mass and VAT mass.

In the m-HCCD group (Panel C), 11 participants lost body mass and one individual gained body mass. The change in body mass ranged from (-4.2 to 0.26 kg). Nine of the 12 participants lost VAT mass while four participants gained VAT mass ranging from (-0.3 to 0.10 kg). The majority of the participants lost body mass and VAT mass. However, two individuals lost body mass, but gained VAT mass and one participant gained both body mass and VAT mass.

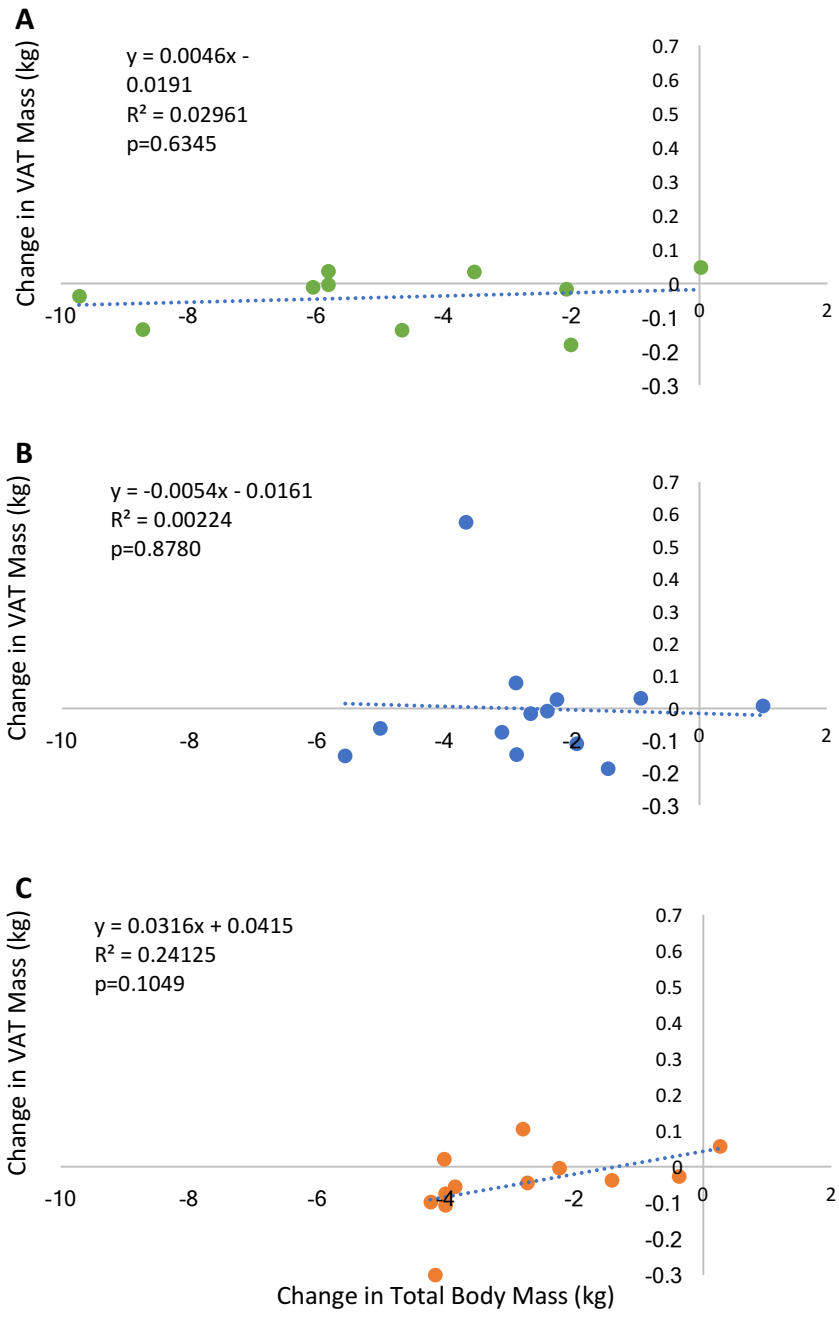


Figure 6. Correlation Between Change in VAT Mass and Change in Total Body Mass.
Panel A represents the VLCD Group, (n=10), Panel B represents the HCCD Group, (n=13), and Panel C represents the m-HCCD Group, (n=12).

Correlation Between Change in VAT Mass and Change in Total Fat Mass

The correlations between change in VAT mass and change in total fat mass are shown in Figure 7. There were no significant correlations between these two parameters in any of the dietary intervention groups. All participants in the VLCD group (Panel A) lost total fat mass. The change in total fat mass ranged from -4.8 to -0.20 kg. Of these participants 7 of the 10 also lost VAT mass while 3 participants gained VAT mass. The change in VAT among these participant's mass ranged from -0.18 to 0.05 kg.

In the HCCD group (Panel B), 12 of the 13 participants lost total fat mass while one participant gained total fat mass. The change in total fat mass in the HCCD group ranged from -4.3 to 0.27 kg. Of the HCCD participants who lost total fat mass, 8 of the 12 participants also lost VAT mass while 4 gained VAT mass. The change in VAT mass ranged from -0.186 to 0.574 kg.

In the m-HCCD group (Panel C), all participants lost total fat mass. The change in total fat mass ranged from -4.8 to -0.22 kg and 9 of the 12 participants also lost VAT mass. The change in VAT mass ranged from -0.3 to 0.10 kg. Overall, total fat mass loss and VAT mass loss were similar among the three groups.

