DIFFERENTIAL EFFECTS OF DIETARY FATS ON METABOLISM AND APPETITE

by

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(Under the Direction of Jamie A. Cooper)

ABSTRACT

In general, high-fat (HF) diets are associated with the development of several metabolic diseases including obesity, diabetes, and cardiovascular disease. However, it is important to consider the composition of fat intake within the diet, due to the differential impact that dietary fatty acids have on physiological function. Current dietary recommendations center on reducing saturated (SFA) and trans-fatty acid intake, and increasing mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) intake. Eating fats high in MUFAs and PUFAs instead of SFA has been linked to reduced disease risk. However, less is known regarding differences in physiological function between MUFA and PUFA intake. The objective of this dissertation is to compare a HF diet rich in MUFAs to a HF diet rich in PUFAs to determine potential differences in metabolism, cholesterol levels, and appetite responses, in normal weight, healthy adults in a single-blind crossover-design. In Manuscript #1, resting substrate oxidation and diet induced thermogenesis (DIT) was measured, acutely and after 5-day HF diets. Acutely, postprandial fat oxidation was lower after a HF PUFA-rich meal compared to a HF MUFA-rich meal. However, after a 5-day HF PUFA-rich diet, there were no differences between MUFAs and PUFAs, meaning fat oxidation increased after a 5-day HF PUFA diet. In Manuscript #2, fasting lipids were measured before and after 5-day HF diets, with significantly more favorable fasting lipid

values observed after the 5-day HF PUFA-rich diet. Postprandial triglycerides were also significantly lower after the 5-day HF PUFA-rich diet compared to the HF MUFA-rich diet. In Manuscript #3, appetite responses were measured acutely and after 5-day HF diets. A 5-day HF PUFA-rich diet led to lower postprandial ratings of hunger and lower concentrations of the hunger hormone ghrelin compared to a 5-day HF MUFA-rich diet. However, no significant differences in energy intake at a buffet meal were observed between diets. There were also no differences in ratings of fullness or concentrations of the satiety hormone PYY between diets. All in all, these results indicate that a HF PUFA-rich diet may have more favorable metabolic outcomes compared to a HF MUFA-rich diet in healthy, normal weight males.

INDEX WORDS: DIETARY FATTY ACIDS, POLYUNSATURATED FATTY ACIDS, MONOUNSATURATED FATTY ACIDS, METABOLISM, SUBSTRATE OXIDATION, APPETITE, DIET, HIGH-FAT, COTTONSEED OIL, OLIVE OIL

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CHAPTER 1

INTRODUCTION

In recent years, a great deal of emphasis in the general population has been put on the types of fats individuals should consume on a daily basis to reduce risks of developing chronic diseases. Recommendations are centered on eating more mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs) while limiting intake of saturated fatty acids (SFAs) and trans fatty acids in the diet (1). Eating oil-based fats (high in MUFAs and PUFAs) instead of solid fats (high in SFA) has been linked to reduced risk of obesity and diabetes (2-4). There are many mechanisms in which unsaturated fats may act to reduce disease risk, some of which may be through their influence on metabolism, cholesterol levels, and appetite in the body.

Fat metabolism is an important factor in maintaining energy balance, and lower fat oxidation has been associated with increased body fat (5) and increased triglyceride concentrations (6). Early animal and human studies have consistently revealed greater oxidation of unsaturated FAs compared to SFAs (7-9); however, differences between unsaturated FAs are not as well characterized, with some studies showing differences between MUFAs and PUFAs (10, 11) and others indicating no differences (12-14). It is also important to note that most studies to date have focused on measuring oxidation rates after acute or single meal challenges. This may be limiting because postprandial oxidation rates following an acute meal may differ based on historical dietary fat consumption and prior body fat composition. A longer-term diet intervention that can influence metabolic changes or adaptations may more accurately define the differential effects of altering dietary FA composition (15).

Another metabolic factor important in the regulation of energy balance is diet induced thermogenesis (DIT), which is the energy expenditure (EE) associated with processing food for use and storage after a meal. Similar to fat oxidation, reductions in DIT have been associated with obesity (16, 17). Studies have consistently reported greater DIT following consumption of unsaturated FAs compared to SFAs (9, 13, 14, 18). Only three studies to date have compared acute meal challenges rich in MUFAs vs. PUFAs with mixed findings (12-14). The longer-term effects of consuming a diet rich in MUFAs vs. PUFAs on DIT or EE have yet to be observed.

Elevated cholesterol levels are a major risk factor for cardiovascular disease (CVD) and it has been shown that the type of fat consumed in the diet plays an important role in modifying these levels (19). Specifically, replacing carbohydrates with saturated fats results in increases in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), while replacing carbohydrates with unsaturated fats is associated with decreases in TC and LDL-C (20). Studies investigating differences between MUFAs vs. PUFAs are harder to interpret due to the inconsistencies in food sources used to derive the FA of interest from (21-23), therefore making it imperative to highlight the source of MUFA and PUFA when interpreting results.

Appetite and satiety responses to different high-fat meals and diets also plays an important role in maintaining energy balance and lowering overconsumption of foods. Acute studies show that MUFAs from a variety of oils induce less satiety than PUFAs in both mice (24) and humans (25, 26). However, little is known regarding the effects on appetite of longer-term ingestion of these two types of fats in humans (27). Therefore, the aim of the current dissertation is to investigate the effects of a 5-day, high-fat diet rich in PUFAs compared to a 5-day, high-fat diet rich in MUFAs on changes in metabolism, cholesterol, and appetite in healthy adult men.

The literature review (Chapter 2) provides an overview of the current body of research surrounding dietary fats and 1) chronic disease risk, 2) metabolism, 3) cholesterol profiles, and 4) appetite. Chapter 3 presents the first manuscript of this dissertation, describing the effects on fasting and postprandial metabolism before and after 5-day high-fat diets rich in either PUFAs or MUFAs. Chapter 4 is the second manuscript, examining the effects on lipid profiles before and after the 5-day high-fat diets rich in either PUFAs or MUFAs. The final manuscript, Chapter 5, describes the effects of both high-fat diets on appetite responses. We hypothesize that a high-fat PUFA-rich diet will lead to higher fat oxidation, better improvements in lipid profiles, and higher satiety than a high-fat MUFA-rich diet.

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CHAPTER 2

REVIEW OF THE LITERATURE

Dietary Fats and Chronic Disease Risk

Increased weight is associated with increases in disease risk factors (1, 2). Therefore, the populations increase in obese and overweight status over the past couple decades has led to a focus on strategies and methods to reduce weight gain and better manage weight balance (3). Consuming diets high in fat (≥40% energy intake) has been associated with weight gain (4), and hence increases in disease risk. However, the fatty acid composition of the diet may differentially influence biological activity, impacting weight management and disease risk outcomes (5).

The most common form of lipid in our diets are triglycerides, with a smaller amount of phospholipids and cholesterol present as well. Triglycerides consist of a glycerol backbone linked with three fatty acids (FAs) via an ester bond. These FAs can vary in chain length and saturation. Short chain FAs are those having a chain length less than 6 carbons long, medium chain FAs consist of 8-14 carbons, and long chain FAs are those having a chain length greater than 14 carbons long. The saturation of FAs depends on the number of double bonds existing in the FA structure. Saturated FAs (SFAs) do not contain double bonds, meaning its structure is completely saturated with hydrogen. Food sources rich in SFAs include dairy products such as butter, milk and cheese, meats such as fatty beef, pork and lard, and palm oil. Unsaturated FAs contain at least one double bond and are separated into mono-unsaturated FAs (MUFAs) containing a single double bond and poly-unsaturated FAs (PUFAs) containing two or more double bonds. Food sources rich MUFAs include olive oil, canola oil, avocados, and nuts such

almonds and cashews. Food sources rich in PUFAs include fish, walnuts, flax seeds, and many vegetable oils such as corn oil, soybean oil, and cottonseed oil. The varying structures of FAs has significant impact on the actions they have within our bodies and it is important to know what types of fats to consume to optimize health and lower disease risk (5, 6).

Dietary fats play important biological and functional roles within our bodies, consequently having a great amount of impact on health and disease risk (5). Research has revealed that different dietary fatty acids have specific actions and it is important not to generalize fats into a single group (5). The type of fat in the diet, rather than the total amount of fat consumed, has been shown to be more important in determining risk for chronic disease (6). The degree of saturation of fatty acids has been recognized as an important characteristic in determining health impact. Unsaturated fats have been associated with a decreased risk of chronic disease (6-8). Early data from the Nurses' Health Study followed over 80,000 women for 14 years and revealed that saturated fat intake was associated with a slight increase in risk for coronary heart disease (CHD), and MUFAs and PUFAs were associated with a lower risk of CHD, particularly PUFAs (6). In general, these original findings have held true over the years, with the most recent 2015 Dietary Guidelines for Americans stating there is still strong evidence to support replacing saturated fats with unsaturated fats, especially PUFAs, in reducing CVD risk (9). However, recently, consumption of omega-6 PUFAs and their association with a lower risk of chronic disease has been a controversial topic. This is due to omega-6 PUFAs potential ability to produce pro-inflammatory eicosanoids (10). However, a recent review and meta-analysis analyzing prospective observational cohort studies concluded that dietary omega-6 PUFA intake, specifically linoleic acid, is still inversely associated with CHD risk (7). Other studies investigating linoleic acid consumption in comparison to other fatty acids have confirmed that

without some other inflammatory stimulus, such as the low-grade inflammation that is associated with obesity, linoleic acid consumption alone does not lead to increases in inflammation and are in themselves not detrimental to health (11).

The mechanisms behind dietary fat intake and their influence on chronic disease may be associated with their impact on metabolism in the body, therefore affecting lipid handling, as well as their influence on overall appetite. Further, determining these differences between the different classes of unsaturated fats may be beneficial to further refine dietary guidelines and increase overall health, lowering disease risk.

Dietary Fats Effect on Metabolism

Both energy balance and macronutrient balance influence body weight regulation and body fat accumulation. Energy balance is the ratio between energy intake and energy expenditure while macronutrient balance is the ratio between the intake of fat, carbohydrate, and protein, and their subsequent oxidation in the body. A chronic imbalance in these equilibriums causes excess weight gain and body fat, leading to an obesogenic state, increasing risk for further diseased states (12). Therefore, studying metabolism, or the rate at which our bodies expend energy, is important in controlling weight maintenance and reducing chronic disease risk. Fats and carbohydrates are the macronutrients that provide most of the dietary energy, with protein accounting for a relatively smaller amount of total dietary energy (13). The body maintains a nearly constant equilibrium of protein content, with protein oxidation matching protein intake (13). Hence, body weight maintenance is primarily controlled by intake and utilization of fats and carbohydrates. According to the Flatt hypothesis, carbohydrate use regulates fat oxidation and storage (13). Carbohydrate stored as glycogen in the body is limited, whereas fat stores in adipose are much higher, making the amount of carbohydrate stored in the body influential over

fat accumulation (13). Therefore, stored glycogen, as well as overall energy balance, dictates fat balance.

It has been shown that a shift from an average diet to a HF diet (>40% energy from fat) does not result in immediate increases in fat oxidation to match the amount of fat intake (14), while adaptation to a high carbohydrate diet happens almost immediately with carbohydrate oxidation rates matching increases in carbohydrate intake within a 24h period (15). The rate of fat oxidation increases slowly over time, taking about 5-7 days to establish fat balance (16-18). This slow rate of metabolic adaptation leads to an imbalance between fat intake and oxidation, increasing storage of fat and leading to positive fat balance for several days before fat balance adaptation occurs. However, the type of fats present in the diet may influence rates of metabolic adaptation, including substrate oxidation and overall energy expenditure, differentially (19).

Substrate Oxidation

The most commonly used technique to measure substrate oxidation is indirect calorimetry; however, early studies have also used stable isotope-labeled fats. The method of using labeled isotopes provides information about the exogenous metabolism of specific fatty acids following high-fat meals, whereas indirect calorimetry measures overall fat oxidation rates, both endogenous and exogenous, after a meal. Early studies using stable isotopes conclude that exogenous unsaturated fats (MUFAs and PUFAs) are oxidized to a greater extent than SFAs (20-23). Two of these studies also looked at oxidation rates between PUFAs and MUFAs. Jones et al. (20) concluded that after a breakfast meal enriched with either PUFAs (specifically omega-6 PUFAs) or MUFAs, MUFAs had the highest oxidation rate, followed by PUFAs in normal weight adults. Delaney et al. (23) showed that following a high fat liquid meal, rich in either

PUFAs or MUFAs, omega-3 PUFAs had the highest oxidation rate, followed by MUFAs, followed by omega-6 PUFAs in normal weight adults.

Studies using indirect calorimetry to measure overall fat oxidation rates have yielded mixed results between unsaturated fats and SFAs. Some show meals/diets rich in unsaturated fats result in higher fat oxidation rates than meals/diets rich in SFAs (24-28), while others do not indicate differences in total rates of oxidation (29-33). Acute meal studies comparing differences between unsaturated fatty acids (MUFAs and PUFAs) are also inconclusive and show little differences (32, 33). Changes in total fat oxidation may be harder to detect than changes in exogenous fat oxidation because total fat oxidation takes into account endogenous fat oxidation as well as exogenous. A previous study by Cooper and colleagues (34) measured total, exogenous, and endogenous fat oxidation rates between high-fat diets rich in SFA or unsaturated fat. After 1 day on the diets, there were no differences in total fat oxidation rates or endogenous fat oxidation; however, exogenous fat oxidation was significantly increased (34). By the fourth day on the diet, the high MUFA diet showed a trend for significance with increasing total fat oxidation compared to the high-fat SFA-rich diet (34). These results demonstrate that a longer-term diet rich in unsaturated fats may be needed to see differences in total fat oxidation rates.

Longer-term diet interventions investigating the effects of fatty acid composition on fat oxidation have centered primarily on investigating differences between MUFAs and SFAs (27, 29-31). Four studies to date have looked at the effects of a 4-week diet on changes in fat oxidation comparing a MUFA-rich diet to a SFA-rich diet with mixed results (27, 29-31). Kien et al. (27) found that healthy adults fed a HF (40% energy from fat) MUFA-rich diet resulted in greater fat oxidation as compared to a HF SFA-rich diet. Following this same diet intervention, Kien and Bunn (31) also found healthy adults fed a HF MUFA-rich diet resulted in greater fat

oxidation compared to a HF SFA-rich diet. However, Piers et al. (30) investigated differences between a HF MUFA-rich diet vs. a HF SFA-rich diet in overweight and obese men and did not find differences in fat oxidation rates between the two diets. Similarly, Lovejoy et al. (29) found no differences in healthy adults fed a MUFA-rich vs. SFA-rich regular fat diet (30% of energy from fat). Recently, our lab has investigated the effects of a 7-day PUFA-rich diet compared to a control diet (higher in MUFA and SFA content), with 35% of energy coming from fat, on healthy adults (28). The 7-day PUFA-rich diet resulted in a significant increase in fat oxidation, where there was no change in fat oxidation seen following the control diet (28). Longer-term studies specifically investigating differences between PUFAs and MUFAs are lacking.

Energy Expenditure

Energy expenditure (EE) and diet induced thermogenesis (DIT) can also be measured with indirect calorimetry. DIT is the increase in EE from the fasted state that is caused by energy spent digesting, transporting, absorbing, metabolizing and storing nutrients in the body (35). DIT is important in the regulation of energy balance (36), and reduced activity of thermogenesis has been associated with obesity (35, 37). Studies to date have consistently reported greater DIT following consumption of unsaturated fats compared to saturated fats (25, 26, 33, 38). Three studies to date have compared MUFA and PUFA with mixed findings (32, 33, 38). Casas-Agustench et al. (33) reported no differences between acute consumption of a MUFA or PUFA rich meal on DIT. Clevenger et al. (38) found that DIT following a PUFA-rich high-fat meal was significantly greater than that of the MUFA or SFA-rich high-fat meals while Jones et al. (32) found that DIT was higher following a MUFA rich meal compared to PUFA.

There are few longer-term studies investigating the effects of fatty acid composition on EE or DIT. Piers et al. (30) investigated differences between a 4-week HF MUFA-rich diet vs. a

HF SFA-rich diet in overweight and obese men and did not find any differences in resting EE or DIT. Following a similar diet intervention, Kien et al. (27) also did not detect any differences in resting EE between a HF MUFA-rich diet and HF SFA-rich diet in healthy adults. However, daily EE (calculated from average energy intake over the diet intervention and change in basal energy from dual x-ray absorptiometry) was significantly decreased following the HF SFA-rich diet, whereas daily EE remained unchanged in the HF MUFA-rich diet (27). Recently in our lab, we investigated the effects of a 7-day PUFA-rich diet compared to a control diet in healthy adults and found no significant changes in EE (28). Studies have yet to observe the longer-term effects of consuming a diet rich in MUFAs vs. PUFAs on EE or DIT.

Dietary Fats Effect on Blood Lipid Profiles

Dyslipidemia is a major risk factor for CVD, with approximately one in every four U.S. adults having elevated levels of cholesterol (39). Dyslipidemia is characterized by elevated levels of total cholesterol (TC) (>200 mg/dL) or low-density lipoprotein cholesterol (LDL-C) (>130 mg/dL) and triglycerides (TG) (>150 mg/dL), or decreased high-density lipoprotein cholesterol (HDL-C) levels (<40 mg/dL) (40). LDL-C are small dense particles present in circulation that primarily consist of cholesterol and account for approximately 60-70% of total serum cholesterol. High levels of circulating LDL-C are a risk factor for atherosclerosis because they can be deposited in the subendothelial space of arteries and become oxidized, contributing to fatty buildup in arteries (41). This raises the risk for heart disease, stroke, and peripheral arterial disease. HDL-C are also present in circulation and, in contrast to LDL-C, work to shuttle cholesterol from peripheral tissues back to the liver for recycling. Thus, increased HDL-C levels are beneficial to health where a decrease is associated with increased risk for disease. There is strong and consistent evidence showing increased TC and LDL-C levels have a direct

relationship to increased rate of development of coronary heart disease (CHD) in adults as assessed by epidemiological studies such as The Framingham Heart Study (42), the Multiple Risk Factor Intervention Trial (MRFIT) (43), and the Lipid Research Clinics (LRC) trial (44). Elevated TG levels have also been correlated with increased risk for CHD (45, 46). Additionally, there is strong evidence to support links between low levels of circulating HDL-C and increases in CHD, demonstrated in epidemiological studies (42, 47). Both diet and lifestyle play important roles in modifying these risk factors and preventing dyslipidemia (48, 49).

In regard to diet, the type of dietary fat consumed has been studied to have influential impact on lipid handling in the body, affecting lipid status (50). In randomized controlled feeding trials, it has been shown that replacing carbohydrates with trans-fat and saturated fat results in increases in LDL-C and TC, whereas replacing carbohydrates with unsaturated fats is associated with decreases in LDL-C and TC (51). Recently, speculation over the food source in which these fatty acids are being consumed, and not just the fatty acids themselves, has become important when evaluating the impact on health. Hence, the whole food itself in which the fatty acids are being derived may influence lipid outcomes differently. For example, it has been suggested that consumption of palm oil, which is high in SFAs, may not produce the same degree of hypercholesterolemic effects that consumption of saturated fats coming from animal fats do (52). In a recent systematic review and meta-analysis of clinical trials, palm oil (high in SFAs) was compared to various other vegetable oils low in saturated fat (53). It was concluded that palm oil, in comparison to other vegetable oils low in saturated fat, still significantly increased total cholesterol and LDL-C levels (53). There have been many randomized controlled clinical trials evaluating the effects of substituting high MUFA oils and high PUFA oils for high SFA oils on cholesterol levels summarized in systematic reviews (54-57). Overall, evidence suggests

substituting high MUFA oils and high PUFA oils for high SFA oils reduces TC and LDL-C levels.

Types of unsaturated fats have been further investigated to determine if there are significant differences between MUFAs and PUFAs in lipid outcomes. When investigating the results of observational cohort studies, many report little beneficial health outcomes associated with MUFA intake when not adjusting for other fat and cholesterol intake (58, 59). MUFAs typically coexist with SFAs in many foods, and as mentioned previously, it may be important to differentiate out the food source in which these MUFAs are being consumed. In observational studies, this is not always separated out or categorized, leading the consumption of MUFAs to seem not as favorable as PUFA consumption. However, in recent years MUFA intake has gained popularity, especially in regard to the Mediterranean diet. Olive oil (OO) is the main food component in the Mediterranean diet that gives the diet its high MUFA intake. Many studies utilizing a Mediterranean diet intervention have consistently reported reductions in TC (60, 61). However, even though the main source of fat in the Mediterranean diet is MUFA from OO, there are also other dietary components such as nuts and seeds (high in PUFA) that may be contributing to these effects. Studies specifically comparing OO to PUFA-rich vegetable oils have identified stronger effects of PUFA-rich oils in modifying cholesterol profiles after 3-4 week interventions (62-65).

In a recent trial, known as the canola oil multi-center intervention trial (COMIT), various oil blends were used to manipulate fatty acid profiles to study differences in various health outcomes to find an optimal oil/fatty acid profile blend (66). Specifically, 5 oils were used including 1) canola oil, 2) high oleic canola oil (omega-9 rich), 3) high oleic canola oil + DHA (omega-9 and very long chain omega-3 rich), 4) blended corn/ safflower oil (omega-6 rich), and

5) blended flax/safflower oil (omega-6 and short chain omega-3 rich) (66). All oils contained relatively similar levels of saturated fat, with the amounts of omega-3, omega-6, and omega-9 varying between them. All oils significantly reduced LDL-C and TC levels after 4 weeks of consumption, with the high oleic canola oil + DHA having the most robust lipid lowering effects (66). These results demonstrate that omega-9 MUFA consumption, combined with very long chain omega-3 consumption, is the most beneficial in promoting positive health outcomes. Further, the COMIT trial found no significant differences between MUFA-rich canola oil vs. PUFA-rich oils in reducing lipid outcomes (66), which is in contrast to studies completed using OO as the high MUFA oil (62-65).