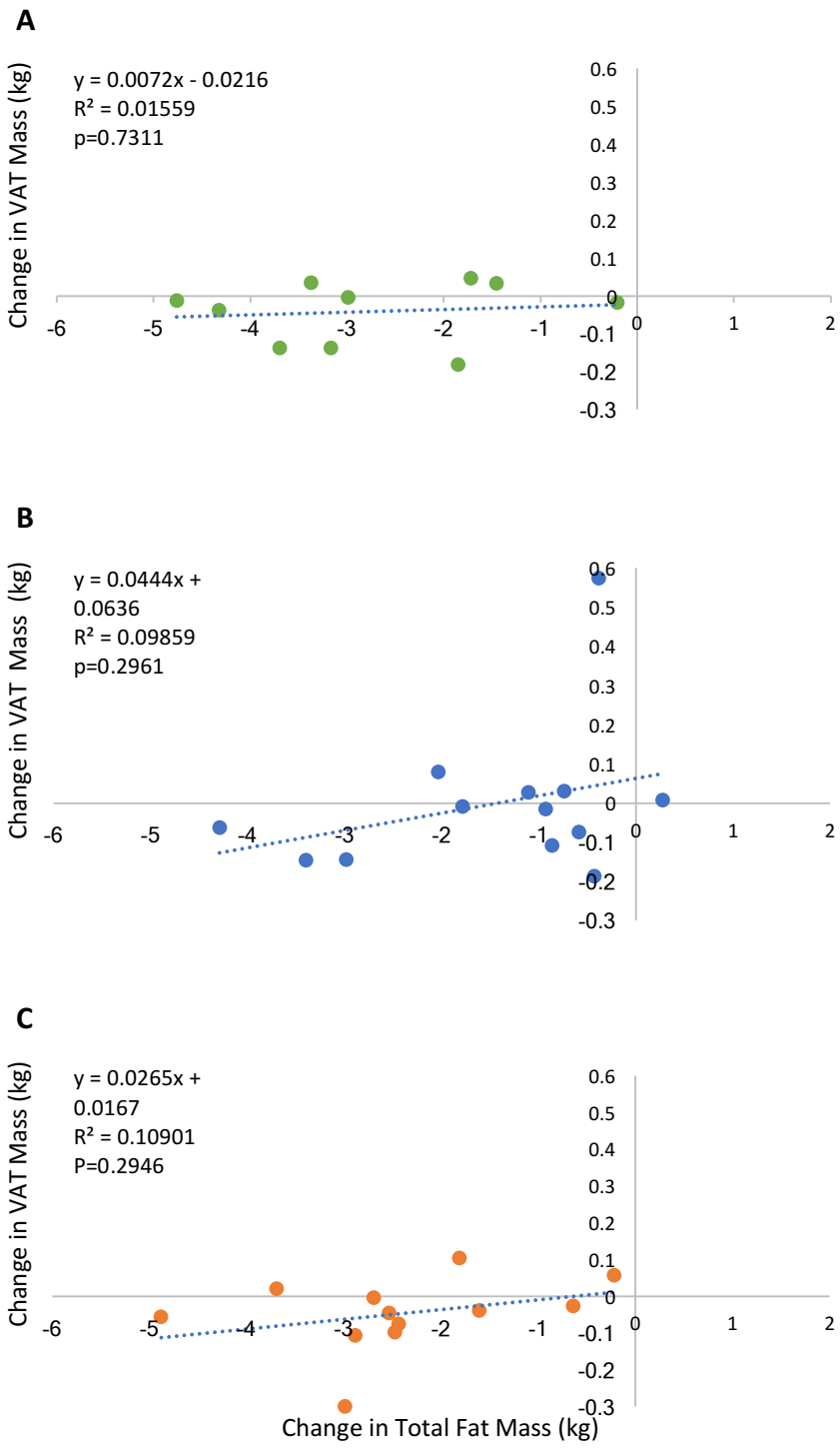


Figure 7. Correlation Between Change in VAT Mass and Change in Total Fat Mass.
Panel A represents the VLCD Group, (n=10), Panel B represents the HCCD Group, (n=13), and Panel C represents the m-HCCD Group, (n=12).

Correlation Between Change in VAT mass and Change in Fasting Serum Leptin Concentrations

As shown in Figure 8, there were no significant correlations between change in leptin concentration (indexed to total fat mass) and change in VAT mass in any of the dietary intervention groups. In the VLCD group (Panel A), all of the participants experienced a reduction in fasting serum leptin concentrations. The change in fasting serum leptin ranged from -0.18 to -0.003 ng/ml · kg. Seven of the ten participants who had a reduction in fasting leptin also lost VAT mass while three individuals gained VAT mass.

In the HCCD group (Panel B), fasting serum leptin concentrations were lower after than before the intervention in 10 of the 13 participants. The change in fasting serum leptin ranged from -0.12 to 0.08 ng/ml·kg. Of the 10 participants who had a reduction in circulating leptin concentration, five individuals lost VAT mass and five individuals gained VAT mass. Among the three individuals who experienced an increase in leptin concentrations after the dietary intervention, each also experienced a slight reduction in VAT mass.

In the m-HCCD group (Panel C), nine of the 12 participants had a reduction in fasting serum leptin concentration while three participants had an increase in leptin concentration. The change in fasting serum leptin ranged from -0.09 to 0.03 ng/ml·kg. Of the participants who experienced a reduction in leptin concentrations, 6 of the 9 individuals also experienced a reduction in VAT mass while three individuals experienced an increase in VAT mass. Similar to the HCCD dietary intervention group, the three individuals who experienced an increase in fasting serum leptin concentrations, also experienced a slight reduction in VAT mass.

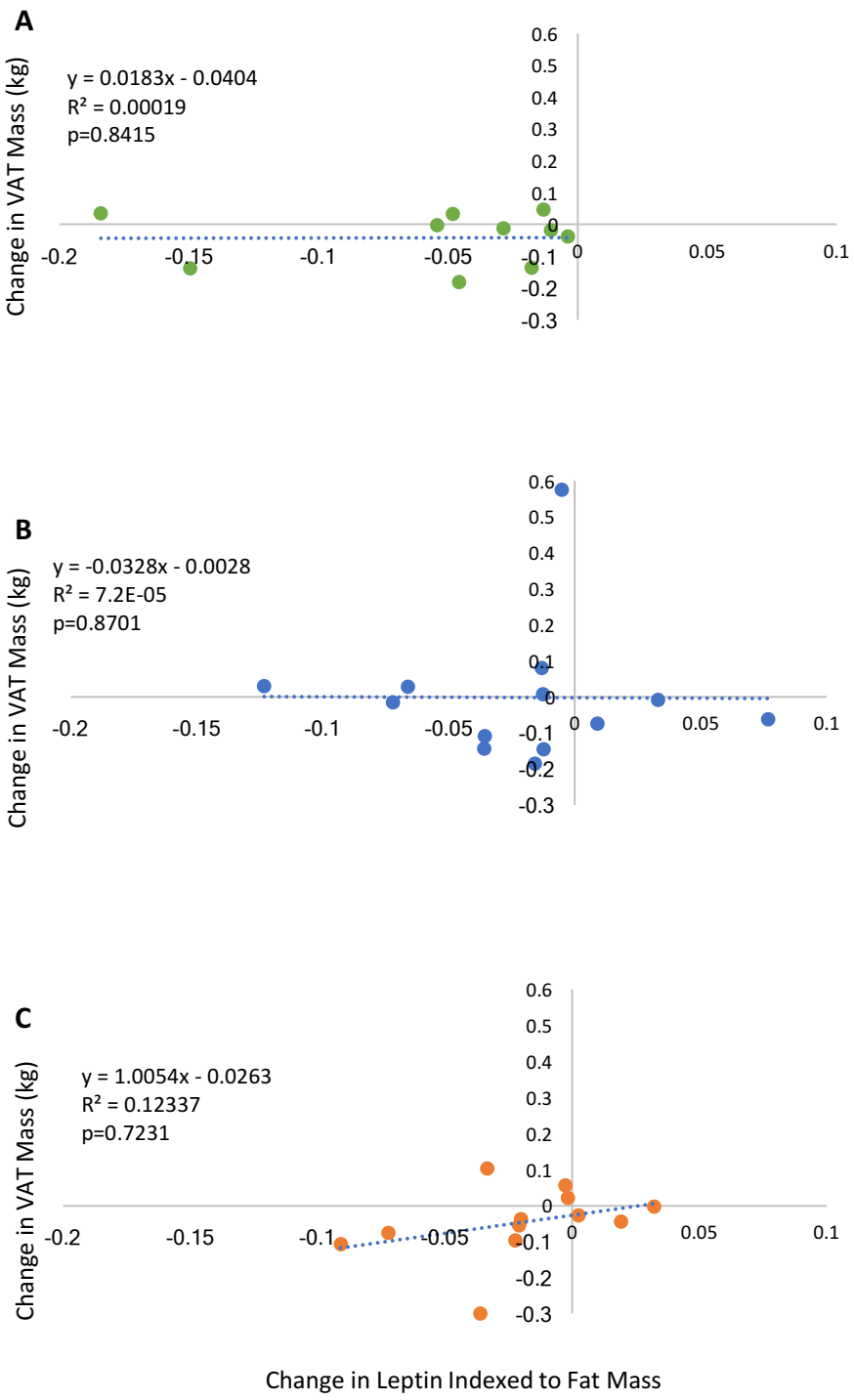


Figure 8. Correlation Between Change in VAT Mass and Change in Fasting Serum Leptin. Panel A represents the VLCD Group, (n=10), Panel B represents the HCCD Group, (n=13), and Panel C represents the m-HCCD Group, (n=12). Leptin indexed to fat mass: ng/ml·kg.

Correlation Between Change in VAT mass and Change in Fasting Plasma hs-CRP Concentrations

Correlation between the change in fasting plasma hs-CRP concentration and change in VAT mass are displayed in Figure 9. The correlation between change in hs-CRP concentration and change in VAT mass in the VLCD group (Panel A) was not significant, (p=0.3236). Three of the participants experienced increases in hs-CRP concentration while six participants experienced decreases in hs-CRP. The change in hs-CRP ranged from -7.6 to 3.4 mg/l. One participant was excluded in this model due to a dramatic increase in hs-CRP concentration at week 6, due to recent injury. Of the six participants who had a reduction in hs-CRP concentration, five of them also lost VAT mass while one individual gained VAT mass. Of the three individuals who had an increase in hs-CRP concentrations, two of them gained VAT mass while one participant had a slight reduction in VAT mass.

In the HCCD group (Panel B), there was no significant correlation between hs-CRP concentration and VAT mass. Eight of the 13 participants had a reduction in hs-CRP concentration while five participants had an increase. The change in hs-CRP ranged from -4 to 4.1 mg/l. Of the eight participants who had a reduction in hs-CRP concentration, seven of the participants also experienced a reduction in VAT mass while one participant gained VAT mass. Of the five participants who experienced an increase in hs-CRP concentration, four participants also gained VAT mass. The fifth participant who demonstrated a slight increase in hs-CRP concentration experienced a slight decrease in VAT mass.

In the m-HCCD group (Panel C), there was a significant correlation between change in hs-CRP concentration and change in VAT mass, (p=0.0201). However, when a Spearman test was conducted the correlation was no longer significant (p=0.5567), suggesting that the correlation was driven by the outlier with a hs-CRP concentration and VAT mass of 7.4 mg/l and -0.3 kg, respectively. Eight of the 12 participants in the m-HCCD group had a reduction in hs-CRP

concentration while four participants had an increase in hs-CRP concentrations. The change in hs-CRP ranged from -3.8 to 7.4 mg/l. Of the eight individuals who had a reduction in hs-CRP concentration, six of them also had a reduction in VAT mass while two of them gained VAT mass. Of the four individuals who had increases in hs-CRP concentrations, one participant had a slight increase in VAT mass while three had reductions in VAT mass.