Recently, an oil high in both omega-6 PUFA and saturated fat has been investigated due to its profound changes on lipid profiles. Consumption of a diet rich in cottonseed oil (CSO) has shown robust lipid lowering effects in both animal and human studies (67-69). In an early animal model, it was discovered that rats fed a high CSO diet, as compared to a high corn oil diet, resulted in reduced TC levels (67). This finding was later confirmed in healthy humans when it was shown that consuming 95g of CSO per day for just 5-days significantly reduced TC and LDL-C (68). This is unique since CSO has higher levels of saturated fat than many other vegetable oils while also having a high omega-6 PUFA content. Therefore, the beneficial effects seen with CSO may be due to other bioactive components in the whole food and not just its fatty acid profile. In a recent mouse model, the authors were able to link the lipid lowering effects of CSO to dihydrosterculic acid, a cyclopropenoid fatty acid found within CSO (69). In this study, mice consuming a HF diet rich in CSO largely protected the animals from HF diet-induced metabolic adaptations compared to a HF diet rich in cocoa butter (high SFA) or safflower oil (high omega-6 PUFA) (69). The livers of mice fed the CSO diet resembled that of mice fed a

chow diet, where the mice fed HF diets rich in cocoa butter or safflower oil displayed increases in liver lipogenic metabolites (69). CSOs protective effect may be due to cyclopropenoid fatty acids which are known to irreversibly inhibit endogenous desaturation of SFA by blocking stearoyl-CoA desaturase-1 (SCD1) activity (70), thus leading to decreases in accumulation of lipids (71). Paton et al. (69) observed increased 16:0/16:1n9 ratios in the mice fed the CSO diet compared to mice fed the safflower oil or cocoa butter diets, indicating decreased SCD1 activity after the CSO diet.

Together, these data describe a unique effect of CSO on lipid/cholesterol profiles, despite its less favorable fatty acid profile. There has only been one study to date investigating the effects of CSO in the diet on cholesterol levels and they were able to detect changes after just 5-days. However, this study did not compare CSO to another oil and this may be important to determine if CSO is more beneficial than other vegetable oils in altering lipid/cholesterol profiles after just 5-days.

Dietary Fats Effect on Appetite

Appetite control is a popular field of study due to the increase in the populations' obese and over-weight status over the past couple decades (3). Increased weight is associated with increases in disease risk factors (1), therefore making it important to target weight management strategies. Research investigating the impact of foods on satiation is an important area of research to refine weight management strategies. Several different terms are used to describe and measure appetite, so it is essential to first define some of these key words. Definitions are adapted from Blundell et al. 2009 (72):

Appetite refers to the whole field of food intake, selection, motivation and preference and specifically to qualitative aspects of eating, sensory aspects or responsiveness to environmental

stimulation that can be contrasted with internal homeostatic view based on eating in response to physiological stimuli, energy deficit, etc.

Hunger refers to the drive to eat and can be inferred from objective conditions such as changes in physical sensations in parts of the body including stomach, limbs, or head.

Satiation is the process that leads to the termination of eating, controlling meal size.

Satiety is the process that leads to inhibition of further eating in the postprandial period.

To examine the impact of foods and to measure foods and diets effects on appetite, hunger, satiation, and satiety, there are a number of methodological approaches to consider. The three most used and popular approaches are questionnaires to assess subjective feelings of hunger and satiety, measurement of food/energy intake, and biological/hormonal control of hunger and satiety.

Subjective Feelings of Hunger and Satiety

Subjective ratings of hunger and satiety can be measured using line, categorical, or numerical scales (72). The most common type is the line visual analogue scale (VAS) (72). Overall, the use of subjective ratings of hunger and satiety to evaluate differences between dietary fat composition in foods has given little insight into their effects, with many studies reporting null findings between SFAs, MUFAs and PUFAs (33, 73-80). There were a few that did detect differences between fats (81-83). In one study, investigators compared the acute effects of isocaloric ileal infusions of SFAs, MUFAs, and PUFAs (81). They found that the MUFA and PUFA infusion resulted in higher ratings of fullness and lower ratings of hunger compared to the SFA infusion (81). In another study, the acute effect of meals rich in SFA, PUFA, MUFA were investigated (82). The MUFA-rich meal resulted in greater feelings of hunger and desire to eat and decreased feelings of fullness compared to the PUFA or SFA rich

meals (82). However, by the end of the day, there were no differences detected between meals (82). In a study performed in our lab, the acute effects of a high-fat liquid meal rich in SFA, MUFA, or PUFA were evaluated (83). Ratings of hunger did not differ between trials; however, ratings of fullness were higher after the high-fat SFA-rich meal compared to the MUFA or PUFA-rich meal (83). There have been three studies to date comparing long-term diet effects of dietary fat composition on subjective feelings of hunger and satiety (79, 80, 84). Two of these showed no differences in subjective ratings of hunger or satiety between diets (79, 80). However, Stevenson et al. (84) did find increased subjective ratings of hunger and decreased ratings of fullness in the PUFA-rich diet compared to a control diet high in MUFA and SFA. The drawback to using self-report appetite scales is that they may not be sensitive enough to evaluate smaller scale comparisons (72). Subjective ratings may also impose more variation due to the potential for environmental, emotional and social factors that may influence mood or behaviors on any given day (85). This is why it may be important to use other techniques, such as measurement of energy intake and hormonal signals, along with VAS questionnaires to evaluate foods effects on appetite and weight maintenance.

Subsequent Energy Intake

Assessing energy intake from ad libitum buffet meals is another measurement used to better understand FA composition effects on appetite, specifically satiation. Further, knowing if subjective ratings actually correspond to energy intake and meal size, is important to consider when translating findings to weight management strategies. Many of the studies that resulted in null findings comparing subjective ratings of hunger and fullness also did not detect differences in subsequent energy intake (33, 73-77, 79). There was one study that did find differences in ratings of hunger and fullness but did not detect differences in energy intake at a subsequent

meal following the intervention meal (81). Conversely, differences in energy intake were detected in a previous study mentioned above which mimicked the differences in ratings of hunger and satiety between acute meal challenges differing in fat composition (82). Energy intake following the MUFA-rich meal was greater compared to the PUFA and SFA-rich meals, which matches the subject rating responses found in the study where the MUFA-rich meal elicited a stronger feeling of hunger and weaker rating of fullness (82). Similar to this finding, another study found that energy intake at a buffet meal following acute intraduodenal infusion of SFA or MUFA resulted in higher energy intake after the MUFA infusion compared to the SFA infusion (78). Longer-term diets assessing effects on energy intake are lacking. There has been one study to date assessing 24h energy intake following 2-week diet interventions high in either MUFAs or PUFAs, with no differences detected between the two diets (79).

It is important to highlight that changes in ratings of hunger and fullness do not always translate to reductions in food intake, making results difficult to interpret. Environmental factors come into play, as well as palatability of food when assessing energy intake, emphasizing the importance of having a well-controlled study design (72). Also, these buffets are given in a lab setting which is often free food and may impact consumption.

Hormonal Control of Hunger and Satiety

Satiety is a complex and highly regulated process involving several centers of the brain, as well as multiple circulating factors that are influenced by meal quantity, frequency, and composition. Several circulating hormones have been implicated in the control of hunger and satiety which directly influence the regulation of energy intake and bodyweight (86). The main regions of the brain involved in hunger and satiety control are the hypothalamus, specifically the arcuate nucleus, as well as the dorsal vagal complex in the brain stem, both containing neurons

that stimulate hunger (orexigenic) or suppress hunger (anorexigenic) (87). The arcuate nucleus contains two subsets of neurons that integrate signals and influence energy homeostasis. The neuropeptide Y (NPY)/ agouti-related peptide (AgRP) neurons are orexigenic (stimulate hunger) and the pro-opiomelanocortin (POMC)/ cocaine-and amphetamine-regulated transcript (CART) neurons are anorexigenic (suppress hunger) (88). The NPY/AgRP neurons have been shown to inhibit POMC neurons by release of the neurotransmitter GABA (88). The neurons in the arcuate nucleus further project to secondary neurons in the hypothalamus (87). Specifically, POMC/CART neurons, stimulated from intake of food, release α-melanocyte-stimulating hormone (α-MSH) which binds to its receptor, melanocortin 4-receptor (MC4R), located on second order neurons in the paraventricular nucleus (PVN) (89). The PVN is the source of corticotrophin releasing factor (CRF), thyrotropin-releasing hormone (TRH), and oxytocin, which all act to stimulate satiety (89). NPY/AGRP neurons release NPY which binds to Y1 and Y5 receptors located on second order neurons, particularly in the lateral hypothalamus (LHA), to stimulate hunger in the fasted state (90). The LHA releases melanin-concentrating hormone (MCH) and orexin A to stimulate hunger (89). NPY/AGRP neurons project to the PVN as well, where they act to antagonise the anorexic action of α-MSH (91). In addition to homeostatic regulation of food intake, it is important to note that hedonic regulation has been linked to influence homeostatic centers and vice versa, meaning that there is neural integration of satiety and reward (92, 93).

Several circulating hunger and satiety hormones including ghrelin, glucagon-like peptide-1 (GLP-1), insulin, leptin, peptide YY (PYY), and cholecystokinin (CCK) are known to target anorexigenic and orexigenic neurons, influencing energy intake, and to a lesser degree, energy expenditure (86). Peripheral hunger and satiety signals such as ghrelin, GLP-1, PYY, and CCK

are involved in short-term regulation of appetite, while central signaling of food intake is primarily conveyed through adiposity signals such as leptin and insulin and contribute to longer-term regulation of appetite, playing a role in overall energy balance. Ghrelin is the primary hunger-stimulating hormone while PYY, GLP-1, CCK, leptin, and insulin all act as satiety-stimulating signals in the body. These hormones regulate appetite through neuroendocrine signaling to stimulate activation in the arcuate nucleus by acting on vagal afferent neurons (VAN) which stimulate neurons of the nucleus of the solitary tract (NTS) and arcuate nucleus (94, 95). The dysfunction and impairment of many of these hormones (or their related mechanism) have been linked to metabolic diseases, including obesity (96).

Ghrelin Hormone

Ghrelin is a unique hormone because it is one of the only hormones in the body that increases hunger by stimulating the orexigenic neurons in the hypothalamus (97). It is released from the X/A-like endocrine cells of the stomach, which are suppressed following nutrient ingestion (97). Studies have shown that ghrelin acts to stimulate NPY/AGRP neurons in the arcuate nucleus by both passage through the blood brain barrier and direct influence on vagal signaling (97). During times of low energy, ghrelin is converted to its active form (acyl-ghrelin) by the enzyme ghrelin O-acyl transferase to stimulate nutrient intake (98). Ghrelin O-acyl transferase is believed to act as a lipid-sensing agent through direct interactions with dietary fats making ghrelin highly influenced by dietary fat consumption (98). There have been few studies evaluating ghrelin concentrations following meals differing in FA composition. Poppitt et al. (99) compared the effects of an acute meal high in unsaturated fats to a meal high in SFAs in healthy males and found no significant differences in ghrelin concentrations between the two meals. Similarly, a recent study evaluated the effects of HF meals rich in either SFA, MUFA, or

PUFA in both men and women with the metabolic syndrome and found no differences in ghrelin concentrations between the meals (100). Contrary to these findings, Stevenson et al. (77) compared the effects of meals rich in SFA, MUFA, or PUFA in obese women and found that both MUFA and PUFA meals suppressed ghrelin concentrations to a greater extent than the SFA-rich meal. There has been one longer-term diet intervention to date assessing the effects of a 3-week diet high in SFA to a diet high in unsaturated fats in males with mild hyperlipidemia and no differences were found in ghrelin concentrations between the diets (101). It is yet to be demonstrated if differences between PUFA vs. MUFA exist after a longer-term diet intervention.

CCK Hormone

CCK is released from endocrine I cells in the duodenum (proximal intestine) in response to the presence of food in the intestine, particularly fat, and binds to CCK-1 receptors on VAN (102). CCK in its bioactive proCCK form has 58 amino acid residues (CCK-58) (103). Endocrine I cells contain CCK-58, -33, -22, and -8, where neurons mainly release CCK-8 (103). CCK is also found in the brain and a majority of CCK receptors are located in the dorsomedial hypothalamus (DMH) where CCK acts to inhibit food intake through suppressing NPY (104, 105). It has also been suggested that CCK is involved in the regulation of PYY and ghrelin by mediating the inhibition of ghrelin and stimulation of PYY in response to intraduodenal fat (106). Infusion of fats differing in fatty acid composition have shown no differences in plasma CCK concentrations between SFA, MUFA, or PUFA acutely (78, 81). One study investigated the acute consumption of HF meals rich in MUFAs, omega-6 PUFAs, omega-3 PUFAs, or SFAs and also showed no overall differences in plasma CCK concentrations; however, the HF, omega-3 PUFA rich meal significantly delayed plasma CCK release (107). There is little to no

knowledge on longer-term diet interventions, differing in fat composition, and their effects on CCK in humans.

PYY Hormone

PYY is released from enteroendocrine L-cells in the distal small intestine and proximal large intestine in response to feeding (86). PYY has two main biologically active circulating forms: PYY1-36 and PYY3-36. PYY3-36 is the product of cleavage of the first two amino acids at the N-terminus of PYY1-36 by dipeptidyl peptidase IV (DPP-IV) (108). PYY3-36 is thought to more actively control food intake by binding to Y2 receptors in the NPY neuron, inhibiting energy intake (108, 109). Dietary fats have been shown to regulate both fasting and postprandial PYY content (86, 109). Infusion with oleic acid (MUFA) was shown to increase PYY content compared with lauric acid (SFA), suggesting a potential regulatory role of fatty acid type in PYY content (78). Our lab previously showed diets high in saturated fat elevate postprandial PYY levels to a greater extent than high-fat diets rich in MUFA (80, 83). Additionally, we have recently shown in two studies that single PUFA-rich meals were more effective than a MUFA-rich meal at stimulating PYY (77, 83).

Summary

Currently, little research exists regarding hormonal and metabolic responses to specific unsaturated fat sources, especially in regards to longer-term consumption of these fats. Further, the food source in which these unsaturated fats are being consumed has been shown to influence biological effects, with CSO (a high PUFA oil) possibly having robust effects on alterations in cholesterol levels compared to other high PUFA oils. In general, recommendations are to increase consumption of unsaturated fats (both MUFA and PUFA) while reducing saturated fat consumption. Expanding our knowledge of unsaturated fats may be important because of the

recent emphasis that has immerged about the types of dietary fats Americans should consume to reduce risk of several diseases. The potential differences in metabolic, cholesterol, and hormonal responses to different unsaturated fats has yet to thoroughly be explored and given the prevalence of obesity and related metabolic diseases, it is important to identify key hormonal and metabolic factors to further refine current recommendations. If it is found that one type of fatty acid is more or less satiating or metabolically beneficial than another, this would be important information to possibly aid in the treatment of chronic disease and minimize disease risk factors.

Methodology Used in Study Design

Indirect Calorimetry

To measure metabolism, indirect calorimetry is often used to assess changes in substrate oxidation and energy expenditure. Specifically, indirect calorimetry measures inspired and expired gases (O_2 consumed and CO_2 produced) by a metabolic cart equipped with gas analyzers. Additionally, the flow rate of those gases is measured, so the composition of the air along with flow rate allows for the determination of the volume of O_2 consumed (VO_2) and volume of CO_2 produced (VCO_2). The principle behind utilizing gases to indirectly measure metabolism assumes that the production of chemical energy is proportional to gas exchange. All energy releasing reactions in humans ultimately depend on O_2 use and produce CO_2 as a byproduct of metabolism. During the metabolic measurement, the subject is asked to lie down on a bed and to remain as still as possible without falling asleep. A hood is placed over their head and sealed tight so that no air can escape. The hood is connected to the metabolic cart by a single tube and gas flow is measured through the cart, detecting changes in CO_2 and O_2. The subject inhales ambient air with a constant composition of 20.93% O_2 and 0.03% CO_2 and the changes in

O2 and CO2 percentages in expired air compared with percentages in the inspired ambient air indirectly reflect the ongoing process of energy metabolism. Therefore, analyzing the volume of air breathed along with the composition of expired air provides a way to measure O_2 consumption. When VO_2 and VCO_2 are measured, we can attain the type and rate of substrate utilization (total fat oxidation, total carbohydrate oxidation, respiratory exchange ratio (RER)) and the energy expenditure (EE). RER is the ratio between the amount of CO_2 produced in metabolism and O_2 used. The closer this ratio is to 1.0, the more carbohydrates that are being oxidized. The closer the ratio is to 0.7, the more fats that are being oxidized. For protein, 0.82 characterizes the RER value for protein oxidation. It is not possible to separate out the contribution of protein using indirect calorimetry, due to the metabolism of protein. The liver first deaminates the amino acid molecule and the body excretes the nitrogen and sulfur fragments in the urine. The remaining keto acid then is oxidized, making it indistinguishable from fat or carbohydrate oxidation. Therefore, the RER calculated from expired air reflects the oxidation of a blend of carbohydrates, fats, and proteins. However, the exact amount of protein oxidized can be calculated from urinary nitrogen analysis. Individual substrate oxidation rates of total carbohydrates and total fats can also be obtained. Frayn et al. (110) developed equations to assess macronutrient oxidation from measuring the volumes of O_2 consumed and CO_2 produced as follows: fat (g min-1) = [1.67 x Vo_2 (l min-1)] – [1.67 x Vco_2 (l min-1)]; and carbohydrate (g min-1) = [4.56 x V_{CO2} (l min-1)] – [3.21 x V_{O2} (l min-1)]. To calculate EE, the Weir equation can be used (111): kcal= [3.94(VO_2)+1.11(VCO_2)]. Diet induced thermogenesis (DIT) can also be calculated from EE by subtracting baseline EE from postprandial EE. For all calculations, the first 10 minutes are discarded for a 30-minute segment to allow participants to enter into a steady

state. When performing measurements throughout the day, measures are taken in 20-minute increments with the first 5-minutes discarded.

To ensure that values obtained from the indirect calorimeter are valid and reliable, methanol burns can be performed. With a methanol burn, a known amount of methanol is burned and since the composition of methanol is also known, we can obtain theoretical values of gas combustion. Comparisons can be made to the values obtained by the cart through CO_2 and O_2 detected during the burn and the accuracy and validity can be determined. In this way, the metabolic cart can be calibrated against methanol burns for each study visit. The percent recoveries for O_2 and CO_2 from each burn are used as correction factors for the corresponding metabolic data for each testing day.

Blood Lipids

To assess plasma triglyceride levels, a manual enzymatic colorimetric assay kit using a spectrophotometer is used (Wako Diagnostics, Wako Pure Chemical Industries, Ltd.). This assay utilizes N-(3-sulfopropyl)-3-methoxy-5-methylanaline (HMMPS) which produces a blue pigment. The amount of triglycerides contained in the sample can be determined by measuring the absorbance of the blue color produced. Specifically, there are 2 reagents that are added to the plasma samples in a 2-step process. In the first step, free glycerol in the sample is decomposed by glycerol kinase (GK), glycerol-3-phosphate oxidase (GPO), and catalase contained within the reagent. In the second step, the triglycerides contained within the sample are hydrolyzed to glycerol and free fatty acids in a reaction catalyzed by lipoprotein lipase (LPL). Glycerol is converted to glycerol-3-phosphate by GK in the presence of ATP that was contained in the first reagent. Glycerol-3-phosphate formed is oxidized by GPO producing hydrogen peroxide (H_2O_2). H_2O_2 causes HMMPS (contained within the first reagent) and 4-aminoantipyrine (contained

within the second reagent) to undergo a quantitative oxidative condensation catalyzed by peroxidase (POD) (also contained within second reagent), producing a blue pigment. The absorbance of the blue color produced can be measured by the spectrophotometer.

We do not measure cholesterol profiles in our lab. Instead, we send samples to Piedmont Athens Regional Medical Center for analysis. However, the same technique can be used, using a manual enzymatic colorimetric assay kit. For the total cholesterol assay, 3,5-Dimethoxy-N_ethyl_N-(2-hydroxy-3-sulfopropyl)-aniline sodium salt (DAOS) is used to produce a blue pigment and the total amount of cholesterol contained in the sample can be determined by measuring the absorbance of the blue color produced. The measurement can be performed in a simple 1 step procedure with one reagent added to the samples. When the reagent is added, cholesterol ester hydrolase (contained within the reagent) catalyzes the reaction in which the cholesterol esters in the plasma are hydrolyzed to free cholesterol and fatty acids. The cholesterol produced and the free cholesterol already present in the plasma sample are oxidized in a reaction catalyzed by cholesterol oxidase (contained within the reagent) that generates H_2O_2. H_2O_2 causes DAOS (contained within the first reagent) and 4-aminoantipyrine (contained within the reagent) to undergo quantitative oxidative condensation catalyzed by peroxidase (POD) (also contained within reagent), producing a blue pigment. The absorbance of the blue color produced can be measured by the spectrophotometer. HDL-C can be measured in a 2-step process using a precipitating reagent and a color reagent. First the precipitating reagent containing phosphotungstate and magnesium salt is added to the sample and the lipoprotein except for the HDL fraction can be precipitated out and removed. The remaining supernatant liquid can then be tested for cholesterol by enzymatic colorimetric assay as described above. Lastly, for calculation of LDL-C the Friedewald formula can be used (112). The principle behind the calculation

assumes that very low-density lipoproteins (VLDL) carry most of the circulating triglycerides, and so VLDL can be estimated reasonably well from measured total TGs (TG/5 mg/L). LDL-C can then be calculated as TC-(HDL-C+VLDL-C). This calculation is used very commonly in laboratories due to the relative convenience of the calculation and the difficulty in directly measuring LDL-C (113). LDL-C can be measured directly through separation of lipoproteins by ultracentrifugation, however the steps involved are tedious, time-consuming, and technically demanding (113). Electrophoresis is another technique that can be used to measure LDL-C, however again, the measurement is labor-intensive and technique-sensitive (113).