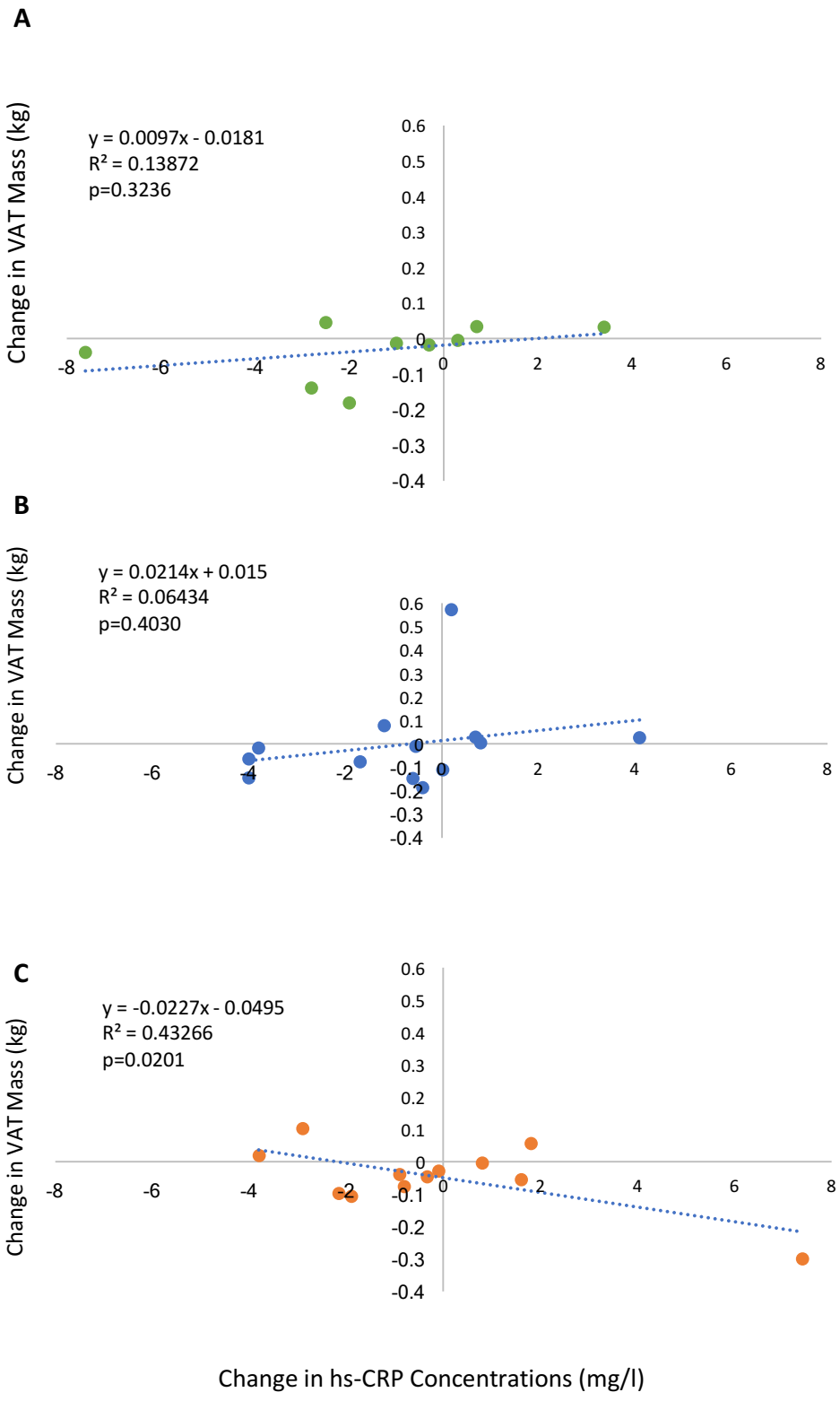


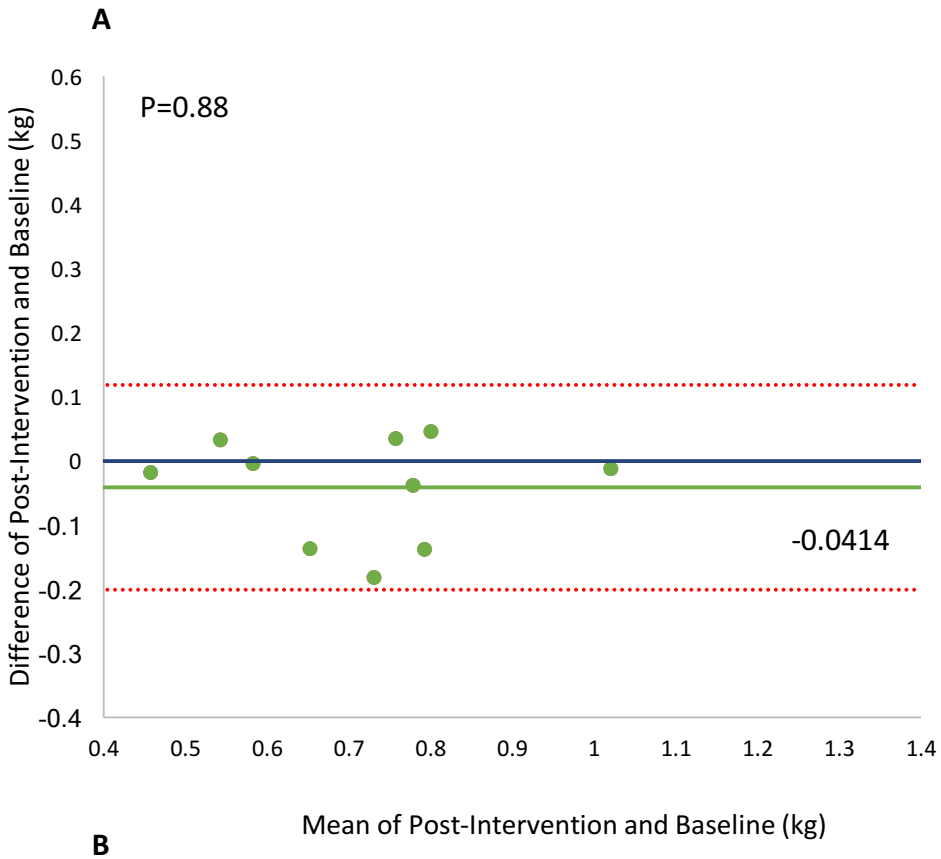
Figure 9. Correlation Between Change in VAT Mass and Change in hs-CRP. Panel A represents the VLCD Group, (n=9), Panel B represents the HCCD Group, (n=13), and Panel C represents the m-HCCD Group, Panel C (n=12).