Radioimmunoassays (RIAs)

RIAs are used to determine circulating concentrations of appetite hormones (EMD Millipore, Billerica, MA, USA). This type of immunoassay uses a radioactive labeled tracer antigen to determine the quantitation of the biomarker of interest. In the procedure a known amount of antibody is added to the plasma samples as well as a fixed concentration of radioactive labeled tracer antigen. The plasma samples contain unlabeled antigen and there is competition between the radioactive labeled tracer antigen and the unlabeled antigen for the limited amount of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen in the plasma sample increases. In a double antibody type RIA a second antibody may be added. This antibody binds to the first antibody/antigen bound complex and is used to separate out the bound and unbound antigen. After centrifugation, the antibody-antigen-antibody complex precipitates to the bottom of the sample forming a small pellet. The unbound tracer antigen is in the remaining supernatant fluid and can be separated out and discarded, leaving only the tracer that is bound to the antibody to be counted.

A gamma counter is used to measure the radioactivity of each sample. The radioactive labeled antigen that will be used in these assays is I-125. I-125 emits gamma rays and the gamma counter contains solid sodium iodide crystals called scintillators that give off a flash of light (scintillation) when they absorb gamma radiation (114). The number of flashes are quantified during the measurement time of each sample, giving counts per minute (CPM). The CPMs can then be calculated to the amount of protein that each tube contains, using the standard curve produced in the assay. The standard curve is set up with increasing amounts of known antigen, and from this curve the amount of antigen in the unknown samples can be calculated.

To maintain quality control throughout the kits utilized to analyze samples, we use pooled plasma run in duplicate with each assay to calculate inter- and intra-assay variability to ensure consistent, reliable results. Each kit obtained also contains quality control samples from the company and these are used with each assay as well to ensure reliable results.

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CHAPTER 3

METABOLIC RESPONSES TO HIGH-FAT DIETS RICH IN MONO-UNSATURATED VERSUS POLY-UNSATURATED FATS[1]

[1]Polley KR, Miller MK, Johnson M, Vaughan R, Paton CM, Cooper JA. Submitted to The British Journal of Nutrition, November 2017.

Abstract

Dietary fatty acid (FA) composition may influence metabolism, possibly affecting weight management. **Purpose:** Compare the effects of a 5-day diet rich in poly-unsaturated FAs (PUFAs) vs. mono-unsaturated FAs (MUFAs). Fifteen normal weight men participated in a randomized cross-over design with two feeding trials (3d lead-in diet, pre-diet visit, 5-day PUFA- or MUFA-rich diet, post-diet visit). The 5d diets (50% fat) were rich in either PUFAs (25% of energy) or MUFAs (25% of energy). At pre- and post-diet visits, subjects consumed breakfast and lunch test meals, rich in the FA for that 5-day diet. Indirect calorimetry was used for 4h after each meal. **Results:** There were no treatment differences in fasting metabolism acutely or following the 5-day diet. For acute meal responses at pre-diet, respiratory exchange ratio (RER) was higher for PUFA vs. MUFA (0.86 ± 0.01 vs. 0.84 ± 0.01, $p < 0.05$), whereas diet induced thermogenesis (DIT) was lower for PUFA vs. MUFA (4.52 ± 0.35 vs. 5.13 ± 0.32 kcals, $p < 0.05$). Following the 5-day diets, the change in RER was different for PUFA vs. MUFA (-0.02 ± 0.01 vs. 0.00 ± 0.01, $p < 0.05$). Similarly, the change in fat oxidation was greater for PUFA vs. MUFA (0.18 ± 0.07 vs. 0.04 ± 0.06 g, $p < 0.05$). **Conclusion:** Acutely, a MUFA-rich meal results in lower RER and greater DIT. However, after a 5-day high-fat diet, the change in metabolic responses were greater in the PUFA diet, showing the metabolic adaptability of a PUFA-rich diet.

Introduction

Dietary fats are implicated in the development of several metabolic diseases including obesity, diabetes, and cardiovascular disease [1]. Overconsumption of energy-dense foods, which is typically associated with a high fat content in foods, is a main contributor to positive energy balance [2]. In recent years, research has suggested that the dietary fatty acid (FA) composition of a high-fat (HF) diet may act differentially on energy usage and storage, effecting weight gain and loss [3; 4].

Decreased fat oxidation has been associated with an increase in body fat [5]. Therefore, it may be important to identify foods and diets that increase fat oxidation for weight management. Early animal and human studies have consistently revealed greater oxidation of unsaturated FAs compared to saturated fatty acids (SFAs) [6; 7; 8], possibly contributing to recommendations centered around increasing unsaturated fat consumption and limiting SFA intake [1]. Differences between rates of fat oxidation amongst various unsaturated FAs also exist. Early human studies using isotope labeling techniques indicate differences in oxidation between MUFAs and PUFAs [9; 10]. Jones et al [9] found that MUFAs were more highly oxidized than omega-6 PUFAs in normal weight men. Delany et al [10] found that again, MUFAs were more highly oxidized than omega-6 PUFAs; however, omega-3 PUFAs were the most highly oxidized FA compared to both MUFA and omega 6 PUFA. More recent research using indirect calorimetry to measure total fat oxidation rates after acute meal challenges have found no differences between these unsaturated fats [11; 12; 13].

Diet induced thermogenesis (DIT) is important in the regulation of energy balance [5], and, similar to fat oxidation, reduced activity of thermogenesis has been associated with obesity [14; 15]. Studies to date have consistently reported greater DIT following consumption of

unsaturated fats compared to saturated fats [8; 12; 13; 16]. Only three studies to date have compared acute meal challenges rich in MUFAs vs PUFAs with mixed findings [11; 12; 13]. Studies have yet to observe the longer-term effects of consuming a diet rich in MUFAs vs. PUFAs on DIT or energy expenditure (EE).

It is important to note that the studies mentioned thus far have all been acute or single meal challenge studies. This may be limiting because postprandial oxidation rates following an acute meal may differ based on historical dietary fat consumption and prior body fat composition. A longer-term diet intervention that can influence metabolic changes or adaptations may more accurately define the differential effects of altering dietary FA composition [3]. Some longer-term diet studies comparing HF diets rich in MUFA vs. SFA [17; 18; 19] and PUFA vs. SFA [6; 20; 21] have been done with significant metabolic differences. However, research exploring the effects of longer-term HF diets rich in PUFAs vs. MUFAs on metabolism is lacking. In our lab, we have recently shown that a diet high in PUFAs resulted in greater fat oxidation after a SFA-rich meal compared to a control diet high in MUFAs [22].

Because of the inconsistent results of previous studies and the lack of research on longer-term ingestion of different types of unsaturated FA-rich diets, the purpose of this study was to compare the effect of a HF diet rich in MUFAs vs PUFAs on metabolism in normal weight men. The primary outcome was to determine the effect of a 5-day diet intervention on fasting and postprandial substrate oxidation and DIT in our PUFA-rich vs. MUFA-rich diets. Based on studies previously performed in our lab [13; 22], we hypothesized that the HF, PUFA-rich diet would lead to greater fasting and postprandial fat oxidation and DIT compared to the HF, MUFA-rich diet. Our secondary outcome was to evaluate the acute meal response, prior to the diet intervention, of consuming a HF meal rich in MUFAs vs. PUFAs on metabolism. Based on

previous findings, we hypothesized that there would be no differences in total fat oxidation and DIT following acute consumption of the PUFA-rich vs. MUFA-rich HF meals.

Methods

Study Design

This study was a single-blind, randomized cross-over design consisting of two different feeding trials. The study protocol included a baseline visit (resting metabolic rate measured), followed by two outpatient feeding trials and is outlined in **Figure 1**. The feeding trials consisted of a 3-day lead in diet, a pre-diet testing visit (*Visit 1),* a 5-day feeding protocol (rich in either MUFA or PUFA), and a post-dieting testing visit (*Visit 2).* Prior to all visits, participants fasted for 8-12 hours and avoided any vigorous exercise for 12h. Following the first trial, there was a 2- to 4-week washout period. Participants then completed trial 2. The only difference between the trials was the type of unsaturated fat provided in the HF diet during the 5-day feeding period. The HF diets were rich in either MUFAs or PUFAs. Participants were randomized to either receive the HF, PUFA-rich diet first or the HF, MUFA-rich diet first following their baseline visit using Research Randomizer (www.randomizor.org). This study was single-blinded, so the participants were not aware of which diet they were receiving first or second.

Participants

Fifteen healthy, normal weight, sedentary adult men were recruited for the study. Inclusion criteria were for males between the ages of 18-45 years, performing less than 3h of structured exercise per week, and normal weight status based on a body mass index (BMI) between 18-24.9 kg/m^2 or body composition analysis using Dual X-Ray Absorptiometry (DXA™; Hologic Inc., Discovery A, Bedford, MA), with a body fat percentage less than 24% to qualify. The exclusion criteria included the following: weight loss or gain exceeding 5% of

body weight in the past 3 months; regular exercise > 3 h per week; plans to lose weight or begin a weight loss program between initiation of the study and final testing; anyone who was vegan or vegetarian; medication use; chronic or metabolic disease, gastrointestinal disorder, or history of medical or surgical events that could affect digestion or swallowing; any supplement use other than a daily multivitamin; blood or plasma donation within 20 days prior to initiation of study; or tobacco use. Subjects were recruited through flyers, campus emails, and word of mouth. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Western Institutional Review Board and written informed consent was obtained prior to beginning study procedures.

Procedures

Baseline Visit

Participants reported to the Human Nutrition Laboratory after an overnight fast (no food or drink except water for 8-12h). Anthropometric measurements (height, weight, and body composition) were taken, followed by resting metabolic rate (RMR) measurement with the ParvoMedics TrueOne® 2400 Canopy System (ParvoMedics, Sandy, UT, USA) for 30 minutes using standardized conditions. Briefly, subjects were asked to stay awake and motionless in a supine position while a plastic hood was placed over their head to measure oxygen consumption and carbon dioxide production. Respiratory gases were used to calculate RMR using the Weir equation [(23)]. Participants' estimated total daily energy needs for the 3-day lead in diet, 5-day diet, and meal challenges at *visits 1* and *2* were based on their calculated RMR multiplied by an average U.S. physical activity value of 1.65 [(24)].

Lead –in diet

For 3 days prior to *Visit 1*, participants were provided with a lead-in diet that was representative of the standard American diet (50% carbohydrate, 35% fat, and 15% protein, **Table 1**). Participants were instructed to consume all foods provided, and no additional foods or beverages besides water were allowed. Total energy content was based on the participant's estimated energy needs calculated from the RMR measurement at the *baseline visit* described above.

Pre-Diet Testing Visit (Visit 1)

Following the 3-day lead-in diet, participants arrived at the laboratory the next morning at 0645h in a fasted state (8-12h fast) and 12h without exercise. Height, weight, blood pressure, waist & hip circumference, and body fat percentage using DXA were measured. Indirect calorimetry was used to measure RMR and fasting substrate oxidation (respiratory exchange ratio (RER) and fat and carbohydrate oxidation) for 30 minutes, as described above. Following indirect calorimetry measurement, participants ingested a HF liquid meal rich in either MUFAs or PUFAs (**Table 2**). The nutrient content of the meal was designed to provide 35% of the participant's estimated total daily energy needs (determined from RMR at *baseline visit*). The liquid meals contained 1% milk, whey chocolate protein, chocolate syrup, and added oil. The oil, either olive oil (OO) or cottonseed oil (CSO), depended on the treatment condition (OO for MUFA or CSO for PUFA). We analyzed the fatty acid composition of each of the oils used in this study. The OO consisted of 19.3% SFA, 67.1% MUFA, and 13.6% PUFA. The CSO consisted of 22.7% SFA, 19.9% MUFA, and 57.4% PUFA. Participants consumed this liquid meal at breakfast (0800h) and another identical meal again 4h later for lunch (1200h).

Following breakfast meal ingestion, respiratory gases were once again measured to determine EE and fuel utilization. Measurements were obtained for a total of 8h postprandially (4h post-breakfast and 4h post-lunch). Specifically, data were collected for a 20-min period followed by a 10-min break. During the 20-min of data collection, participants remained awake but were instructed not to move. During the 10-min break, participants remained seated with minimal movement. Participants were also given 4oz of water in each hour.

Dietary Intervention

Following *Visit 1*, participants began the 5-day, HF feeding trial rich in either MUFA or PUFA (**Table 1**). The order of the feeding trials was randomized. On days 1-5, participants reported to the laboratory between 0700h and 1000h to receive their breakfast, which was given as a shake. Following the meal, participants were then given the rest of their food and beverages for the day and instructed to consume all the food provided and abstain from any other food or drink aside from water. All food items were weighed and prepared by research personnel. The 5-day diet consisted of 50% of total energy from fat, 35% from carbohydrates, and 15% from protein. For the HF portion, diets were prepared using either cottonseed oil (for the PUFA treatment) or olive oil (for the MUFA treatment). For the PUFA treatment, 50% of total fat content was derived from PUFA. For the MUFA treatment, 59% of total fat content was derived from MUFA, with differences reflecting the FA composition of those oils. The total energy intake was prescribed to equal each participant's total estimated daily energy needs (determined from RMR at *baseline visit*).

Post-Diet Testing Visit (Visit 2)

The day following the 5th day of the feeding period, participants reported to the laboratory under fasted (8-12h) and unexercised (12h) conditions. All procedures that took place

during *Visit 1* were repeated. This included consumption of the same HF meals, and metabolic and anthropometric measurements.

The duration of the lead-in diet through *Visit 2* represented *Trial 1*. The participant then underwent 2- to 4-weeks of washout during which time they returned to their normal dietary patterns. After the washout period, the participants underwent *Trial 2* (including lead-in diet, *Visit 1* test-day, a 5-day high PUFA or MUFA dietary intervention, and *Visit 2* test-day). The only difference between the trials were the foods for each 5-day trial and corresponding HF meal challenges during the testing visits (MUFA vs. PUFA).

Calculations

Respiratory gases were used to calculate EE using the Weir equation [23] and macronutrient oxidation using the following equations developed by Frayn [25]: fat (g min^{-1}) = [1.67 x Vo_2 (l min^{-1})] – [1.67 x Vco_2 (l min^{-1})]; and carbohydrate (g min^{-1}) = [4.56 x VCO_2 (l min^{-1})] – [3.21 x VO_2 (l min^{-1})]. VO_2 and VCO_2 represent O_2 consumption and CO_2 production, respectively, in liters per minute. RER was calculated as the ratio of VCO_2 to VO_2. For these calculations, the first 5 min of each 20 min segment were discarded to allow participants to enter into a steady state. The DIT was calculated from postprandial EE subtracted by baseline EE.

Additionally, the metabolic cart was calibrated against methanol burns throughout the duration of the study [26]. Burns were performed in the morning and afternoon of each testing visit. The percentage recoveries from each burn were used as correction factors for the corresponding metabolic data for each testing day. The average correction factors were 99.15% and 98.57% for O_2 and CO_2, respectively.

Statistical analysis

To determine if our sample size was appropriate, a within subject, repeated measure ANOVA was used to assess trial effects. With an effect size of 0.67 (determined from the mean postprandial change between PUFA vs. MUFA for RER from this study), we would require a total sample size of 8 subjects, assuming 80% power (beta) and an alpha of 0.05. The number of subjects required was determined using G*Power 3.1.9.2. SAS version 9.4 statistical package (SAS Institute Inc, Cary, NC, USA) was used for all statistical analyses. To address our primary outcome of the effect of the 5-day intervention diet, change from Visit 1 (pre-diet) to Visit 2 (post-diet) was calculated and a within subjects repeated measures ANOVA was used to detect main effects (time, treatment) and interaction effects. A secondary analysis was performed to determine differences in acute meal response at Visit 1, prior to the intervention diet between MUFAs and PUFAs using a within subject repeated measures ANOVA. If significance was found, post hoc analyses were performed using Tukey's test. Data are presented as means ± SEM. Statistical significance was set at $p \leq 0.05$.

Results

Subjects

Fifteen normal weight male participants completed both feeding trials. Physical characteristics of the participants are shown in **Table 3**. Participants were between 18 and 45 years of age and were of normal weight based on body mass index (BMI= 18-24.9 kg/m^2) or DXA body fat percentage (14 - 24%). All participants were also free from any known metabolic or chronic disease and were not taking any medications.

Acute metabolic responses

There were no significant differences at fasting/baseline for the pre-diet visit (Visit 1) between MUFAs and PUFAs for any outcome variable (RER, fat and carbohydrate oxidation, and EE) (**Table 4**). When examining the time-course meal responses, as expected, there was a significant time effect for all substrate oxidation (RER, fat, and carbohydrate oxidation) and energy expenditure data (DIT) ($p < 0.001$). There were no treatment by time interactions; however, several treatment effects were found. For RER, there was a significant treatment effect (**Figure 2a**), with a significantly higher RER for the PUFA pre-diet visit compared to the MUFA pre-diet visit (post-prandial average of 0.86 ± 0.01 vs. 0.84 ± 0.01, $p < 0.05$, **Figure 2b**). For fat oxidation, there also was a significant treatment effect (**Figure 3a**), with lower fat oxidation for the PUFA pre-diet vs. MUFA pre-diet visits (post-prandial average of 1.20 ± 0.12 vs. 1.33 ± 0.09 g, $p < 0.05$, **Figure 3b**). For carbohydrate oxidation, there was a significant treatment effect (**Figure 4a**), with higher carbohydrate oxidation for the PUFA pre-diet vs. MUFA pre-diet visit (post-prandial average of 3.30 ± 0.17 vs. 2.97 ± 0.18 g, $p < 0.05$, **Figure 4b**). For DIT, there was a significant treatment effect (**Figure 5a**), with lower DIT for the PUFA pre-diet visit vs. MUFA pre-diet visit (postprandial averages of 4.52 ± 0.35 vs. 5.13 ± 0.32 kcals, $p < 0.05$, **Figure 5b**).

Metabolic responses to 5-day HF diets

There were no significant differences in fasting values from pre- to post- diet in any outcome variable (RER, fat and carbohydrate oxidation, and EE) (**Table 4**). When examining the change in time-course meal responses, as expected, there was a significant time effect for all substrate oxidation (RER, fat, and carbohydrate oxidation) and energy expenditure data (DIT) ($p < 0.001$). There were no treatment by time interactions; however, there were several treatment effects for substrate oxidation. For change in RER, there was a significant treatment effect, with

the decrease in RER from pre- to post- PUFA diet being significantly different from MUFA (post-prandial average change of -0.02 ± 0.01 vs. 0.00 ± 0.01, $p < 0.05$, **Figure 6a**).

Similar to RER, there was a significant treatment effect for change in total fat oxidation from pre- to post-diet. The increase in the change in fat oxidation was significantly different from the change in MUFA (post-prandial average change of 0.18 ± 0.07 vs. 0.04 ± 0.06 g, $p < 0.05$, **Figure 6b**). For change in carbohydrate oxidation, there was also a significant treatment effect. The decrease in carbohydrate oxidation from pre- to post-PUFA diet intervention was significantly different than the change in MUFA (post-prandial average change of -0.38 ± 0.18 vs. 0.00 ± 0.17 g, $p < 0.05$, **Figure 6c**). Finally, to look at the meal response EE, we calculated DIT and compared the change in DIT for PUFA vs. MUFA. Unlike substrate oxidation, there were no significant treatment effects for change in DIT **(Figure 6d)**.

Discussion

In this study, we found differences in the metabolic response to acute high-fat meal challenges compared to longer-term HF diet responses rich in PUFAs or MUFAs. In regard to acute meal consumption, our results indicate that intake of a HF meal rich in MUFAs leads to greater fat oxidation, and lower carbohydrate oxidation compared to a HF meal rich in PUFAs. Cottonseed oil, which was our PUFA treatment oil is rich in omega-6 FAs, especially linoleic acid. A prior stable isotope study by Jones et al. (1985) also found that a meal high in oleic acid (MUFA) resulted in higher fat oxidation than a meal high in linoleic acid (omega-6 PUFA). Further, Delany et al. (2000) also showed that with labeled isotopes, a meal high in oleic acid (MUFA) resulted in higher fat oxidation than a meal high in linoleic acid (PUFA). More recent studies have not detected a metabolic difference between acute PUFA- vs. MUFA-rich meals using indirect calorimetry, and this may be due to PUFA-rich meals having mixed contents of

omega-3 and omega-6 PUFAs [12; 13]. Delany et al. (2000) also demonstrated that a meal high in alpha-linolenic acid (omega-3 PUFA) resulted in the greatest amount of fat oxidation compared to both oleic and linoleic acid, indicating that the type of PUFA may be important. Our study used an oil that was specifically high in linoleic acid with little alpha-linolenic acid, which could have impacted our significant findings compared to the null findings between MUFAs and PUFAs of other studies which used HF meals with significant amounts of omega-3 PUFA [12; 13].

The mechanisms behind greater fat oxidation acutely following a MUFA-rich, HF meal compared to a PUFA-rich, HF meal are largely unknown. McCloy et al. [27] demonstrated that acutely, linoleate is preferentially esterified to plasma phospholipids and cholesterol esters compared to oleate and α-linolenate, which would lead to preferential retention and sequestration of linoleate rather than oxidation. However, whether this decreased oxidation of linoleate is due to a greater preference for glycerolipid synthesis, a reduced preference for β-oxidation, or perhaps a combination thereof, is not known. Hodson et al. [28] also demonstrated preferential incorporation of linoleate into plasma phospholipids and cholesterol esters compared to oleate following a HF meal. This again could imply that more oleate is available for oxidation, and may explain our results of greater fat oxidation following a single MUFA-rich HF meal compared to a PUFA-rich HF meal.

Our findings also suggest that a more chronic or longer-term effect on metabolism from either PUFA vs. MUFA consumption is different than that of the acute meal effect. After the 5-day HF diet period, significant changes in substrate oxidation only occurred following the HF PUFA-rich diet, which led to substrate oxidation being similar between our PUFA and MUFA groups post-diet. This finding is important because it shows the adaptability of the PUFA-rich, HF diet after just 5 days. Acutely, MUFAs may have more metabolically favorable outcomes

with higher fat-oxidation than PUFAs; however, after 5 days of either a HF MUFA- or PUFA-rich diet, both result in favorable metabolic measures (higher fat oxidation and lower carbohydrate oxidation). This adaptability of our PUFA-rich diet may be due to a limited capacity to sequester linoleic acid into phospholipids and thus be diverted to oxidation once this pool is full, which would be detectable with a longer-term diet [28]. Acutely, the partitioning of linoleic acid may result in preferential incorporation of these dietary fats into phospholipids, hence lower oxidation after a single meal [27; 28]. However, longer-term consumption of a diet rich in linoleic acid, such as our PUFA diet, is able to reveal changes and adaptations over a few days. PUFAs are also potent regulators of lipogenic gene expression, and diets rich in linoleic acid result in down regulation of genes encoding enzymes of lipid synthesis [29], thus affirming our findings of increased fat oxidation after a 5-day, PUFA-rich diet. Finally, the cottonseed oil used to enrich the PUFA diet in this study may contain a unique component that could be contributing to the increase in oxidation seen after our 5-day diet. Cottonseed oil contains cyclopropenoid FAs, specifically dihydrosterculic acid (DHSA) [30]. DHSA may play an important role in reducing stearoyl-CoA desaturase-1 (SCD-1) activity [30; 31], therefore reducing overall triglyceride formation and channeling free FAs into β-oxidation [32]. This illustrates a unique component that may be present in CSO. Taken together, the significant increase in fat oxidation from pre- to post- PUFA-rich HF diet may have been due to the fatty acid content as well as a unique component in the cottonseed oil used in the diet.

Other longer-term studies have investigated the metabolic effects of PUFA vs. SFA [6; 20; 21] and MUFA vs. SFA [17; 18; 19]. Additionally, our lab previously showed that a 7-day, PUFA-rich diet resulted in higher fat oxidation following a SFA-rich meal compared to a control diet (containing a higher percentage of fat from MUFA) [22]. In contrast, we did not see differences

between our MUFA- and PUFA-rich diets after 5-days, leading us to reject our hypothesis that a PUFA-rich HF diet would lead to greater fat oxidation than a MUFA-rich HF diet. This could be due to the relatively high fat content of our diets which could mask the FA effect that was detectable in our previous study with a lower percentage of energy in the diet was fat [22]. Our previous PUFA-rich, 7-day diet intervention also had a higher percentage of PUFAs coming from omega-3 fats, which may have also contributed to differences between the two study outcomes [22].

Our acute results for DIT showed that a HF, MUFA-rich meal resulted in greater DIT compared to a HF, PUFA-rich meal. In agreement with these results, Jones et al. (2008) found that a HF meal enriched in MUFAs induced greater DIT compared with a HF meal enriched in PUFAs. However, in contrast to our findings, Clevenger et al. (2014) found that a HF meal rich in PUFAs increases postprandial thermogenesis compared to a MUFA-rich meal in females. Potential metabolic differences between males and females may contribute to the contradicting results of the current study. Other previous studies have not detected differences in DIT between MUFAs and PUFAs [12; 33]. It is also important to note that while the change in DIT was not different between MUFA vs. PUFA for the 5-day diet period, DIT was similar between PUFA and MUFA after the 5-day diet intervention period.

Limitations of the current study include self-reported meal compliance during the study which could impact the outcomes measured. Our participants were free-living, making it possible for them to consume other foods and beverages throughout the day. However, participants came into the lab every morning to consume breakfast and were reminded each day to only eat and drink the food that was provided to them. Another limitation of the study is that we used oils high in either PUFA or MUFA to enrich each diet type, and such oils may contain other

beneficial nutrients or components in them that could be attributable to the metabolic differences. For example, OO contains polyphenols that may inhibit lipogenesis, therefore possibly contributing to its effect in greater fat oxidation [34]. However, because the study design was matched except for differences in oils used, we can narrow differences down to the specific food source (CSO or OO). Future studies are needed to determine if these differences are specific to the FAs in the oils used, due to other nutrients within the oils, or a combination of a synergistic effect of the nutrients and types of FAs in the oil as a whole. Another limitation is that the percentage of fat from our fatty acid of interest (MUFA for OO, PUFA for CSO) differed because of the composition of the oils (67.1% MUFA in OO, 57.3% PUFA in CSO). However, one of the strengths of the current study is that we used a whole foods approach, mimicking real-life eating patterns. Since we do not eat FAs, matching the percent of total energy from a specific fatty acid group is not always feasible. Our study also contained diets that were above the upper limits of dietary recommendations for total fat and PUFA content which was by design to establish proof of principle. This may, however, limit clinical application of the diets used, and future studies are needed to explore the metabolic responses to lower fat diets. Lastly, the study sample included apparently healthy men with normal body fat percentages, so these results may not be extrapolated to other populations.

In conclusion, the present findings indicate that an acute HF meal rich in MUFAs from OO results in greater acute total fat oxidation and lower total carbohydrate oxidation and RER compared to a HF meal rich in PUFAs from CSO. However, consumption of a 5-day HF diet rich in PUFA leads to increases in total fat oxidation and decreases in total carbohydrate oxidation and RER making substrate oxidation after 5-day HF diets rich in these different fats equal. Because greater fat oxidation has been associated with lower body fat mass, rates of fat

oxidation can have implications for weight management. Our results indicate that after a 5-day period, both sources of unsaturated FAs may have a similar metabolic impact on weight management since fat oxidation rates were similar between the diets. Finally, DIT followed the same trend as seen with substrate oxidation, being higher after the acute MUFA-rich meal compared to PUFA. While the change over the 5-day HF diet intervention was not significantly different between treatments, DIT was similar between the treatments after adaptation occurred with the PUFA-rich diet. Future studies are needed to investigate the mechanisms behind differences seen acutely between PUFAs and MUFAs, and whether further differences would be seen with chronic consumption of these two diets.

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Table 3.1 Nutrient content for each HF diet

Composition	Lead-in diet	PUFA-rich HF diet	MUFA-rich HF diet
Percentage of total energy from			
Protein	15.0	15.0	15.0
Carbohydrate	50.0	35.0	35.0
Fat	35.0	50.0	50.0
Percentage of energy from fatty acid of interest			
MUFA	16.0	9.7	31.5
PUFA	7.6	26.7	6.6
n6 PUFA	*7.6*	*26.3*	*5.7*
n3 PUFA	*0.0*	*0.4*	*0.9*
SFA	11.4	13.4	10.4

HF high fat, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid, *n6 PUFA* omega-6 polyunsaturated fatty acid, *n3 PUFA* omega-3 polyunsaturated fatty acid

Table 3.2 Nutrient content for each HF test meal

Composition	PUFA-rich HF meal	MUFA-rich HF meal
Percentage of total energy from		
Protein	15.5	15.4
Carbohydrate	28.1	28.3
Fat	56.7	56.3
Percentage of energy from fatty acids		
MUFA	11.5	36.7
PUFA	31.1	7.4
n6 PUFA	*30.9*	*6.8*
n3 PUFA	*0.2*	*0.6*
SFA	14.2	12.3

HF high fat, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid, *n6 PUFA* omega-6 polyunsaturated fatty acid, *n3 PUFA* omega-3 polyunsaturated fatty acid

Table 3.3 Participant characteristics

Characteristic	Value
Age (y)	21.67 ± 2.58
Height (cm)	177.74 ± 6.95
Weight (kg)	77.24 ± 13.61
Body mass index (kg/m^2)	24.27 ± 2.81
Body fat (%)	16.49 ± 4.85
Waist circumference (cm)	83.61 ± 9.16
Hip circumference (cm)	98.44 ± 8.54

Note: All values are means ± SD (n=15)

Table 3.4 Fasting Metabolic Measurements

	PUFA-rich HF diet		MUFA-rich HF diet	
	Pre-diet	Post-diet	Pre-diet	Post-diet
RER	0.84 ± 0.02	0.83 ± .01	0.83 ± 0.01	0.83 ± 0.01
Fat Oxidation (g/15 min)	1.06 ± 0.13	1.15 ± 0.07	1.14 ± 0.11	1.12 ± 0.09
Carbohydrate Oxidation (g/15 min)	2.65 ± 0.27	2.33 ± 0.19	2.18 ± 0.23	2.32 ±0.20
Energy Expenditure (kcal/15 min)	19.23 ± 0.82	19.67 ± 0.70	18.65 ± 0.82	19.33 ± 0.68

PUFA polyunsaturated fatty acid, *HF* high fat, *MUFA* monounsaturated fatty acid, *RER* respiratory exchange ratio

Values are presented as means ± SEM; no significant differences from pre- to post-diet or between diets

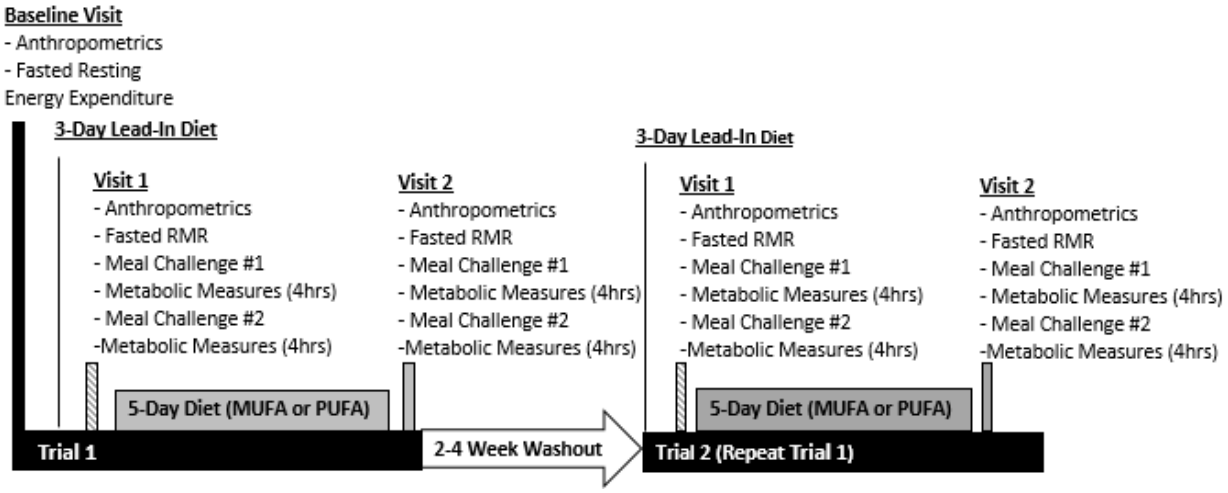

Figure 3.1 Time-course of study visits and measurements taken at each visit. RMR = resting metabolic rate, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

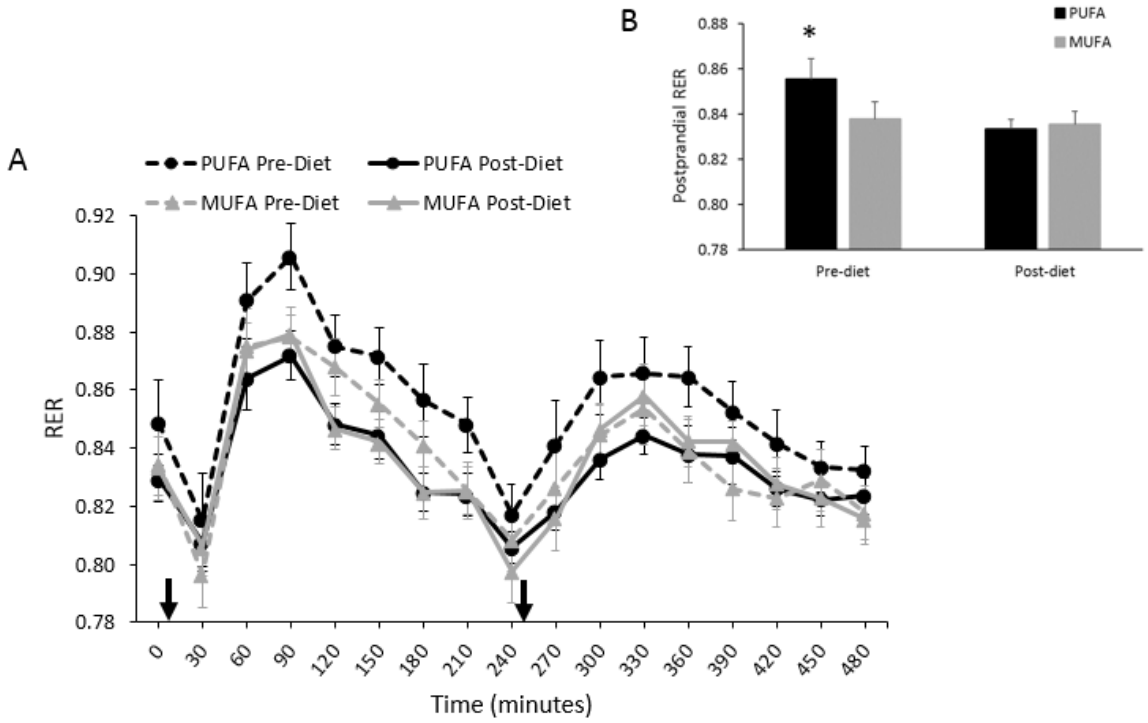

Figure 3.2 Mean ±SE, (**A**) Fasting and postprandial RER for each of the diets, pre- and post-diet intervention. Subjects consumed the HF meal immediately after time 0 and time 240 (black arrows). (**B**) Average postprandial RER for pre- and post-diet interventions. * Significantly different RER values between PUFA pre-diet and MUFA pre-diet visits, ($p<0.05$). MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

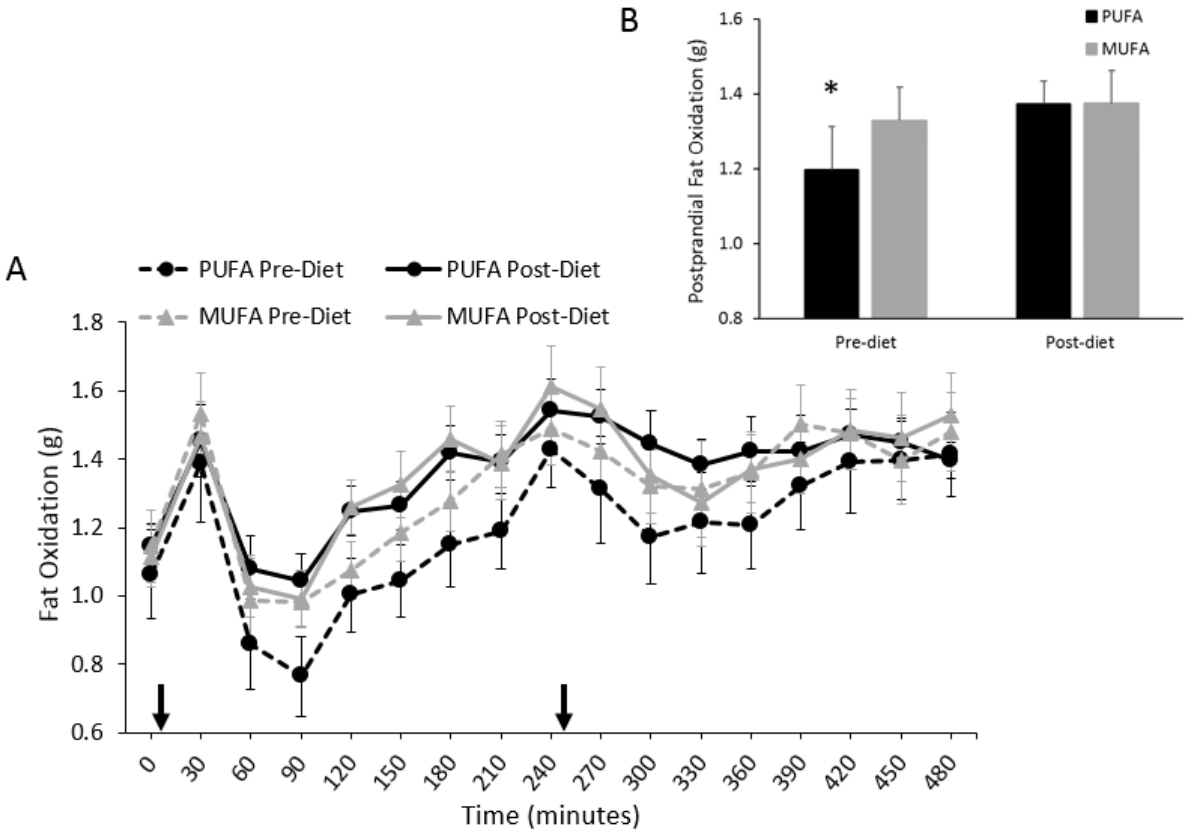

Figure 3.3 Mean ±SE, (**A**) Fasting and postprandial fat oxidation for each of the diets, pre- and post-diet intervention. Subjects consumed the HF meal immediately after time 0 and time 240 (black arrows). (**B**) Average postprandial fat oxidation for pre- and post-diet interventions. *Significantly different fat oxidation values between PUFA pre-diet and MUFA pre-diet visits, ($p<0.05$). MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

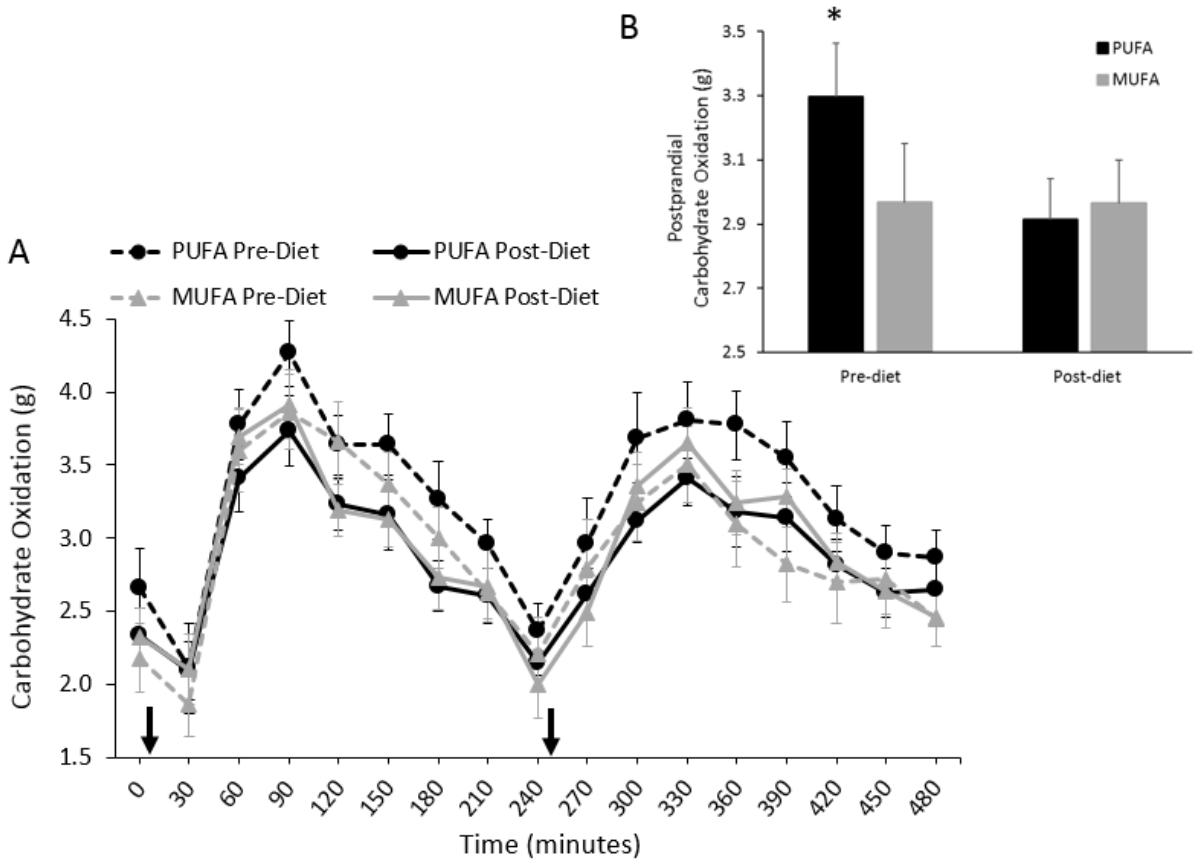

Figure 3.4 Mean ±SE, (**A**) Fasting and postprandial carbohydrate oxidation for each of the diets, pre- and post-diet intervention. Subjects consumed the HF meal immediately after time 0 and time 240 (black arrows). (**B**) Average postprandial carbohydrate oxidation for pre- and post-diet interventions. * Significantly different carbohydrate oxidation values between PUFA pre-diet and MUFA pre-diet visits, ($p<0.05$). MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