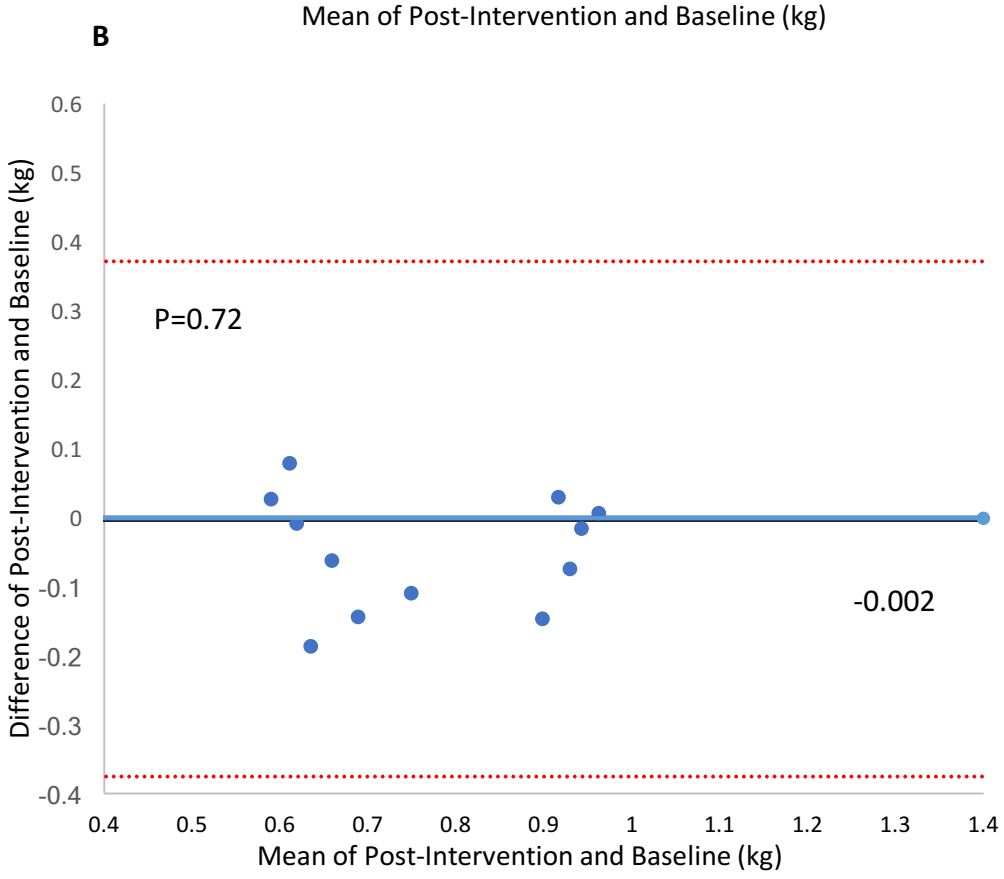
Bland Altman Plots

Bland Altman plots were used to visually assess agreement between baseline and post-intervention VAT mass (kg) in each dietary intervention group, as presented in Figure 10. The graph is a scatterplot in which the Y axis represents the difference between post-intervention and baseline and the X axis represents the average of the post-intervention and baseline VAT mass values for each participant. The graph also shows the upper and lower limits of agreement that represent ± 2 standard deviations from the mean difference of the 2 time points. A Spearman test was used to assess correlation between the average of post-intervention and baseline values and the difference of post-intervention and baseline values.

In the HCCD group, on average there was a slight reduction in VAT mass (-0.002 kg, 95% CI: -0.375, 0.371) with no significant variation in baseline to post-intervention amounts (p=0.7208). Eight participants lost VAT mass, while 5 participants gained VAT mass.

In the m-HCCD group, on average there was a slight reduction in VAT mass (-0.047 kg, 95% CI: -0.244, 0.151) with no significant variation from baseline to post-intervention amounts (p=0.6681). Nine of the twelve participants lost VAT mass while 3 participants gained VAT mass. In the VLCD group, there was a reduction of -0.0414 kg, 95% CI: -0.201, 0.119 in mean VAT mass with no significant variation from baseline to post-intervention (p=0.8810). Seven of the ten participants lost VAT mass and 3 participants gained VAT mass.


A
P=0.88
-0.0414
Difference of Post-Intervention and Baseline (kg)
Mean of Post-Intervention and Baseline (kg)
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4


B
P=0.72
-0.002
Difference of Post-Intervention and Baseline (kg)
Mean of Post-Intervention and Baseline (kg)
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4

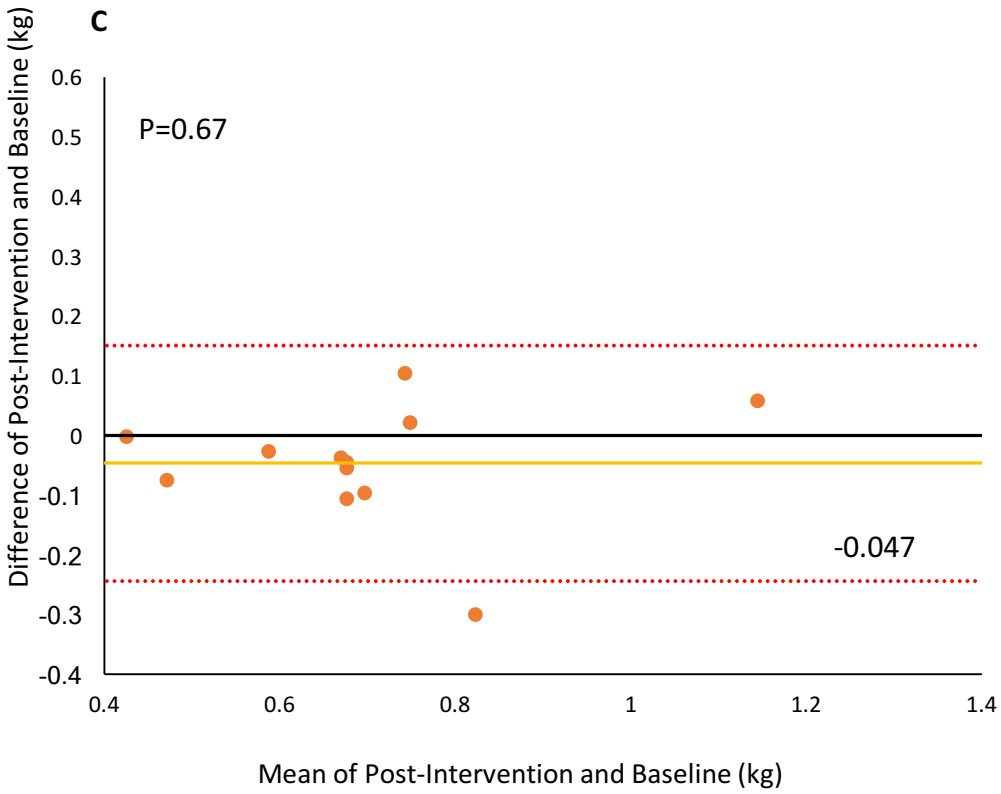


Figure 10. Bland Altman Plots of Visceral Adipose Tissue Mass (kg) Before and After Dietary Intervention. Panel A represents the VLCD Group, (n=10), Panel B represents the HCCD Group, (n=13), and Panel C represents the m-HCCD Group, (n=12). Dotted lines represent 95% upper and lower limits of agreement. Solid lines represent observed average agreement
The lines through zero represent perfect average agreement.

Chapter 5

Discussion

The results of this study confirm the relationship between consumption of high complex-carbohydrate diets and very low-carbohydrate diets and change in VAT mass in overweight and obese individuals as reported by others. The most significant finding of this study was that over a period of six weeks, in all dietary intervention groups, there were significant reduction in mean weight, BMI, total fat mass, total percent fat mass, and fasting serum leptin concentrations. However, there were no significant reduction in mean abdominal VAT mass or fasting hs-CRP concentrations.

We predicted that abdominal VAT mass would be lower after the six-week dietary intervention in each diet group and that VAT mass loss would be correlated with total body mass loss and total fat mass loss. However, this was not observed in any of the dietary intervention groups, separately or combined together. Although VAT mass loss was similar between dietary intervention groups, participants in the VLCD and m-HCCD groups showed more similarity than those in the HCCD group. This pattern was also observed in change in VAT volume, VAT area, total fat mass and BMI. The pattern observed between the VLCD group and m-HCCD group may be due to the similar reduction in energy intake among participants in the VLCD group and the m-HCCD group compared to the HCCD group. In each group, the minimal reduction in VAT mass may explain the lack of association between change in VAT mass and change in total body mass or total fat mass.