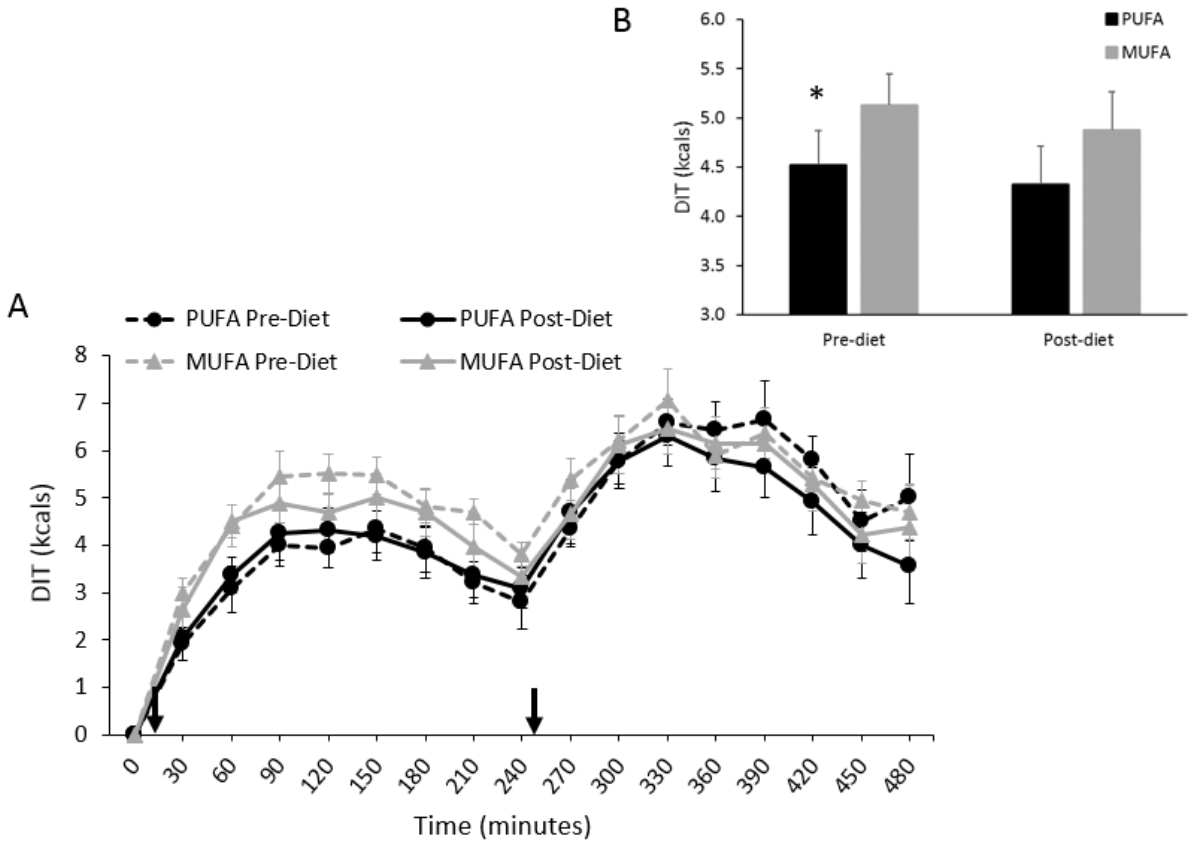

Figure 3.5 Mean ±SE, (**A**) Diet induced thermogenesis (DIT) for each of the diets, pre- and post-diet intervention. Subjects consumed the HF meal immediately after time 0 and time 240 (black arrows). (**B**) Average DIT for pre- and post-diet interventions. * Significantly different DIT values between PUFA pre-diet and MUFA pre-diet visits. MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

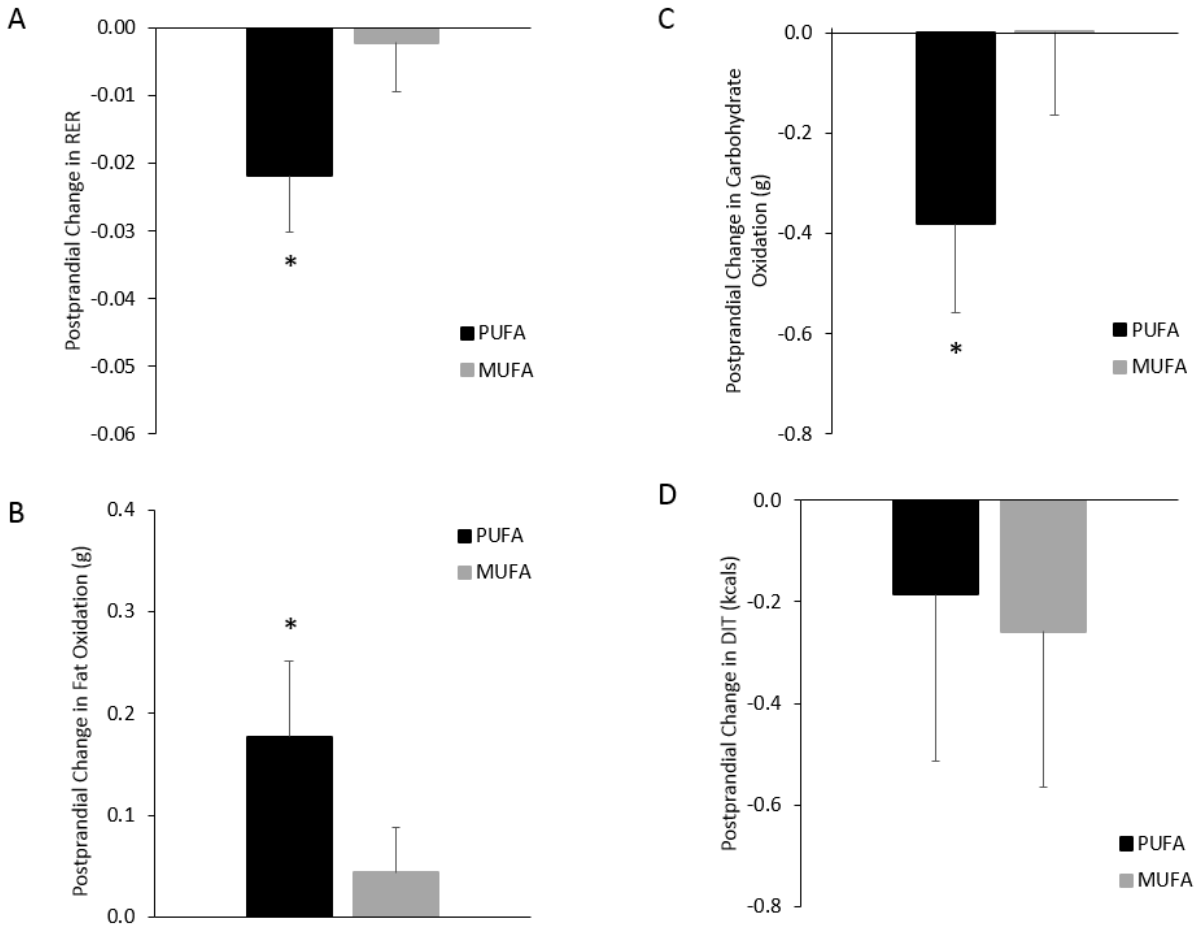

Figure 3.6 Mean ±SE, (**A**) Pre- to post-diet change in RER for each diet. (**B**) Pre- to post-diet change in fat oxidation for each diet. (**C**) Pre- to post-diet change in carbohydrate oxidation for each diet. (**D**) Pre- to post-diet change in DIT for each diet. * Significantly different between PUFA and MUFA diets, ($p<0.05$). MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, RER = respiratory exchange ratio, DIT = diet induced thermogenesis

CHAPTER 4

CHANGES IN LIPID PROFILES FOLLOWING A HIGH-FAT DIET RICH IN COTTONSEED OIL VERSUS OLIVE OIL[2]

[2]Polley KP, Oswell NJ, Pegg RB, Paton CM, Cooper JA. Submitted to Nutrients, May 2018.

Abstract

Background: Modifying the type of dietary fat consumed is important for minimizing risk factors for cardiovascular disease and modulating dyslipidemia.

Objective: To compare the effects of a 5-day, high-fat diet rich in cottonseed oil (CSO) to a diet rich in olive oil (OO) on changes in lipid profiles.

Methods: Fifteen normal weight men participated in a randomized cross-over design with two controlled feeding trials (3d lead-in diet, pre-diet visit, 5d CSO- or OO-rich diet, post-diet visit). The 5d diets (50% fat) were rich in either CSO (44% of total energy) or OO (44% of total energy). At pre- and post-diet visits, subjects consumed test meals at breakfast and lunch rich in the oil that coincided with their 5-day diet. Fasting and postprandial blood draws for 4h after each meal were performed to determine changes in lipid profiles.

Results: Fasting total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were significantly lower following CSO diet intervention (TC: 148.40±6.39 to 135.93±6.31 mg/dL; LDL-C: 92.20±5.57 to 78.13± 5.60mg/dL; TG: 80.11±4.91 to 56.37±5.46 mg/dL, for pre- to post-diet, respectively; $p<0.05$). High-density lipoprotein cholesterol (HDL-C) significantly increased following CSO diet intervention (HDL-C: 46.67±2.41 to 50.24±2.20 mg/dL, for pre- to post-diet, respectively; $p<0.05$). Conversely, no changes in blood lipids were found following OO diet although there was a trend for lower fasting TG (74.51 ± 8.38 vs. 64.08 ± 6.87 mg/dL, $p=0.11$). Postprandial TG levels were significantly lower following CSO diet (AUC of 954.28±56.90 vs. 722.16±56.15mg/dL/8h, for pre- vs. post-diet, respectively; $p < 0.01$) but did not change for OO diet (AUC average of 913.65 ± 77.46 vs. 801.17 ± 62.72 mg/dL/8h, $p = 0.09$).

Conclusions: A 5-day CSO-rich diet resulted in significant improvements in cholesterol profiles and TG levels, while no changes were observed after a 5-day OO-rich diet in the same participants.

Introduction

Dyslipidemia is a major risk factor for cardiovascular disease with one in every four U.S. adults having elevated levels of triglycerides (>150 mg/dL) (1). Diet and lifestyle changes play an important role in minimizing this modifiable risk factor, and many studies focus on various diet and exercise interventions to prevent dyslipidemia (2-4). Of these strategies, modifying the type of dietary fat consumed has proven to be an important and effective area of research (5). Current dietary recommendations center on reducing saturated and trans-fatty acid intake, and increasing mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) intake (6). Eating oil-based fats (high in MUFAs and PUFAs) instead of solid fats (high in saturated fat) has been linked to reduced disease risk (7-9). Most research to date focuses on the fatty acid class or family to describe generic functional roles or health impacts (10). However, it is important to consider the whole food in which these fatty acids are derived from, along with other components of the food itself which may affect physiological function.

Cottonseed oil (CSO) is rich in omega-6 PUFAs, especially linoleic acid (18:2n6). In early studies in animal models, research has shown a lipid lowering effect with consumption of a diet rich in CSO (11, 12). This is unique since CSO also has higher levels of saturated fat than many other vegetable oils along with its high linoleic acid content. Therefore, despite CSO's higher levels of saturated fat, which are typically associated with dyslipidemia (13-15), it has a more positive effect on lipid profiles than other high linoleic oils, such as corn oil and safflower oil (11, 12). To further demonstrate CSO's lipid-lowering effects, a recent study in human subjects showed significant reductions in total cholesterol and LDL-C after just 5 days of supplementation with 95g per day of CSO that was incorporated into each person's regular diet (16).

Olive oil (OO) is rich in MUFAs, specifically oleic acid, and has been repeatedly linked to reductions of chronic disease risk factors when substituted for saturated fats or carbohydrates (17). OO is also the main fat consumed in the Mediterranean diet, and many studies utilizing a Mediterranean diet intervention have consistently reported reductions in total cholesterol (18, 19). However, studies comparing OO to PUFA-rich vegetable oils have revealed stronger effects of PUFA-rich oils in modifying cholesterol profiles after 3-4 week diet interventions (20-23). OO has never been compared to CSO, and because of CSO's unique fatty acid profile, with high amounts of both saturated fats and PUFAs, it would be important to know whether this oil has more robust changes in lipid profiles than OO; and further, if differences between the oils are detectable after just 5 days of diet intervention.

To further investigate the effects of CSO, and compare CSO to OO, the aim of the current study was to evaluate the effect of a 5-day, high-fat (HF) diet rich in CSO to a 5-day, HF diet rich in OO on fasting and postprandial lipid profiles in healthy, normal weight men in a cross-over design. Based on previous human and animal models, we hypothesized that the HF, CSO-rich diet would lead to lower fasting and postprandial lipid levels to a greater extent than the HF, OO-rich diet.

Methods

Study Design

This study was a single-blind, randomized cross-over design consisting of two different feeding trials. The study protocol included a baseline visit (resting metabolic rate measured), followed by two outpatient feeding trials (outlined in **Figure 1**). The feeding trials consisted of a 3-day lead-in diet, a pre-diet testing visit (*Visit 1)*, a 5-day outpatient feeding protocol (HF diet rich in either CSO or OO), and a post-diet testing visit (*Visit 2)*. Prior to all visits, participants

fasted for 8-12h and avoided any vigorous exercise for 12h. Following the first trial, there was a 2- to 4-week washout period. Participants then completed trial 2. The only difference between the trials was the type of oil provided in the HF diet during the 5-day feeding period. The HF diets were rich in either CSO or OO.

Participants

Fifteen healthy, normal weight, sedentary (< 3h of exercise per week) men between the ages of 18 and 45 years were recruited for the study. Normal weight status was determined based on a body mass index (BMI) between 18-24.9kg/m^2 or body composition analysis using Dual X-Ray Absorptiometry (DXA™; Hologic Inc., Discovery A, Bedford, MA, USA), with a body fat percentage less than 24% to qualify. The exclusion criteria included the following: weight loss or gain exceeding 5% of body weight in the past 3 months; regular exercise > 3h per week; plans to lose weight or begin a weight loss program between initiation of the study and final testing; anyone who was vegan or vegetarian; medication use; chronic or metabolic disease, gastrointestinal disorder, or history of medical or surgical events that could affect digestion or swallowing; any supplement use other than a daily multivitamin; blood or plasma donation within 20 days prior to initiation of study; or tobacco use. All experimental procedures were approved by the Western Institutional Review Board and written informed consent was obtained prior to beginning study procedures.

Procedures

Baseline Visit

Participants reported to the Human Nutrition Laboratory after an overnight fast (no food or drink except water for 8-12h). Anthropometric measurements (height, weight, and body composition) were taken, followed by resting metabolic rate (RMR) measurement with the

ParvoMedics TrueOne® 2400 Canopy System (ParvoMedics, Sandy, UT, USA) for 30 minutes using standardized conditions (24). Briefly, subjects were asked to stay awake and motionless in a supine position while a plastic hood was placed over their head to measure oxygen consumption and carbon dioxide production. Respiratory gases were used to calculate RMR using the Weir equation (25). Participants' estimated total daily energy needs for the 3-day lead-in diet, 5-day intervention diet, and meal challenges at *visits 1* and *2* were based on their calculated RMR multiplied by an average U.S. physical activity value of 1.65 (26).

Lead-in diet

For 3 days prior to *Visit 1*, participants were provided with a lead-in diet that was representative of the standard American diet (50% carbohydrate, 35% fat, and 15% protein, **Table 1**). Participants were instructed to consume all foods provided, and no additional foods or beverages besides water were allowed. Total energy content was based on the participant's estimated energy needs calculated from the RMR measurement at the *baseline visit* described above.

Pre-Diet Testing Visit (Visit 1)

Following the 3-day lead-in diet, participants arrived at the laboratory the next morning at 0645h in a fasted state (8-12h fast) and 12h without exercise. Height, weight, blood pressure, waist & hip circumference, and body fat percentage using DXA were measured. An intravenous catheter was inserted in to the antecubital vein for intermittent blood draws and a 15mL fasted blood sample was obtained to assess blood lipids. The line was kept patent with 0.9% normal saline, and blood was drawn into EDTA vacutainers (Greiner Vacuette, Monroe, NC, USA) and immediately centrifuged at 3,000rpm for 15 min at 4ºC. Five mL of sample was analyzed for a complete lipid panel including total cholesterol (TC), low density lipoprotein cholesterol (LDL-

C), and high-density lipoprotein cholesterol (HDL-C) (Athens Regional Laboratory; Athens, GA, USA). Following the fasted blood draw, participants ingested a HF liquid meal rich in either CSO or OO (**Table 2**). The nutrient content of the meal was designed to provide 35% of the participant's estimated total daily energy needs (determined from RMR at *baseline visit*). The liquid meals contained 1% milk, whey chocolate protein, chocolate syrup, and added oil. The oil, either olive oil (OO) or cottonseed oil (CSO), depended on the treatment condition. Participants were given 5 min to consume the entire liquid meal. One meal was consumed at breakfast (0800h) and another identical meal consumed 4h later for lunch (1200h).

Following breakfast meal ingestion, a 10mL blood sample was taken at minutes 30, 60, 90, 120, 150, 180, and 240 for measurement of triglyceride (TG) concentrations. After the 240 min blood draw, participants were fed another HF liquid meal that was identical to the breakfast meal. Blood draws continued to be collected at minutes 270, 300, 320, 350, 380, 420 and 480. Therefore, measurements were obtained for a total of 8h postprandially (4h post-breakfast and 4h post-lunch). Participants were also given 4oz of water each hour. Blood samples were immediately spun at 3,000rpm for 15min at 4ºC. Plasma was aliquoted and stored at -80ºC until assayed. The intravenous catheter was removed after 8h.

Dietary Intervention

Following *Visit 1*, participants began the 5-day, HF outpatient feeding trial rich in either CSO or OO (**Table 1**). The order of the feeding trials was randomized. On days 1-5, participants reported to the laboratory between 0700h and 1000h to consume their breakfast, which was given as a shake. Following the meal, participants were given the rest of their food and beverages for the day and instructed to consume all the food provided and abstain from any other food or drink aside from water. All food items were weighed and prepared by research personnel. The 5-

day diet consisted of 50% of total energy from fat, 35% from carbohydrates, and 15% from protein. For the HF portion, diets were prepared using either CSO (44% of total energy) or OO (44% of total energy). The total energy intake was prescribed to equal each participant's total estimated daily energy needs (determined from RMR at *baseline visit*).

Post-Diet Testing Visit (Visit 2)

The day following the 5th day of the feeding period, participants reported to the laboratory under fasted (8-12h) and unexercised (12h) conditions. All procedures that took place during *Visit 1* were repeated. This included anthropometric measurements, consumption of the same HF meals, and blood draws.

The duration of the lead-in diet through *Visit 2* represented *Trial 1*. The participant then underwent 2- to 4-weeks of washout during which time they returned to their normal dietary patterns. After the washout period, the participants underwent *Trial 2* (including lead-in diet, *Visit 1* test-day, a 5-day high CSO or OO dietary intervention, and *Visit 2* test-day). The only difference between the trials were the foods for each 5-day trial and corresponding HF meal challenges during the testing visits (CSO vs. OO).

Plasma fatty acid analysis

Fasting and 120-minute postprandial blood samples were used to determine plasma fatty acid concentrations from each of the testing visits for both diets. Of specific interest was 16:1n9 fatty acid profiles, which is predominately generated by endogenous desaturase activity, to determine a potential mechanism behind lipid changes in the body after the diets given. Lipid extractions were performed by modified Folch method (chloroform:methanol 2:1 with butylated hydroxytoluene) (27). Mass of each lipid was determined relative to heptadecanoic acid internal standard. Fatty acid methyl esters (FAME) were derivatized using BF3/CH3OH prior to GC-FID

analysis. FAME identification was determined using GC-FID (Agilent 6890N) using a Supelco SP-2560 highly polar biscyanopropyl column (Bellefonte, PA, USA) and by retention time mapping against GLC-463 FAME standard mixture (Nu-Chek Prep., Inc., Elsyian, MN, USA).

Statistical analysis

SAS version 9.4 statistical package (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. A within subject one-way ANOVA was used to determine differences between fasting lipids (TC, HDL-C, and LDL-C) and 16:1n9 fatty acid profiles. A mixed effects model repeated measures ANOVA was used for TG meal responses. If significance was found, a post hoc analysis was performed using the Tukey's test. Data are presented as means ± SEM. Statistical significance was set at $p \leq 0.05$.

Results

Subjects

Fifteen normal weight male participants completed both feeding trials (**Figure 2**). Physical characteristics of the participants are shown in **Table 3**. Participants were between 18 and 45 years of age and were of normal weight based on body mass index (BMI= 18-24.9 kg/m^2) or DXA body fat percentage (14-24%). All participants were also free from any known metabolic or chronic disease and were not taking any medications.

Fasting and Postprandial Lipids

Fasting TC decreased significantly from pre-to post-diet following the CSO diet intervention (average change -12.47±3.31mg/dL, $p<0.05$) while there was no change in TC following the OO diet intervention (average change -5.69±3.97mg/dL, *ns*, effect size=0.35, **Figure 3a**). Fasting HDL-C increased significantly from pre- to post-CSO diet (average change 3.57±1.01mg/dL, $p<0.05$). No change in HDL-C was observed with the OO intervention

(average change 1.28±0.97mg/dL, *ns*, **Figure 3b**). Similar to TC, fasting LDL-C also decreased significantly from pre-to post-CSO diet (average change -14.07±3.16mg/dL, $p < 0.05$), while no changes were observed following the OO diet intervention (average change -4.54±3.54mg/dL, *ns*, effect size=0.22, **Figure 3c**). Finally, fasting TG levels decreased significantly with the CSO diet (average change -23.74±5.47mg/dL, $p<0.05$), and there was a trend in a decrease in TG for the OO diet (average change -11.40±5.23, $p = 0.11$, effect size=0.34, **Figure 3d**).

For the meal challenges, there was no difference in fasting TG levels between CSO vs. OO at the pre-diet visit. When examining the time-course meal responses for total TGs, there was a significant time effect ($p < 0.001$), visit effect ($p < 0.001$), and treatment by visit interaction ($p < 0.001$) (**Figure 4a**). Acutely (at the pre-diet visit - *visit 1*), there were no postprandial differences between the HF CSO meal vs. OO meal pre-diet. The significant effects were lower postprandial TG levels from pre- to post-CSO diet (area under the curve (AUC) average of 954.28±56.90 to 722.16±6.15mg/dL/8h, $p<0.01$). There was no significant change in postprandial TG for the OO diet; however, there was a trend for a decrease in TG from pre- to post-OO diet (AUC average of 913.65±77.46 vs. 801.17±62.72mg/dL/8h, $p=0.09$, effect size=0.42, **Figure 4b**).

Plasma fatty acid profiles

At fasting, fatty acid 16:1n9, which is predominately generated by endogenous desaturase activity, was significantly lower post-CSO diet compared to pre-CSO diet (0.86±0.10 vs. 1.07±0.11%, for post- vs. pre-diet, respectively; $p<0.05$). At time 120-min post-meal 16:1n9 was also significantly lower post-CSO diet compared to pre-CSO diet (0.73±0.07 vs. 1.00±0.08%, for post- vs. pre-diet, respectively; $p<0.05$, **Figure 5a**). No change in 16:1n9 was detected for the OO diet intervention at fasting (1.02±0.10 vs. 0.99±0.14%, for pre- vs. post-diet, respectively,

ns) or 120-min postprandial time points (1.07±0.08 vs. 1.29±0.16%, for pre- vs. post-diet, respectively, *ns*, **Figure 5b**).

Discussion

The results of this study reveal significant improvements in cholesterol profiles and both fasting and postprandial TG levels after a 5-day HF diet rich in CSO. Conversely, the 5-day HF diet rich in OO did not change either cholesterol profiles or TG levels significantly. Importantly, based on our study design, the results can be solely attributed the type of oil that was consumed. The cross-over study design exposed all participants to both diets in a random order, and diets were matched for all other foods and energy content allowing for isolation of the effect of the oils in our study. These results demonstrate that a CSO-rich diet over a 5-day period has a more beneficial effect on cholesterol profiles and TG levels than an OO-rich diet.

The improvements in lipid profiles with CSO-enriched diets is not surprising given that previous studies have shown improvements in lipids with CSO consumption (11, 12, 16). In an early animal model, it was discovered that rats fed a high CSO diet, as compared to a high corn oil diet, resulted in reduced TC levels (11). At the time, this was an interesting finding since CSO has higher levels of saturated fat, which are typically associated with dyslipidemia (13-15), and high levels of omega-6 PUFAs. This finding was later confirmed in healthy humans when it was shown that consuming 95g of CSO per day for a 5-day period significantly reduced TC and LDL-C with no change in HDL or TG (16). Similarly, we also incorporated a 5-day intervention in healthy adults; however, in addition to improvements in TC and LDL-C, we also saw an increase in HDL-C and lower fasting and postprandial TGs. Interestingly, these changes were only observed in subjects on the CSO-rich diet, while no changes were found in the same subjects during the OO-rich diet. There was a trend for a decrease in fasting TG with the OO

diet. It should be noted that the decreases in fasting TG levels after both diets may be due in large part to the lower carbohydrate content of the diets (and higher fat content) which decreases hepatic TG formation (28). Our study also incorporated CSO as a percentage of each participants' estimated energy needs (44% of energy), provided all foods for the 5-day period, and included only men whereas the previous study by Davis et al. (16) used a set gram amount of CSO for all participants, provided most of the food for the intervention, and included both men and women. These slight differences in study design could explain why we were able to detect changes in HDL-C and TG.

The lack of change with the OO-enriched diet is not necessarily unexpected, since the participants in this study had healthy cholesterol profiles at baseline. Considering these were healthy subjects, a lack of change with OO does not mean OO is unhealthy; rather, our findings highlight the substantial benefits of CSO on cholesterol profiles. Based on the very limited past literature on CSO consumption and blood lipid responses, we did hypothesize to observe improvements in blood lipids. What was somewhat surprising was the magnitude of change in fasting blood lipids. TC, LDL-C, and TG levels decreased by an average of 8, 15, and 30% respectively, and HDL-C increased by 8% after just 5 days on a CSO-rich diet. Since this was an outpatient feeding study where all foods were provided to participants, it could be argued that another component in the diet aside from the CSO led to the improved lipids. However, with the cross-over design, those same subjects received the exact same foods during the OO treatment. The only difference in the diet between the two treatments was the type of oil used. Therefore, our findings can be attributed specifically to the CSO.

Traditionally, measurements in the fasted state are used for identifying disease risk. However, the postprandial period is now also recognized as an important period in which disease

development can be exacerbated (29, 30). This is especially important in today's western society, since a majority of individuals spend approximately two-thirds of the day in a postprandial state (31). The western diet also tends to include meals that are higher in fat, which can lead to postprandial hyperlipidemia (32). This postprandial rise in TGs is linked to the occurrence of increased inflammation and oxidative stress within the body, ultimately resulting in endothelial dysfunction and diseased states (32, 33). In the current study at the first visit, there were no differences in postprandial TG levels after a single CSO vs. OO meal. However, following the 5-day HF diet intervention, postprandial TG levels decreased significantly in the CSO treatment but not in the OO treatment. There are no previous studies looking at longer-term diet interventions with CSO on postprandial TG levels although one previous study showed a diet rich in omega-6 PUFA oils lowered postprandial TG levels (34). Contrary to the lack of data on CSO, several studies have examined changes in postprandial TGs in longer-term diet interventions utilizing OO, mainly from Mediterranean diet interventions (35, 36). These studies consistently report lower levels of postprandial TGs after consumption of a high OO Mediterranean diet, although they are not compared to a PUFA-rich or CSO-rich diet (35, 36). However, these studies are difficult to compare to ours because Mediterranean diet interventions consist of several changes to overall diet, including increases in consumption of nuts (high in PUFAs). Therefore, based on the lack of previous data on CSO enrichment, showing reductions in postprandial TG is both novel and clinically relevant for overall health.

In most human clinical intervention studies, mechanisms of action behind physiological changes are extremely difficult to ascertain. However, in the present study, we are able to describe a potential mechanism by which favorable lipid changes are observed after a HF, CSO-rich diet. The overall improvements in fasting and postprandial lipids may not only be due to the

major fatty acid content of the diets (omega-6 PUFA/saturated fat vs. MUFA), but more specifically to a unique component of CSO. In a recent mouse model, consuming a HF diet rich in CSO largely protected the animals from HF diet-induced metabolic adaptations compared to a HF diet rich in cocoa butter (high saturated fat) or safflower oil (high linoleic acid) (12). The livers of mice fed the CSO diet resembled that of mice fed a chow diet, where the mice fed HF diets rich in cocoa butter or safflower oil displayed increases in liver lipogenic metabolites (12). The authors were able to link the protective effect of CSO to a cyclopropenoid fatty acid found within CSO, namely dihydrosterculic acid (12). Cyclopropenoid fatty acids are known to irreversibly inhibit endogenous desaturation of saturated fat by blocking stearoyl-CoA desaturase-1 (SCD1) activity (37), thus leading to decreases in accumulation of triglycerides (38). Paton et al. (12) observed increased 16:0/16:1n9 ratios in the mice fed the CSO diet compared to mice fed the safflower oil or cocoa butter diets, indicating decreased SCD1 activity after the CSO diet. In the current study, to elucidate this mechanism in humans, plasma fatty acids were analyzed before and after both diet interventions. Of special interest was the 16:1n9 fatty acid content which is primarily produced endogenously in the liver via SCD1. There was a significant decrease in 16:1n9 from pre to post CSO-rich diet. These findings confirm what has been shown in mice and allude to a decrease in SCD1 activity, contributing to the significant decreases in TG levels that occurred in our study after the CSO-rich diet.

There are some strengths and limitations to the current study. The outpatient feeding and cross-over design are strengths of the study while relying on self-report for meal compliance could be a limitation. Our participants were free-living, making it possible for them to consume other foods and beverages. However, breakfast was consumed in the lab each morning with daily reminders to follow the given diet to try to maximize compliance. Another strength of the current

study is the use of a diet intervention rather than simply an acute meal challenge. Yet, the diet intervention was only 5 days, so it limits our abilities to extrapolate the results to a longer-duration. Future studies are needed to determine if these favorable changes in lipid profiles are present or even further exacerbated after a long-term diet intervention. Lastly, our test meals at pre- and post-diet intervention contained the oil that matched the diet intervention the participant was undergoing. Since we did not use the same test meal during both the CSO and OO diet interventions, we were not able to compare differences in plasma fatty acid profiles *between* diets. However, we were able to compare *within* diet changes, allowing us to describe a potential mechanism behind CSO's favorable lipid changes.

In conclusion, a 5-day, HF diet rich in CSO greatly improved cholesterol profiles and TG levels in healthy adult males, whereas the OO-rich diet had no significant impact on these measures. Further, we were able to link a mechanism behind CSO's beneficial effects on lipid metabolism in healthy, normal weight male adults. These findings are important and identify CSO as a potential intervention to reduce fasting and postprandial TGs to modulate disease risk for conditions such as obesity, type 2 diabetes, and non-alcoholic fatty liver disease. Future studies should focus on the potential for CSO to be used in diseased populations to lower disease risk associated with dyslipidemia.

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Table 4.1. Nutrient content of the diets

Composition	Lead-in diet	PUFA-rich HF diet	MUFA-rich HF diet
Percentage of total energy from			
Protein	15.0	15.0	15.0
Carbohydrate	50.0	35.0	35.0
Fat	35.0	50.0	50.0
Percentage of total energy from fatty acid of interest			
MUFA	16.0	9.7	31.5
PUFA	7.6	26.7	6.6
n6 PUFA	*7.6*	*26.3*	*5.7*
n3 PUFA	*0.0*	*0.4*	*0.9*
SFA	11.4	13.4	10.4

HF high fat, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid, *n6 PUFA* omega-6 polyunsaturated fatty acid, *n3 PUFA* omega-3 polyunsaturated fatty acid

Table 4.2. Nutrient content for each High-Fat test meal

Composition	PUFA-rich HF meal	MUFA-rich HF meal
Percentage of total energy from		
Protein	15.5	15.4
Carbohydrate	28.1	28.3
Fat	56.7	56.3
Percentage of energy from fatty acids		
MUFA	11.5	36.7
PUFA	31.1	7.4
n6 PUFA	*30.9*	*6.8*
n3 PUFA	*0.2*	*0.6*
SFA	14.2	12.3

HF high fat, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid, *n6 PUFA* omega-6 polyunsaturated fatty acid, *n3 PUFA* omega-3 polyunsaturated fatty acid

Table 4.3. Participant characteristics

Characteristic	Value
Age (y)	21.67 ± 2.58
Height (cm)	177.74 ± 6.95
Weight (kg)	77.24 ± 13.61
Body mass index (kg/m^2)	24.27 ± 2.81
Body fat (%)	16.49 ± 4.85
Waist circumference (cm)	83.61 ± 9.16
Hip circumference (cm)	98.44 ± 8.54

Note: All values are means ± SD (n=15)

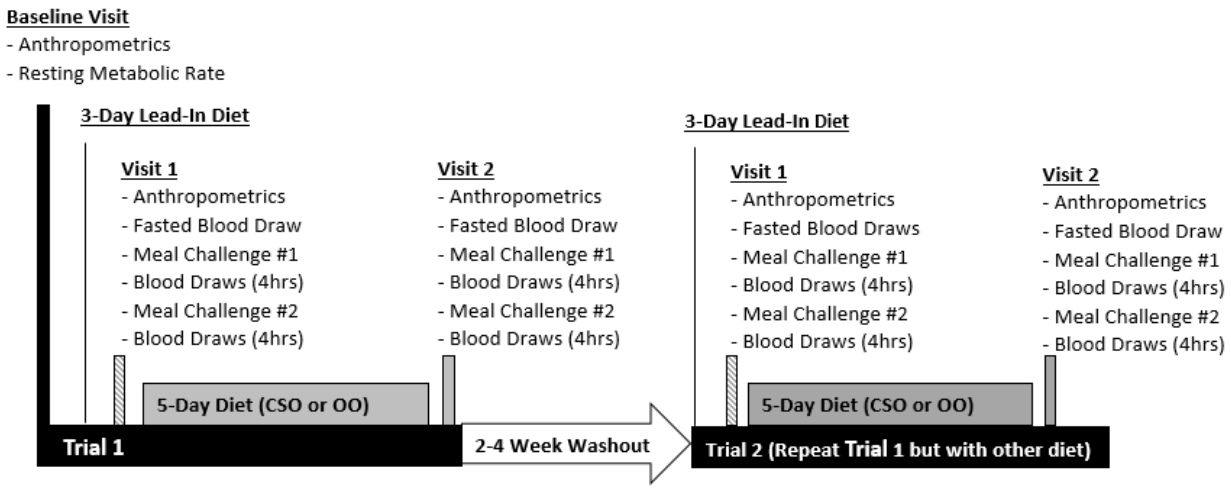

Figure 4.1 Time-course of study visits and measurements taken at each visit. CSO = cottonseed oil, OO = olive oil

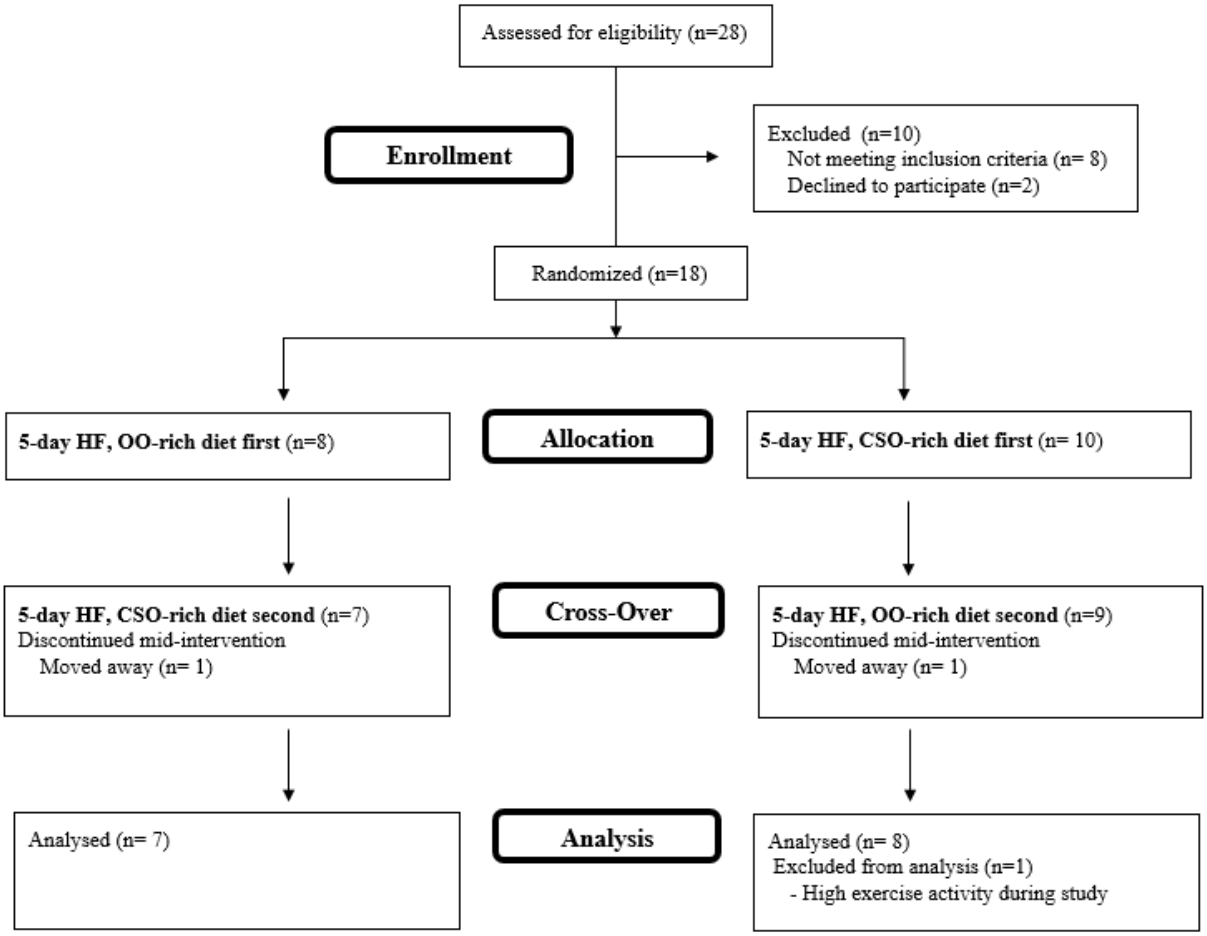

Figure 4.2 CONSORT diagram through completion of both diet interventions of participants who received either the HF, CSO-rich diet first or the HF, OO-rich diet first in a randomized crossover design. CSO = cottonseed oil, OO = olive oil

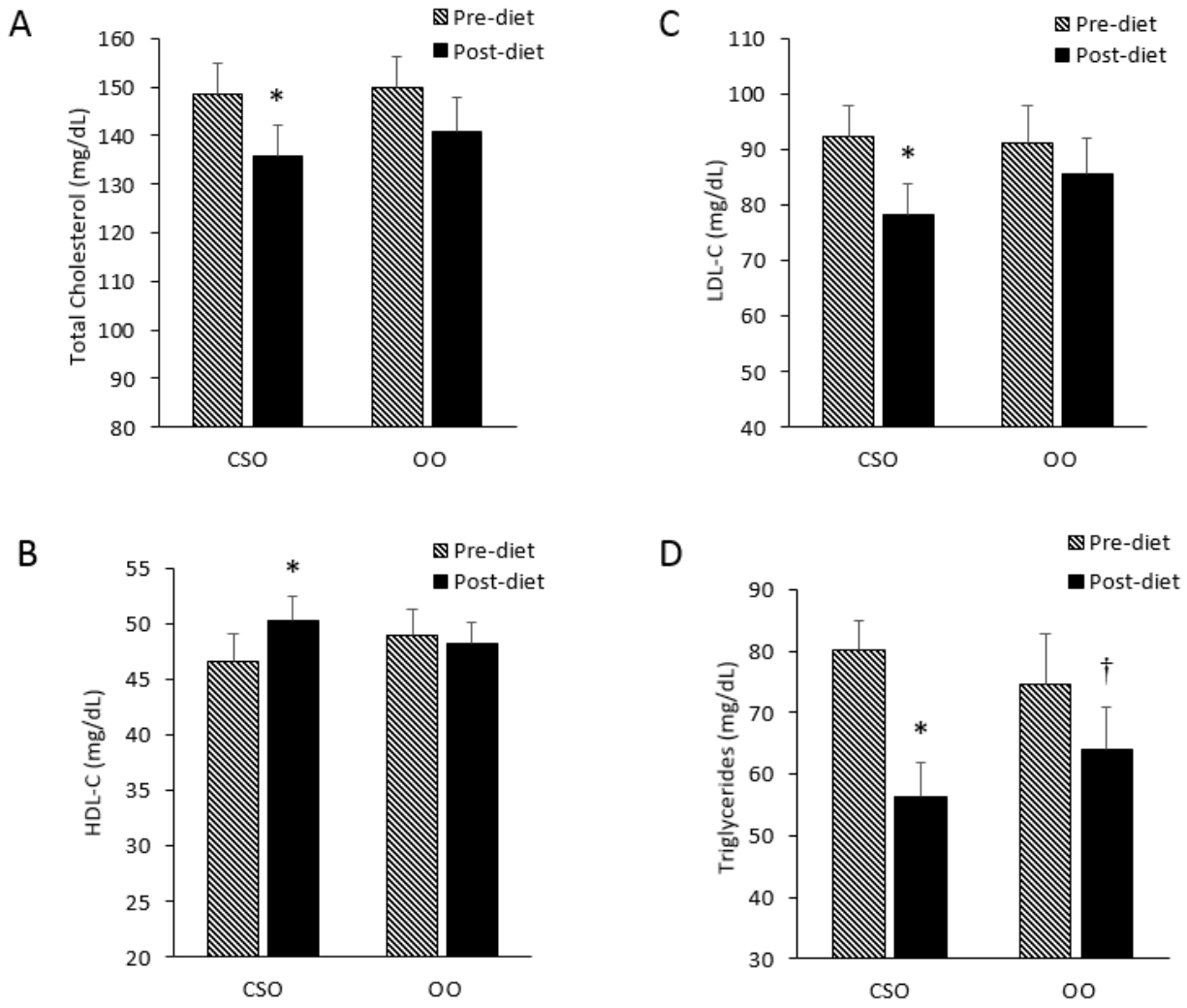

Figure 4.3 Mean ±SE, (**A**) Fasting total cholesterol for each of the diets, pre- and post-diet intervention visits. (**B**) Fasting HDL-C for each of the diets, pre- and post-diet intervention visits. (**C**) Fasting LDL-C for each of the diets, pre- and post-diet intervention visits. (**B**) Fasting triglycerides for each of the diets, pre- and post-diet intervention visits. * Significantly different values from CSO pre-diet to CSO post-diet, ($p<0.05$). †Trend towards different triglyceride values from OO pre-diet to OO post-diet, ($p=0.11$). HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, CSO = cottonseed oil, OO = olive oil