We chose to evaluate additional body composition parameters to achieve a more comprehensive understanding of how HCCD, VLCD and m-HCCD interventions impact the body fat stores. After six-weeks of dietary intervention the participants in the VLCD group (-4.95 ± 0.91 kg), HCCD group (-2.81 ± 0.54 kg), and m-HCCD group (-3.08 ± 0.52 kg) lost similar amounts

of weight (p=0.13). Our results are different from other studies that investigated weight loss among participants consuming a high or low carbohydrate diet [26]. These studies typically observed greater weight loss in the low carbohydrate dietary intervention groups during short dietary intervention periods. However, unlike our study, energy intake was typically not matched between dietary intervention groups. Foster et al. compared weight loss, after 3 months, in 63 obese men and women who were counseled to consume either a low carbohydrate (~20 g/day) or a conventional diet composed of ~60% carbohydrate [26]. Although not reported as absolute mean weight loss, the participants in the low carbohydrate group lost significantly more weight, as a percent of baseline weight, compared to those in the conventional dietary intervention group (-6.8 ± 5.0 vs. -2.7 ± 3.7 % change in body weight, p=0.001). Mean weight among those in the low carbohydrate diet group remained significantly lower after six-months compared to those in the conventional diet group, but differences in weight were no longer significant after 1 year.

In this study, after the six-week dietary intervention, the VLCD (-2.75 ± 0.44 kg), HCCD (-1.48 ± 0.38 kg), and the m-HCCD (-2.40 ± 0.36 kg) groups all lost significant amount of total fat mass; however, mean fat mass loss was not significantly different between groups (p=0.08). Other studies have also reported significant loss of total fat mass from baseline to post-intervention without a significant difference between dietary intervention groups [32]. Veum et al., compared the consumption of a very high fat-low carbohydrate diet to a low-fat, high carbohydrate diet for 12 weeks in 46 obese men [32]. There was a significant reduction in mean total fat mass (-10.3 kg 95% CI: -11.5, -9.2 kg, p<0.001) from baseline to 12 weeks when the data was pooled, however there was no significant difference between dietary groups. The low fat, high carbohydrate diet group had a mean reduction of total fat mass from baseline (37.5 ± 9.8) to 12 weeks (26.9 ± 8.7 kg), while participants in the high fat, low carbohydrate diet group had a

mean reduction of total fat mass from baseline (38.7 ± 6.8 kg) to 12 weeks (28.6 ± 6.4 kg). Hall et al., found similar results when conducting a 2-week metabolic balance feeding study on 19 obese men and women [34]. For five days, the participants consumed a eucaloric baseline diet then consumed 30% fewer calories during the dietary intervention period. Participants either consumed a carbohydrate restricted or fat restricted diet for six days. Those in the carbohydrate-restricted group (-0.53 ± 0.13 kg) and fat-restricted group (-0.58 ± 0.14 kg) lost a significant amount of fat mass from baseline to post-intervention but there was no significant difference between the groups. With the combination of these results, we conclude that total fat mass loss can be achieved over a short duration of energy restriction regardless of dietary composition.

Contrary to our predictions, VAT area was not significantly reduced from baseline to post-intervention in the VLCD group (-8.47 ± 5.31 cm^2; p=0.12), the HCCD group (-0.69 ± 11.04 cm^2; p=0.95) or the m-HCCD group (-9.88 ± 6.02 cm^2; p=0.11) and change was not different between groups (p=0.76). Veum et al., found similar results when comparing consumption of a very high fat-low carbohydrate diet to a low-fat, high carbohydrate diet for 12 weeks in 46 obese men [32]. From baseline to 12 weeks, participants in the low fat, high carbohydrate diet group had a reduction of VAT area, measured using CT imaging, from baseline (198 ± 52.5 cm^2) to 12 weeks (153 ± 48.3 cm^2), while the high fat, low carbohydrate diet group had a similar reduction of VAT area from baseline (196 ± 48.0 cm^2) to 12 weeks (139 ± 26.9 cm^2). These values were not significantly different which is inconsistent with our results. When the data was pooled, the authors observed a significant change from baseline to 12 weeks (-57.2 CI: (-65.2, -49.1); p<0.001). However, this study population was conducted in men during a 12-week intervention period so the findings are not completely transferable.

The relationship between changes in fasting serum leptin concentration (indexed to total fat mass) and changes in VAT mass were evaluated in this study. As we predicted, mean fasting serum leptin concentration was significantly lower after the 6-week dietary intervention period in each group but the mean change was not significantly different between groups. However, when leptin was indexed to total fat mass, the change from baseline to 6 weeks was no longer significant in the HCCD group. This may be a result of the HCCD group showing the least amount of change in total fat mass and fasting serum leptin concentrations during the 6-week intervention period. Contrary to our predictions, there was no correlation between change in VAT mass and change in leptin concentrations (indexed to fat mass) in any of the dietary intervention groups. However, a cross-sectional study that investigated the relationship between serum leptin concentrations and VAT volume at one time point, found contradicting results in 452 obese men and women with sleep apnea [48]. VAT volume, measured through MRI, was significantly correlated with serum leptin concentrations ($p<0.001$), even after adjustment for gender ($p<0.001$). However, VAT volume was less strongly associated with serum leptin concentrations than subcutaneous adipose tissue. Although the data was not reported, the authors mentioned that serum leptin concentrations were twice as high in the women than the men. The women also had higher subcutaneous adipose tissue mass compared to the men. Consistent with our results, although we had more women participants than men, the women in this study (n=30) had mean fasting serum leptin concentrations three times higher than the men (n=5) at baseline. Although VAT mass was similar at baseline among the men and women, the women had a total percent fat mass 11% greater than the men. This may suggest that the women had more subcutaneous adipose tissue mass compared to men. Our results suggest that the significant reduction in fasting serum leptin concentration was more likely due to reduction in total fat mass or possible SAT mass rather than VAT mass.

The relationship between change in VAT mass and change in plasma hs-CRP concentration was also analyzed. Unlike fasting serum leptin concentrations, hs-CRP concentrations were not significantly lower after the 6-week intervention period than before nor were they significantly different between treatment groups. At post-intervention, the mean hs-CRP concentration was 4.37 ± 0.73 mg/L in the VLCD group, 4.14 ± 0.83 mg/L in the HCCD group and 3.92 ± 0.85 mg/L the m-HCCD group. Other studies reported similar results for example, in a cross-sectional study of healthy sedentary men (n= 68) and women (n=64), abdominal VAT was measured by DEXA and CT and plasma hs-CRP concentrations were measured [56]. Mean VAT area in men and women were 104.5 ± 5.7 cm^2 and 59.6 ± 4.3 cm^2, respectively, while mean hs-CRP concentrations were 3.2 ± 0.3 mg/l and 4.8 ± 0.6 mg/l, respectively. There was a significant association between VAT area and hs-CRP concentration in men; however, similar to our results, there was no significant association between VAT area and hs-CRP concentration in the women.