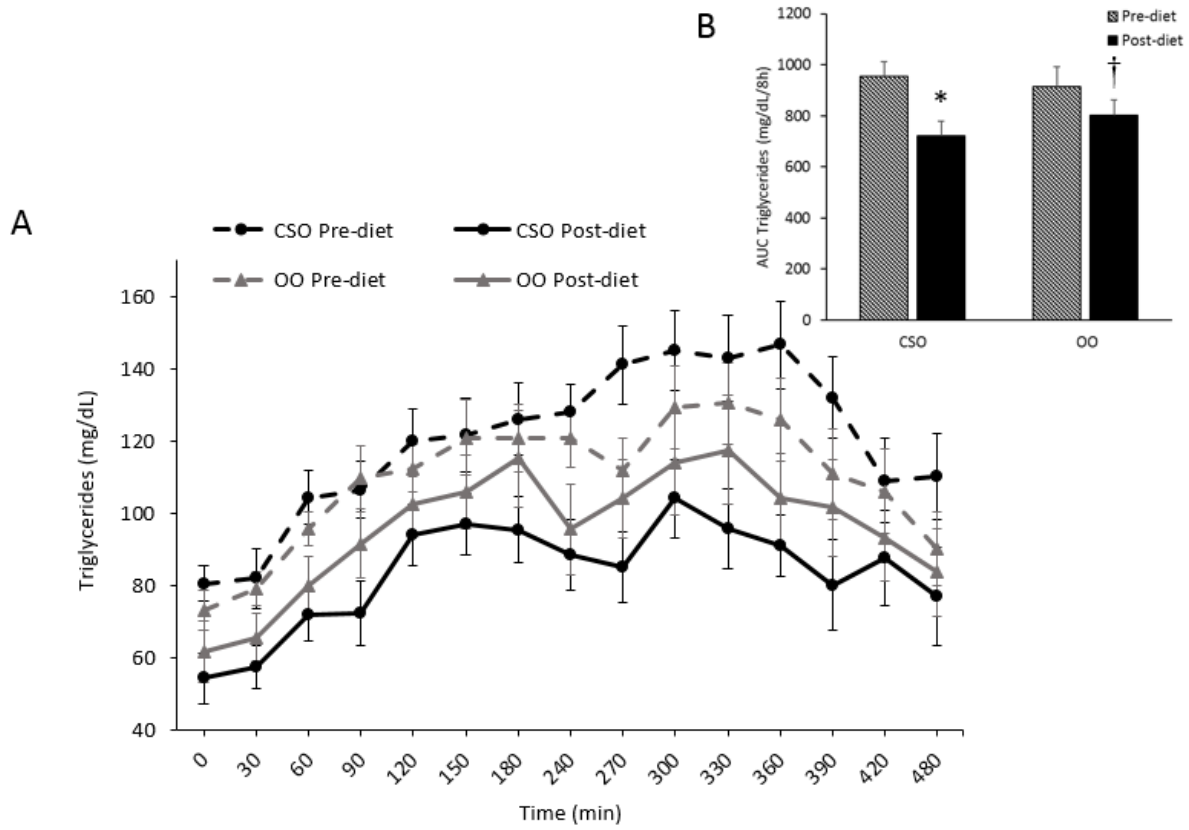

Figure 4.4 Mean ±SE, (**A**) Fasting and postprandial triglyceride values for each of the diets at the pre- and post-diet intervention visits. Subjects consumed the high-fat meals immediately after time 0 and time 240. (**B**) AUC for pre- and post-diet interventions. *Significantly different triglyceride values from CSO pre-diet to CSO post-diet, ($p<0.05$). †Trend towards different triglyceride values from OO pre-diet to OO post-diet, ($p=0.09$). AUC = area under the curve, CSO = cottonseed oil, OO = olive oil

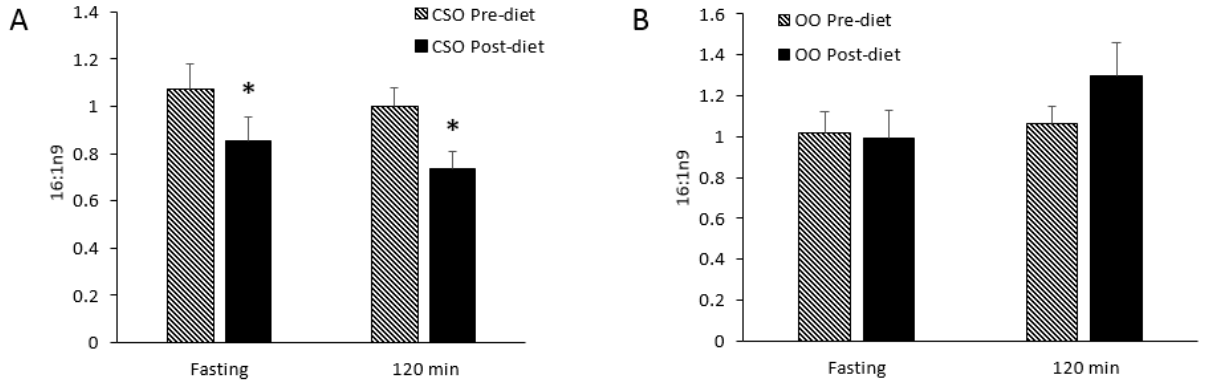

Figure 4.5 Mean ±SE. Fatty acid composition was determined by GC-MS and expressed as g fatty acid/100g lipid. (**A**) Fasting and postprandial (time point 120min) 16:1n9 values for the CSO-rich diet at pre- and post-diet intervention visits. (**B**) Fasting and postprandial (time point 120 min) 16:1n9 values for the OO-rich diet at pre- and post-diet intervention visits. * Significantly different 16:1n9 values from both fasting and 120min time points for CSO pre-diet to CSO post-diet ($p<0.05$). CSO = cottonseed oil, OO = olive oil

CHAPTER 5

APPETITE RESPONSES TO HIGH-FAT DIETS RICH IN MONO-UNSATURATED VERSUS POLY-UNSATURATED FATS[3]

[3]Polley KP, Kamal F, Paton CM, Cooper JA. Submitted to Physiology and Behavior, June 2018.

Abstract

Background: Modifying the type of dietary fat consumed may impact appetite, therefore having implications in weight management.

Objective: To compare the effects of a 5-day, high-fat diet rich in poly-unsaturated fatty acids (PUFAs) to a diet rich in mono-unsaturated fatty acids (MUFAs) on markers of appetite.

Methods: Fifteen normal weight men participated in a randomized cross-over design with two controlled feeding trials (3d lead-in diet, pre-diet visit, 5d PUFA- or MUFA-rich diet, post-diet visit). The 5d diets (50% fat) were rich in either PUFA (25% of total energy) or MUFA (25% of total energy). At pre- and post-diet visits, subjects consumed test meals at breakfast and lunch, rich in the FA that coincided with their 5-day diet. Fasting and postprandial subjective ratings of appetite were determined using visual analogue scale (VAS) questionnaires for 4h after each meal. Additionally, fasting and postprandial blood draws were performed for 4h after each meal to determine changes in appetite hormones. An ad libitum buffet meal was given at the end of the day at pre- and post-diet visits.

Results: Following the diet interventions, PUFA resulted in lower hunger ratings vs. MUFA (iAUC: -274.46±41.47 vs. 217.91±42.15 mm/8h, for PUFA vs. MUFA, respectively; $p<0.05$). Similarly, for the hunger hormone ghrelin, post-diet PUFA resulted in lower concentrations vs. MUFA (iAUC: -305.47±48.05 vs. -223.47±42.64 pg/ml/8h, for PUFA vs. MUFA, respectively; $p<0.01$). The satiety hormone CCK corresponded to these findings, with a significant treatment effect (PUFA resulted in higher concentrations vs. MUFA, $p<0.05$). However, energy intake, ratings of fullness, or the satiety hormone PYY did not differ between treatments following the diet interventions (ns).

Conclusions: A 5-day, PUFA-rich diet suppressed ratings of hunger and ghrelin and raised CCK compared to a MUFA-rich diet. However, this did not translate to differences in subsequent energy intake between the two diets.

Introduction

Due to the growing prevalence of obesity over the past few decades (1), determining methods in which to better manage appetite control has become an important area of research. Investigating the impact of foods or specific diets on satiation is a critical component in refining weight management strategies. Specifically, consuming diets high in fat (≥40% energy intake) has been associated with weight gain (2). However, the fatty acid (FA) composition of the diet may differentially influence appetite responses in the body, impacting weight management and possibly dietary recommendations (3).

There are several different measurement tools to assess appetite, including appetite hormones, subjective ratings, and/or ad libitum food intake (4). Appetite hormones play a significant role in controlling energy balance, sensing food that enters the gut and subsequently signaling information about meal quality and quantity to the hypothalamus (5, 6). Three hormones that are involved in short-term regulation of appetite are cholecystokinin (CCK), peptide YY (PYY), and ghrelin. Ghrelin is the primary hunger-stimulating hormone, released from the stomach, activating orexigenic neurons in the hypothalamus (7). In contrast to ghrelin, PYY acts as a satiety-stimulating signal. It is released primarily from the distal small intestine and proximal colon in response to meal consumption and acts to reduce energy intake by inhibiting orexigenic neurons in the hypothalamus (8). CCK is released from the proximal intestine following nutrient ingestion, particularly fat, and acts as a satiety signal by suppressing orexigenic neurons (9, 10). CCK has also been suggested to be involved in the regulation of PYY and ghrelin, mediating the inhibition of ghrelin and stimulation of PYY in response to intraduodenal fat (11). Our lab has previously shown that a single poly-unsaturated fat (PUFA)-rich meal is more effective than a mono-unsaturated (MUFA)-rich meal at stimulating PYY

release (12, 13). Additionally, we have showed that a 7-day, PUFA-rich diet resulted in higher fasting and postprandial PYY levels, and lower fasting ghrelin levels (14). Together, this data suggests that PUFAs may be effective for decreasing appetite; however, additional longer-term diet studies to confirm these findings are needed.

Several acute meal challenge studies have also examined subjective ratings or ad libitum food consumption in response to high-fat (HF) meals varying in FA composition, with equivocal findings. Many have not detected differences between meals rich in saturated fats (SFAs), MUFAs, or PUFAs (12, 15-19), while a few have shown differences based on FA composition (13, 20, 21). Fewer studies (only three to date) have examined the chronic diet effect of FA composition on appetite ratings and energy intake, but all three showed no chronic effect based on FA composition when pre-diet differences were accounted for (14, 22, 23).

To investigate the longer-term effects of PUFAs compared to MUFAs on appetite responses, the aim of this study was to evaluate the impact of a 5-day, HF diet rich in PUFAs versus MUFAs on fasting and postprandial appetite measures in healthy, normal weight men in a crossover design. Specifically, we evaluated subjective ratings, ad libitum food intake, and biological hormones utilizing a tightly controlled feeding trial. Based on previous studies in our lab, we hypothesized that the HF, PUFA-rich diet would lead to greater increases in fasting and postprandial measurements of satiety (and less hunger) compared to the HF, MUFA-rich diet.

Methods

Study Design

This study was a single-blind, randomized cross-over design consisting of two different outpatient feeding trials. The study protocol consisted of a baseline visit (resting metabolic rate measured), followed by two outpatient feeding trials and is outlined in **Figure 1**. The feeding

trials consisted of a 3-day lead in diet, a pre-diet testing visit (*Visit 1)*, a 5-day feeding protocol (rich in either MUFA or PUFA), and a post-dieting testing visit (*Visit 2).* Prior to all visits, participants fasted for 8-12h and avoided any vigorous exercise for 12h. Following the first trial, there was a 2- to 4-week washout period (average of 19.8 days). Participants then completed trial 2. The only difference between trials 1 and 2 was the type of unsaturated fat provided during the 5-day HF diet. The HF diets were rich in either MUFAs or PUFAs. Participants completed the diets in a random order using Research Randomizer (www.randomizor.org).

Participants

Fifteen apparently healthy, normal weight, sedentary adult men were recruited for the study. Inclusion criteria were for males between the ages of 18-45 years, performing less than 3h of structured exercise per week, and normal weight status based on a body mass index (BMI) between 18-24.9 kg/m^2 or body composition analysis using Dual X-Ray Absorptiometry (DXA™; Hologic Inc., Discovery A, Bedford, MA), with a body fat percentage less than 24% to qualify. The exclusion criteria included the following: weight loss or gain exceeding 5% of body weight in the past 3 months; regular exercise > 3h per week; plans to lose weight or begin a weight loss program between initiation of the study and final testing; anyone who was vegan or vegetarian; medication use; chronic or metabolic disease, gastrointestinal disorder, or history of medical or surgical events that could affect digestion or swallowing; any supplement use other than a daily multivitamin; blood or plasma donation within 20 days prior to initiation of study; or tobacco use. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Western Institutional Review Board, and written informed consent was obtained prior to beginning study procedures.

Procedures

Baseline Visit

Participants reported to the Human Nutrition Laboratory after an overnight fast (no food or drink except water for 8-12h). Anthropometric measurements (height, weight, and body composition) were taken, followed by resting metabolic rate (RMR) measurement with the TrueOne® 2400 Canopy System (ParvoMedics, Sandy, UT, USA) for 30 minutes using standardized conditions. Briefly, subjects were asked to stay awake and motionless in a supine position while a plastic hood was placed over their head to measure oxygen consumption and carbon dioxide production. Respiratory gases were used to calculate RMR using the Weir equation (24). Participants' estimated total daily energy needs for the 3-day lead in diet, 5-day diet, and meal challenges at *visits 1* and *2* were based on their calculated RMR multiplied by an average U.S. physical activity value of 1.65 (25).

Lead –in diet

For 3 days prior to *Visit 1*, participants were provided with a lead-in diet that was representative of the standard American diet (50% carbohydrate, 35% fat, and 15% protein, **Table 1**). Participants were instructed to consume all foods provided, and no additional foods or beverages besides water were allowed. Total energy content was based on the participant's estimated energy needs calculated from the RMR measurement at the *baseline visit* described above.

Pre-Diet Testing Visit (Visit 1)

Following the 3-day lead-in diet, participants arrived at the laboratory the next morning at 0645h in a fasted state (8-12h fast) and 12h without exercise. Height, weight, blood pressure, waist & hip circumference, and body fat percentage using DXA were measured. An intravenous

catheter was inserted in to the antecubital vein for intermittent blood draws and a fasted blood sample was obtained to assess appetite hormones. The line was kept patent with 0.9% normal saline, and blood was drawn into EDTA vacutainers (Greiner Vacuette, Monroe, NC, USA) containing 10µl of DPPIV inhibitor and 1mg/ml of AEBSF hydrochloride (EMD Millipore, Billerica, MA, USA). Blood was immediately centrifuged at 3,000rpm for 15 min at 4ºC. Following the fasted blood draw, participants ingested a HF liquid meal rich in either MUFAs or PUFAs (**Table 2**). The nutrient content of the meal was designed to provide 35% of the participant's estimated total daily energy needs (determined from RMR at *baseline visit*). The liquid meals contained 1% milk, whey chocolate protein, chocolate syrup, and added oil. The oil, either olive oil (OO) or cottonseed oil (CSO), depended on the treatment condition (OO for MUFA or CSO for PUFA). We analyzed the FA composition of each oil used in this study. The OO consisted of 19.3% SFA, 67.1% MUFA, and 13.6% PUFA. The CSO consisted of 22.7% SFA, 19.9% MUFA, and 57.4% PUFA. Participants consumed this liquid meal at breakfast (0800h) and another identical meal again 4h later for lunch (1200h).

Following breakfast meal ingestion, blood samples was taken at minutes 30, 60, 90, 120, 150, 180, and 240 for measurement of appetite hormones. After the 240 min blood draw, participants were fed the lunch HF liquid meal, and blood draws continued to be collected at minutes 270, 300, 320, 350, 380, 420 and 480. Therefore, measurements were obtained for a total of 8h postprandially (4h post-breakfast and 4h post-lunch). Participants were also given 4oz of water each hour. Blood samples were immediately spun at 3,000rpm for 15min at 4ºC. Plasma was aliquoted and stored at -80ºC until assayed.

Immediately following the 8h of testing, participants were given an ad libitum buffet-style meal. The buffet meal consisted of cold and hot meal choices and a variety of beverages.

An assortment of sandwiches, chips, crackers, and fruit were provided, as well as beverages such as water, soda, and diet soda. The same amount of food and beverages given in excess were provided at all study visits. Participants were instructed to eat as much or as little as they chose until they were comfortably full, and all subjects remained in the room for a 30-minute time period. Meal items were weighed before consumption and the remaining meal items were weighed after. The difference in weight was calculated (which represented the amount that had been consumed) to determine energy and macronutrient consumption.

Dietary Intervention

Following *Visit 1*, participants began the 5-day, HF feeding trial rich in either MUFA or PUFA (**Table 1**). On days 1-5, participants reported to the laboratory between 0700h and 1000h to consume their breakfast, which was given as a shake. Following the meal, participants were given the rest of their food and beverages for the day and instructed to consume all the food provided and abstain from any other food or drink aside from water. All food items were weighed and prepared by research personnel. The 5-day diet consisted of 50% of total energy from fat, 35% from carbohydrates, and 15% from protein. For the HF portion, diets were prepared using either CSO (for the PUFA treatment) or OO (for the MUFA treatment). For the PUFA treatment, 50% of total fat content was derived from PUFA. For the MUFA treatment, 59% of total fat content was derived from MUFA, with differences reflecting the FA composition of those oils. The total energy intake was prescribed to equal each participant's total estimated daily energy needs (determined from RMR at *baseline visit*).

Post-Diet Testing Visit (Visit 2)

The day following the 5th day of the feeding period, participants reported to the laboratory under fasted (8-12h) and unexercised (12h) conditions. All procedures that took place

during *Visit 1* were repeated. This included consumption of the same HF meals, and metabolic and anthropometric measurements.

The duration of the lead-in diet through *Visit 2* represented *Trial 1*. The participants then underwent 2- to 4-weeks of washout during which time they returned to their normal dietary patterns. After the washout period, the participants underwent *Trial 2* (including lead-in diet, *Visit 1* test-day, a 5-day high PUFA or MUFA dietary intervention, and *Visit 2* test-day). The only difference between the trials were the foods for each 5-day trial and corresponding HF meal challenges during the testing visits (MUFA vs. PUFA).

Satiety Hormone Analyses

Active ghrelin, and total PYY levels were measured using radioimmunoassays (RIAs) (EMD Millipore, Billerica, MA, USA). Each participant's total number of samples were run within the same assay. The intra- and inter-assay coefficients of variation for active ghrelin were 8.94% and 9.12% and were 5.31% and 9.96% for total PYY. CCK-8 levels were also measured using RIAs (Phoenix Pharmaceuticals, Burlingame, CA, USA). Each participant's total number of samples were run within the same assay. The intra- and inter-assay coefficients of variation were 6.75% and 6.99% for CCK-8.

Statistical analysis

R version 3.4 statistical package (R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analyses. Fasting and postprandial change from baseline was calculated for subjective ratings of appetite and appetite hormones. Incremental area under the curve (iAUC) was calculated for 0-4h and 4-8h periods, with 0-4h representing postprandial breakfast time points, and 4-8h representing postprandial lunch time points. For the 0-4h iAUC, the value at 0 was taken as baseline and for the 4-8h iAUC the value at 4h was taken as baseline.

Total energy and macronutrient intake was calculated at the buffet meal before and after each trial. A within subjects repeated measures ANOVA was used to detect main effects and interaction effects. If significance was found, post hoc analyses were performed using Tukey's test. Data are presented as means ± SEM. Statistical significance was set at $p \leq 0.05$.

Results

Subjects

Fifteen normal weight male participants completed both feeding trials. Physical characteristics of the participants are shown in **Table 3**. Participants were between 18 and 45 years of age and were of normal weight based on body mass index (BMI= 18-24.9kg/m^2) or DXA body fat percentage (14 - 24%). All participants were also free from any known metabolic or chronic disease and were not taking any medications.

Subjective ratings of appetite

Fasting Data. Subjective VAS measures for hunger, fullness, and prospective consumption were evaluated. Fasting values are represented in **Table 4**. There was no difference between treatments at the pre-diet visit for any of the VAS measures. For hunger ratings, there was no treatment effect; however, there was a trend toward a main effect of visit for fasted hunger ratings (p=0.07), which was driven by PUFA (64.31±4.40 vs. 75.56±5.12 mm, for pre- vs. post-diet, respectively). For VAS fullness responses, there was a main effect of visit ($p<0.05$), which was a decrease in fullness ratings from pre- to post-diet visits, and the decrease was similar between treatments. Finally, there were no significant effects for prospective consumption ratings (ns).