Study Strengths and Limitations

There were many strengths to this study. Our study was a very well controlled feeding study that also controlled for physical activity. The subjects were provided all meals and returned all uneaten food; therefore, we know the content and quantity of foods consumed. Our study design included a three-week weight maintenance phase on a standardized diet prior to the intervention. We also included three dietary intervention groups, two of which consumed their assigned diets under *ad libitum* conditions and one that was energy matched to the VLCD, to account for the reduced energy intake observed in low-carbohydrate diet groups previously identified in pilot studies conducted at OHSU. We also were able to measure VAT through DEXA scans before and after the dietary intervention and collect fasting blood samples for analysis.

One limitation of our study was that our population was mainly middle aged white women; therefore, our findings are not generalizable to other subgroups of the population. Also, we were not able to determine abdominal SAT mass through our DEXA analysis. In addition, our study sample of 35 adults may have limited the power of the study to detect differences in VAT mass between groups, particularly due to the division into three treatment groups. Furthermore, the intervention period of 6-weeks may have been too short to see significant change, especially in VAT mass; thus, we are not able to determine the long-term impact of these dietary interventions on VAT mass.

Our results suggest that energy restriction, but not dietary macronutrient composition influences change in weight, body mass, BMI, total fat mass, total percent fat mass, and fasting serum leptin concentrations. Our findings also suggest that neither energy restriction nor differences in macronutrient composition during our 6-weeks intervention significantly altered abdominal VAT mass or fasting plasma hs-CRP concentrations. Finally, our results imply that changes in VAT mass are not correlated with changes in total body mass, total fat mass or fasting serum leptin and plasma hs-CRP.

Future Research Recommendations

Future research should investigate these findings in a diverse and larger study sample including both men and women. Our results indicate average VAT mass is greater in women older than 50 years of age; therefore, researchers should consider investigating how diet impacts VAT mass in postmenopausal women. In our study, participants were instructed to maintain their typical exercise routine. Future research should investigate the synergistic role of diet and exercise on reducing VAT mass in men and women, including postmenopausal women.

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Appendix:

Evidence Table

Low-and High Carbohydrate Diets and Change in Body Composition and Fat Distribution

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes
Veum 2017 (32)	• Randomized control trial	• 12 weeks	• n=46, men • Aged 20-50 y/o • BMI > 29 kg/m^2 • Normal fasting blood glucose	• Body composition was measured by bioelectrical impedance. • Fat distribution was quantified with computed tomography imaging	• Very high-fat, low-carbohydrate diet (73% fat and 10% carbohydrate) • Or a low-fat, high carbohydrate diet (30% fat and 53% carbohydrate) • The diets were equal in energy (8750 kJ/d or 2090 kcal)	• In the very high-fat, low carbohydrate group, there was a significant reduction from baseline to 12 weeks in BMI (34.1 ± 2.4 to 30.6 ± 3.3 kg/m^2), fat mass (38.7 ± 6.8 to 28.6 ± 6.4 kg), and visceral fat area (196 $\pm$ 48.0 to 139 $\pm$ 26.9 cm^2) P<0.001 for all measurements. • In the low-fat, high carbohydrate group, there was a significant reduction from baseline to 12 weeks in BMI (33.6 ± 3.6 to 29.9 ± 3.3 kg/m^2), fat mass (37.5 $\pm$ 9.8 to 26.9 $\pm$ 8.7 kg) and visceral fat (198 $\pm$ 52.5 to 142 $\pm$

						45.2 cm^2) P<0.001 for all measurements. • Changes were similar between groups.
De Souza 2012 (29)	• Randomized control trial of four weight loss diets.	• 6 months and 2 years	• n= 424 (used DEXA) • n=165 (used CT)	• Body fat and lean mass were measured by DEXA. • Abdominal fat was measured by CT	• 20% fat, 15% protein, 65% CHO (Average Protein, Low Fat) • 20% fat, 25% protein, 55% CHO (High Protein, Low Fat) • 40% fat, 15% protein, 45%CHO (Average Protein, High Fat) • 40% fat, 25% protein, 35% CHO (High Protein, High Fat)	• At 6 months: • Participants lost 4.2 $\pm$ 0.3 kg fat mass (p<0.0001). • Participants lost a significant amount of subcutaneous fat (1.5 $\pm$ 0.2 kg) and visceral fat (0.9 $\pm$ 0.1 kg; p<0.0001). • No differences in fat mass, subcutaneous fat or visceral fat loss between groups. • By 2 years: • Participants regained 40% of their losses. • However, a net loss of 1.3 ± 0.2 kg total abdominal fat remained (p<0.0002).

Hall 2015 (34)	• Inpatient metabolic balance, cross over, feeding study	• 6 days in a metabolic chamber	• n=10 obese males • n=9 obese women	• A pair of two-week inpatient periods separated by a 2-4-week washout period. • Two Isocaloric diets in random order during two inpatient stays. • Fat mass measured by DEXA.	• Reduced carbohydrate diet (29% carbohydrate) • Reduced fat diet (71.2% carbohydrate) • Protein intake unchanged • Each group consumed a 30% reduction in energy intake from baseline.	• There was a significant reduction in body weight in the reduced carbohydrate diet group (-1.85 ± 0.15 kg) and the reduced fat diet group (-1.3 ± 0.16 kg). • Reduced carbohydrate group lost more weight compared to the reduced fat group (p=0.022) • There was a significant reduction in fat mass in the reduced carbohydrate group (-0.52 ± 0.13 kg) and the reduced fat diet group (-0.58 ± 0.14 kg). • Fat mass loss was similar between groups.

Foster 2003 (26)	• Multi-center randomized control trial	• 3, 6 and 12 months	• n=43 obese women • n=20 obese males • Mean age 44 y/o • Mean BMI 34 kg/m^2	• Change in body weight measured by scale.	• Low-carbohydrate diet (n=33) • High-carbohydrate, low-fat, low-calorie (conventional) diet (n=30)	• Participants on the low-carbohydrate diet lost more weight than the conventional diet at 3 months (6.8 ± 5.0 vs 2.7 ± 3.7 percent of body weight; p=0.001). • At 6 months participants on the low-carbohydrate diet lost more weight than the conventional diet (7.0 ± 6.5 vs 3.2 ± 5.6 percent of body weight; p=0.02) • At 12 months, there was a similar reduction in weight (4.4 ± 6.7 vs. 2.5 ± 6.3 percent body weight; p=0.26).

Leptin, Visceral Adipose Tissue and Dietary Intervention

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes
Kohara 2011 (47)	• Case-control	n/a	• n=303 men • n=479 women • Japanese • n=133 visceral obese men • n=133 visceral obese women • Mean age of 65 y/o • Mean BMI 24 kg/m^2	• To determine the relationship between visceral obesity and plasma levels of leptin. • Four groups based on presence or absence of either visceral obesity. • Visceral fat was measured by computed tomography. • Visceral obesity was defined as a visceral fat area >100 cm^2 in both men and women.	• Cases: participants with visceral obesity • Controls: participants without visceral obesity	• The men in the visceral obese group had significantly greater body weight (65.7 ± 7.2), BMI (24.6 ± 2.3) and visceral fat area (154.2 ± 40.5) compared to the normal weight group, ($p<0.001$ for all variables). • Reported values for weight, BMI, and visceral fat area in women with visceral obesity were identical to the men, which is likely a mistake. • Overall, women had significantly less visceral fat area (78.4 ± 51.6 cm^2) compared to

						the men (128.4 ± 64.8 cm^2; p<0.001). • Participants with visceral obesity had significantly greater leptin concentrations (4.3 ± 2.3 µg/l) compared to the normal weight group (1.9 ± 2.3 µg/l; p<0.001).
Arnardottir 2013 (48)	• Cross-sectional	n/a	• n=377 men • n=75 women • With untreated obstructed sleep apnea • Mean age 54.3 ± 10.6 y • Mean BMI 32.7 ± 5.3 kg/m^2	• Fasting serum leptin concentrations were measured. • Visceral fat measured by magnetic resonance imaging.	n/a	• Pearson correlation showed a significant association between leptin concentrations and total abdominal fat volume, subcutaneous fat volume, BMI and visceral fat volume (cm^3), all p values <0.0001. • Subcutaneous fat was more associated with leptin concentrations (r=0.67) compared

						to visceral fat (r=0.24).
Weigle 2003 (51)	• Feeding Study	• 16 weeks	• n=18 healthy adults • n=2 men • n=16 women • Mean age 45.3 y.o. • Mean BMI 27.1 kg/m^2	• Leptin concentrations were measured. • Weight by scale was measured.	• First 2-weeks: baseline diet (35% fat, 45% carbohydrate, and 20% protein) to stabilize body weight. • All meals were provided. • Subjects were instructed to consume all food provided. • Next 2 weeks: isocaloric low fat diet (15% fat, 65% carbohydrate and 20% protein) • The next 12 weeks: participants remained fixed on the low-fat diet ad libitum.	• Leptin concentrations were not significantly different among the weight maintaining 35% fat and 15% fat conditions 17.8 ± 1.9 ng/ml vs. 17.2 ± 2.0 ng/ml. • However, while consuming the ad libitum diet, leptin was significantly different from both weight maintenance diets 12.2 ± 1.8 ng/ml; ($p<0.001$). • There was also significant weight loss during the ad libitum conditions 70.8 ± 2.7 kg compared to the weight maintenance conditions (15%: 74.6 ± 2.4 kg, 35%: 74.9 ± 2.4kg, $p<0.001$).

C-Reactive Protein, Visceral Adipose Tissue and Dietary Intervention

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes
Faber 2012 (55)	• An ongoing single centered prospective cohort study	n/a	• n=1729 men • n=681 women • Ages 58-60 y • BMI from 24-30 kg/m^2	• Subcutaneous and visceral fat masses were measured with ultrasonography • C-reactive protein was measured	• Participants separated by quartile of intra-abdominal fat. • Determined the relationship between CRP concentrations and different amounts of visceral adipose tissue.	• Participants within the highest intra-abdominal fat quartile, median CRP concentrations were 2.4 (1.3-4.4) mg/l compared to 1.1 (0.5-2.6) mg/l in the lowest quartile. • Intra-abdominal fat and BMI in both men and women was significantly correlated with CRP concentrations.

Maki 2011 (56)	• Baseline results from a multi-center clinical trial	n/a	• n=68 men • n=64 women	• Fat distribution measured by dual- energy x-ray absorptiometry and abdominal computed tomography • CRP concentrations were measured	• Determined the relationship between CRP and total fat mass, subcutaneous fat mass and visceral fat mass.	• CRP concentrations were 3.2 ± 0.3 mg/L and 4.8 ± 0.6 mg/L in men and women. • Abdominal visceral adipose tissue was greater in men than women (104.5 ± 5.7 vs. 59.6 ± 4.3 cm^2). • Women had greater subcutaneous adipose tissue compared to men (334.6 ± 11.6 vs 285.0 ± 13.4 cm^2, $p<0.01$). • In men, there was a significant association between CRP and total fat mass ($p<0.01$), VAT ($p<0.05$ and SAT ($p<0.01$).

						• In women, significant association only shown between CRP and total fat mass.
Noakes 2005 (58)	• Randomized control trial	• 12 weeks	• n=100 women • Mean BMI: 32 kg/m^2 • Mean age: 50 y.o	• Body weight measured by scale • Dual-energy x-ray absorptiometry was performed at weeks 0 and 12 • Attended individual diet counseling every 4 weeks • CRP was measured on weeks 0 and 12	• Randomly assigned to 1 of 2 isocaloric (1338 kcal) dietary interventions • High protein (34% protein, 20% fat, and 46% carbohydrate) • High carbohydrate (17% protein, 20% fat and 64% carbohydrate)	• Overall, CRP significantly decreased from baseline to 12 weeks ($p<0.001$). • In the high protein group CRP significantly decreased (6.6 ± 0.7 mg/L to 4.9 ± 0.6 mg/L). • In the high carbohydrate group CRP significantly decreased (4.8 ± 0.5 mg/L to 4.0 ± 0.4 mg/L). • The reduction was similar among groups ($p=0.447$). • In the high protein group, there was a

						significant mean change in total fat mass -5.7 ± 0.6 kg and midriff fat mass -0.9 ± 0.1 kg; (p<0.01). • In the high carbohydrate group, there was a significant mean change in total fat mass -4.5 ± 0.5 kg and midriff fat -0.7 ± 0.1 kg; (p<0.01). • The change was similar among groups (p-value not provided).