Meal Response Data. The time course of postprandial change from baseline for VAS ratings for hunger, fullness, and prospective consumption can be found in **Figure 2a-c**. For

hunger, there was a visit effect ($p<0.05$) and treatment by visit interaction ($p<0.05$). This interaction was a decrease in hunger ratings from pre- to post-diet in PUFA (iAUC: -172.06±40.59 vs. -274.46±41.47 mm/8h, for pre- vs. post-diet, respectively; $p<0.01$; **Figure 2d**) while no change occurred in MUFA. Additionally, the PUFA post-diet visit also had lower hunger ratings compared to the MUFA post-diet visit (iAUC: -274.46±41.47 vs. 217.91±42.15 mm/8h, for PUFA vs. MUFA, respectively; $p<0.05$; **Figure 2d**). For fullness, there was a visit effect ($p<0.05$) which was an increase in ratings from pre- to post-diet visits (**Figure 2e**). The increased fullness ratings were not different between treatments. There were no significant changes in ratings of prospective fullness (**Figure 2f**).

We further analyzed iAUC for postprandial breakfast (PBF) time points (0-4h) and postprandial lunch (PLN) time points (4-8h) to examine the individual meal responses (**Figure 3**). For PBF, hunger ratings decreased from pre- to post-diet in PUFA (iAUC: -85.24±18.78 vs. -139.06±21.65 mm/4h, for pre- vs. post-diet, respectively; $p<0.03$; **Figure 3a**), with no change in MUFA (iAUC: -105.31±22.73 vs. -106.69±20.20 mm/4h, for pre- vs. post-diet, respectively; ns). For PLN, no significant differences were found for hunger ratings (**Figure 3d**) indicating that the overall changes for hunger were driven by responses to the breakfast meal. There were no significant differences for PBF or PLN for fullness or prospective consumption (**Figure 3b-c, e-f**).

Appetite hormones

Fasting Data. Fasting concentrations from pre- to post-diet intervention for ghrelin, PYY, and CCK are shown in **Table 4**. There was no difference between treatments at the pre-diet visit for PYY or CCK. For Ghrelin, there was a main effect of treatment ($p<0.01$), but no visit effect or treatment by visit interaction (ns). The treatment effect was higher fasting ghrelin

concentrations in PUFA compared to MUFA (**Table 4**). This was surprising given that the fasting differences at the pre-diet visit occurred following administration of the same lead-in diet but prior to any diet or meal intervention.

Meal Response Data. The time course of postprandial change from baseline for plasma ghrelin, PYY, and CCK is shown in **Figure 4**. For ghrelin, there was a treatment effect ($p<0.01$) and treatment by visit interaction ($p<0.05$). Acutely, at the pre-diet visit, the PUFA-rich meal resulted in lower ghrelin concentrations compared to MUFA (iAUC: -350.85±60.70 vs. -233.16±61.42 pg/ml/8h, for PUFA vs. MUFA, respectively; $p<0.001$; **Figure 4a, d**). Following the diet interventions, PUFA again resulted in lower ghrelin compared to MUFA (iAUC: -305.47±48.05 vs. -223.47±42.64 pg/ml/8h, for PUFA vs. MUFA, respectively; $p<0.01$). Similarly, for CCK there was also a treatment effect ($p<0.05$) showing higher CCK concentrations in PUFA compared to MUFA (**Figure 4c, f**). Finally, for PYY, no significant differences were found (ns, **Figure 4b, e**).

We further analyzed iAUC for the same PBF and PLN responses in ghrelin and PYY (**Figure 5**). Due to limited plasma, fewer CCK time points were analyzed, therefore assessment of individual meal responses was not able to be calculated. For PBF ghrelin concentrations, there was a treatment effect ($p<0.05$) for lower ghrelin concentrations for PUFA vs. MUFA (**Figure 5a**). For PLN, no differences were found for ghrelin (**Figure 5c**), once again showing that breakfast was the driving force for overall differences in ghrelin. For PBF PYY concentrations, there were no significant differences (**Figure 5b**). For PLN, however, there was a significant visit effect ($p<0.05$), which was an increase in PYY from pre- to post-diet in both MUFA and PUFA (**Figure 5d**).

Buffet meal

Total energy and macronutrient intake was assessed at the ad libitum buffet meal given 4h after the lunch test meal at the pre- and post-diet test visits (**Figure 6**). For total energy intake, there was a main effect of visit ($p<0.05$), but no treatment effect or visit by treatment interaction (ns). The visit effect was a decrease in energy intake from the pre to post-diet visits for both MUFA and PUFA (**Figure 6a**) which was driven by a decrease in carbohydrate intake from pre- to post-diet in both MUFA and PUFA ($p<0.05$) (**Figure 6b**). There were no significant differences in fat or protein intake (ns, **Figure 6c-d**).

Discussion

Overall, our findings suggest that the PUFA-rich diet suppresses postprandial subjective ratings, and hormonal concentrations, of hunger to a greater extent than the MUFA-rich diet, and these differences were driven primarily by the breakfast meal response. The PUFA-rich diet also resulted in greater concentrations of the satiety hormone, CCK, compared to MUFA while no treatment differences were found for PYY. Additionally, the hormonal and subjective rating differences for appetite (ghrelin, CCK, and VAS hunger) did not differentially influence subsequent energy intake at a buffet meal.

For VAS, acutely (pre-diet visit), there were no postprandial differences between PUFA vs. MUFA. This is similar to many previous findings that show no differences in subjective ratings for acute PUFA- vs. MUFA-rich meals (12, 13, 15-19). However, following our intervention, postprandial hunger decreased with PUFA (driven by PBF), and this resulted in lower overall hunger ratings for PUFA vs. MUFA post diet. No changes from pre- to post-diet occurred in MUFA, which confirms findings from Lawton et al. (20) showing with a PUFA-rich meal, postprandial hunger ratings decreased to a greater extent than a MUFA-rich meal.

Three previous studies utilizing longer-term diet interventions comparing dietary FA composition on ratings of hunger have reported no differences between diets when accounting for baseline or pre-intervention group differences (14, 22, 23). The lack of differences observed in those studies compared to our significant findings could be due to differences in the macronutrient composition of the diets, the food source of PUFAs (and specific FAs within those sources), or participant characteristics. A strength to the current study design was that our PUFA and MUFA-rich diets incorporated one food source that was specifically high in the FA's of interest (CSO for PUFA, OO for MUFA). Therefore, any differences seen between the two diets can be attributable to these two food sources. Our data would suggest that CSO could have a more positive effect on decreasing hunger long-term. Interestingly, in the current study, postprandial fullness ratings increased from following *both* diet interventions. This change occurred despite the fact that the study was designed to keep participants in energy balance, and this was confirmed by a lack of change in body weight over the intervention period. The greater fullness following both diets indicates that a high fat content of a diet itself may raise postprandial fullness. A previous study investigating the effects of HF diets on fullness ratings after 4-weeks reported decreased postprandial fullness (26), which is in contrast to our findings. However, French et al. (27) found that after 2 weeks on a HF-diet, postprandial fullness ratings increased. Therefore, it may be that a shorter duration HF diet, such as our 5-day intervention, could affect fullness and may be masked with a longer-term diet intervention.

With respect to appetite hormones, this is the first study to show that PUFAs stimulate postprandial CCK more than MUFAs. These diet effects for CCK are different from previous acute meal challenge (28) or fat infusion studies (21, 29), but since longer term-diet interventions are lacking, these results are both novel and quite promising with respect to a clinical

application. Additionally, postprandial ghrelin concentrations were lower both acutely, and after the 5-day diet for PUFA vs. MUFA. Our meal analysis revealed that the PBF concentrations were driving this response. Therefore, the higher postprandial CCK concentrations corresponded with ghrelin suppression, which was not surprising given that CCK has been shown to influence ghrelin concentrations in the plasma postprandially (11). This lower ghrelin post-diet in PUFA vs. MUFA also corresponded to the lower post-diet subjective ratings of hunger.

While ghrelin and CCK responded similarly to the diets (higher CCK, lower ghrelin for PUFA), postprandial PYY was not different between or within groups from pre- to post-diet when the full 8h postprandial period was analyzed. When data were divided into separate breakfast and lunch meal responses, however, PYY increased following lunch from pre- to post-diet in both groups. This response is similar to the increase in fullness ratings from pre- to post-diet in both groups. A high PUFA meal (12, 13) and diet (14) has previously been linked to higher PYY concentrations. Since we observed this increase in both PUFA and MUFA, it may be an effect of the HF diet itself which could have blunted potential FA differences between our diets. Although more research is needed to confirm this hypothesis, a HF diet alone has been shown to increase postprandial fullness ratings (27).

The mechanisms behind PUFA's suppression of hunger to a greater extent than MUFAs has not been fully elucidated. However, PUFAs have been shown to specifically activate G protein-coupled receptor 120 (GPR120) (30), which is a free fatty acid-binding G-protein coupled receptor expressed in the gastrointestinal tract and has been shown to mediate the effect of FAs on the secretion of CCK (31). It has been demonstrated that CCK is involved in the regulation of ghrelin and PYY by mediating the inhibition of ghrelin and stimulation of PYY in response to intraduodenal fat (11). Therefore, increased consumption of PUFAs could lead to

increased activation of GPR120, increasing CCK concentrations and ultimately decreasing ghrelin concentrations and ratings of hunger. The lack of treatment differences in PYY was surprising, but again, the overall high fat content of the diet may have blunted potential FA effects. Another mechanism may be related to the rate of digestion and absorption of different FAs. An early study demonstrated higher absorption rates of a MUFA-rich meal compared to PUFA-rich meals in humans based on postprandial chylomicron appearance in plasma (32). A slower absorption rate for PUFAs would mean that they are in contact with the endocrine cells that release hunger and satiety hormones for longer periods of time, therefore affecting the magnitude of response to a greater extent than MUFAs.

Lastly, even though we found significant differences in our hormonal and subjective measures of hunger in PUFA vs. MUFA, we did not see treatment differences when measuring energy intake at a buffet meal. It is possible that a buffet setting, which provides free and unlimited food, overrides other hunger or fullness cues (4). Energy intake did decrease in both groups post intervention, which was driven by a decreased intake of carbohydrates. Our data does correspond to the higher postprandial fullness ratings and PLN PYY that was observed in both treatments. The increase in PYY was stronger after the second meal (lunch), therefore possibly driving the lower energy intake observed at the dinner buffet meal. This highlights the importance of looking at the responses to meals separately, as well as looking at multiple meals, since that mimics human eating patterns and can provide better insight into understanding total appetite regulation. It is equally possible that outside factors, such as the participants' knowledge of the termination of the diet intervention (so they could eat any foods they desired after this post-diet meal) or a decrease in the palatability of the food after already consuming the same buffet meal 5 days prior, influenced energy intake.

Some limitations to the current study include the measurement of total PYY instead of active PYY. However, previous studies have shown that measures of total PYY track similarly to active PYY with respect to postprandial responses (33). Another limitation was having fewer postprandial time-points for CCK measurement. Despite this, we were still able to detect significant differences and examine complete meal responses. While we contend that it is a strength to assess multiple measures of hunger and satiety, utilizing a free buffet meal in a laboratory setting to measure appetite may not be the best indicator of energy intake or appetite regulation, especially over time. Finally, our study contained diets that were above the dietary recommendations for total fat and PUFA content. This was by design to establish proof of principle and due to the popularity of HF diets, such as a ketogenic or low-carb diet. Conversely, this may somewhat limit clinical application and may have blunted the FA effect of the diets.

In conclusion, this is the first study to find differences in appetite responses between a PUFA- vs. MUFA-rich diet in healthy adult males. Both ghrelin and subjective ratings of hunger were lower, and CCK higher, following the PUFA-rich vs. MUFA-rich diet. Subjective ratings of fullness and post-lunch PYY were increased, and energy intake decreased, to the same degree following both diets. Therefore, FA composition differences in the diets impacted hunger ratings and ghrelin (lower for PUFA vs. MUFA), while other effects on fullness and energy intake seemed to be driven by the high fat content of the diet rather than the FA composition itself. Future research should focus on the potential of PUFAs to suppress hunger responses and the possibility of its use as a weight management strategy, especially in diets with a lower fat content. Studies should also focus on the mechanisms behind PUFAs ability to stimulate greater CCK and suppress hunger to a greater extent than MUFAs, with specific focus on omega-6 PUFAs which was the primary source of PUFA utilized in this study.

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Table 5.1. Nutrient content of the diets

Composition	Lead-in diet	PUFA-rich HF diet	MUFA-rich HF diet
Percentage of total energy from			
Protein	15.0	15.0	15.0
Carbohydrate	50.0	35.0	35.0
Sugars		*12.8*	*12.7*
Fiber	*4.2*	*2.7*	*2.7*
Fat	35.0	50.0	50.0
Percentage of total energy from fatty acid of interest			
MUFA	16.0	9.7	31.5
PUFA	7.6	26.7	6.6
n6 PUFA	*7.6*	*26.3*	*5.7*
n3 PUFA	*0.0*	*0.4*	*0.9*
SFA	11.4	13.4	10.4

HF high fat, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid, *n6 PUFA* omega-6 polyunsaturated fatty acid, *n3 PUFA* omega-3 polyunsaturated fatty acid

Table 5.2. Nutrient content for each High-Fat test meal

Composition	PUFA-rich HF meal	MUFA-rich HF meal
Percentage of total energy from		
Protein	15.5	15.4
Carbohydrate	28.1	28.3
Fat	56.7	56.3
Percentage of energy from fatty acids		
MUFA	11.5	36.7
PUFA	31.1	7.4
n6 PUFA	*30.9*	*6.8*
n3 PUFA	*0.2*	*0.6*
SFA	14.2	12.3

HF high fat, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid, *n6 PUFA* omega-6 polyunsaturated fatty acid, *n3 PUFA* omega-3 polyunsaturated fatty acid

Table 5.3. Participant characteristics

Characteristic	Value
Age (y)	21.67 ± 2.58
Height (cm)	177.74 ± 6.95
Weight (kg)	77.24 ± 13.61
Body mass index (kg/m^2)	24.27 ± 2.81
Body fat (%)	16.49 ± 4.85
Waist circumference (cm)	83.61 ± 9.16
Hip circumference (cm)	98.44 ± 8.54

Note: All values are means ± SD (n=15)

Table 5.4. Fasting values for subjective ratings of appetite and appetite hormone concentrations

	PUFA-rich HF diet		MUFA-rich HF diet	
	Pre-diet	Post-diet	Pre-diet	Post-diet
Hunger (mm)	64.31±4.40	75.56±5.12*	67.37±4.51	69.94±4.40
Fullness (mm)	18.75±4.54	12.94±3.31*	22.37±4.86	12.91±2.30*
Prospective Consumption (mm)	67.15±3.64	66.38±5.17	67.00±4.39	68.31±5.73
Ghrelin (pg/ml)	115.46±10.73†	114.46±10.60†	100.42±9.72	102.98±10.52
PYY (pg/ml)	104.64±5.87	108.59±7.77	110.04±10.26	108.31±8.31
CCK (pg/ml)	35.36±3.22	32.47±3.42	37.53±5.42	38.36±1.95

HF high fat, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid
*Significant difference from pre- to post-diet ($p<0.05$) †Significant difference between PUFA vs. MUFA ($p<0.05$)

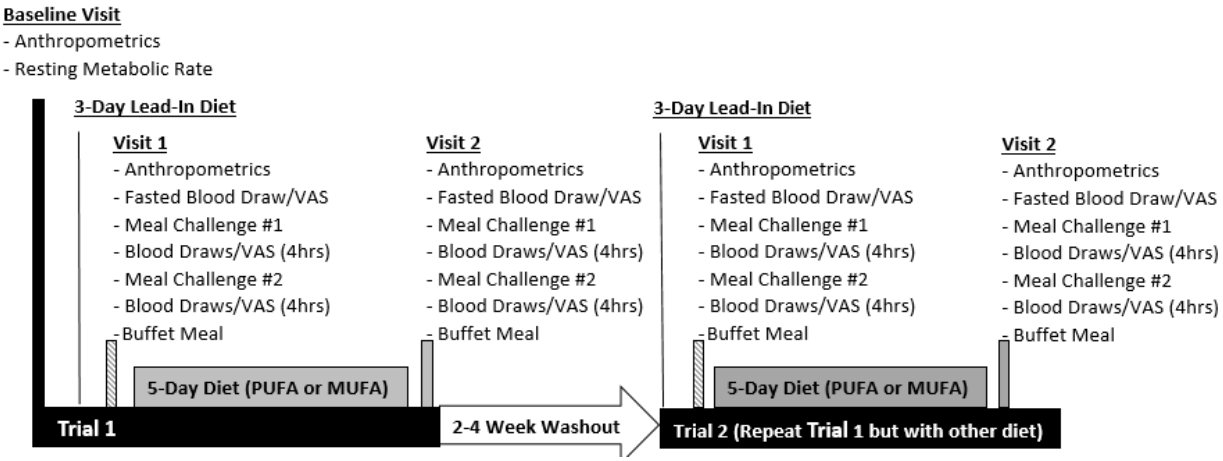

Figure 5.1 Time-course of study visits and measurements taken at each visit. MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, VAS = visual analogue scale questionnaire

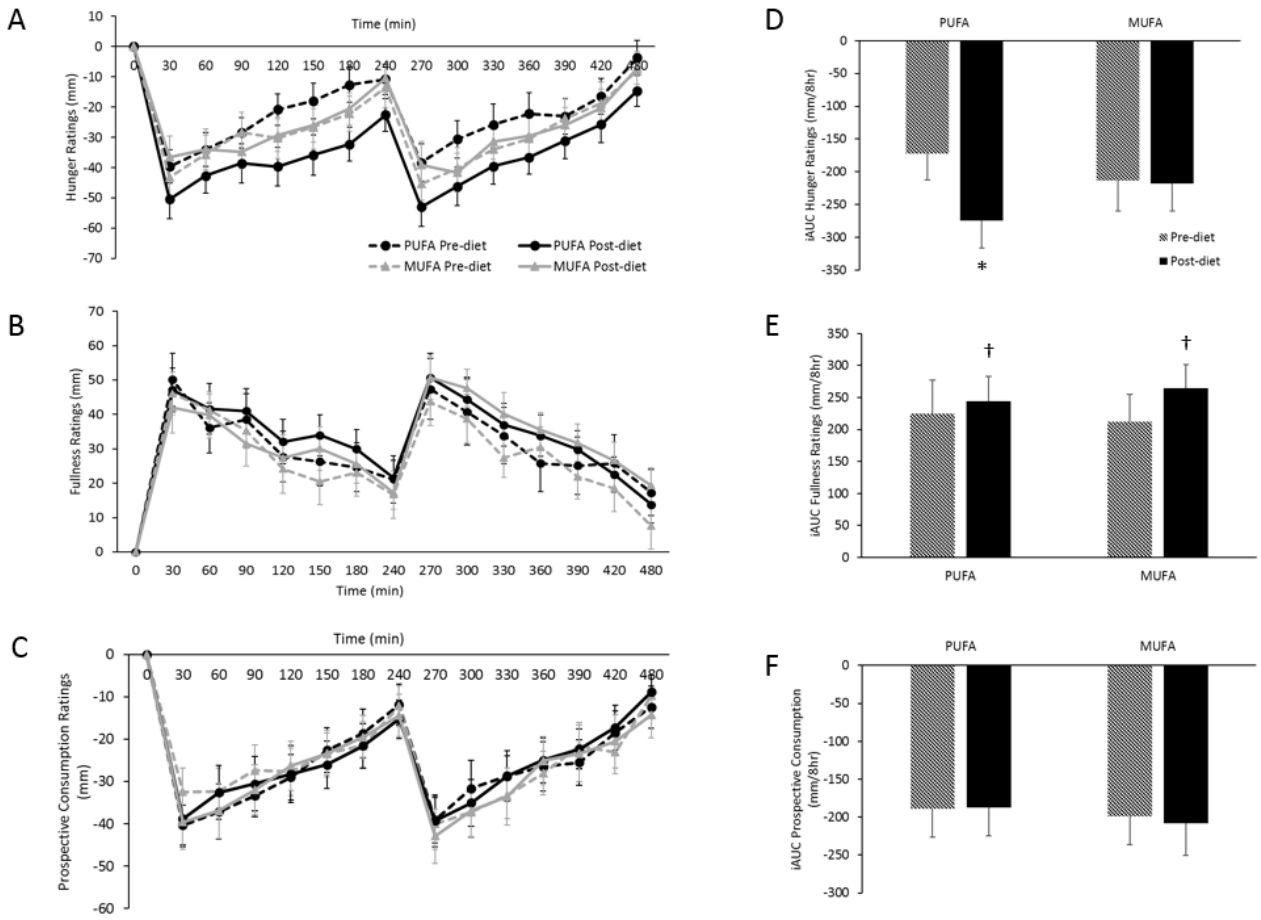

Figure 5.2 Mean ±SE, (**A-C**) Change from baseline for each of the diets, pre- and post-diet intervention. Subjects consumed the HF meal immediately after time 0 and time 240. (**D-F**) Incremental area under the curve (iAUC) for each of the diets, pre- and post-diet interventions. (**A, D**) Hunger ratings. (**B, E**) Fullness ratings. (**C, F**) Prospective consumption ratings. *Significantly lower hunger ratings for PUFA pre-diet vs. both MUFA pre- and post-diet visits, ($p<0.05$). †Significant visit effect for greater fullness ratings at post- vs. pre-diet visits, ($p<0.05$). MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

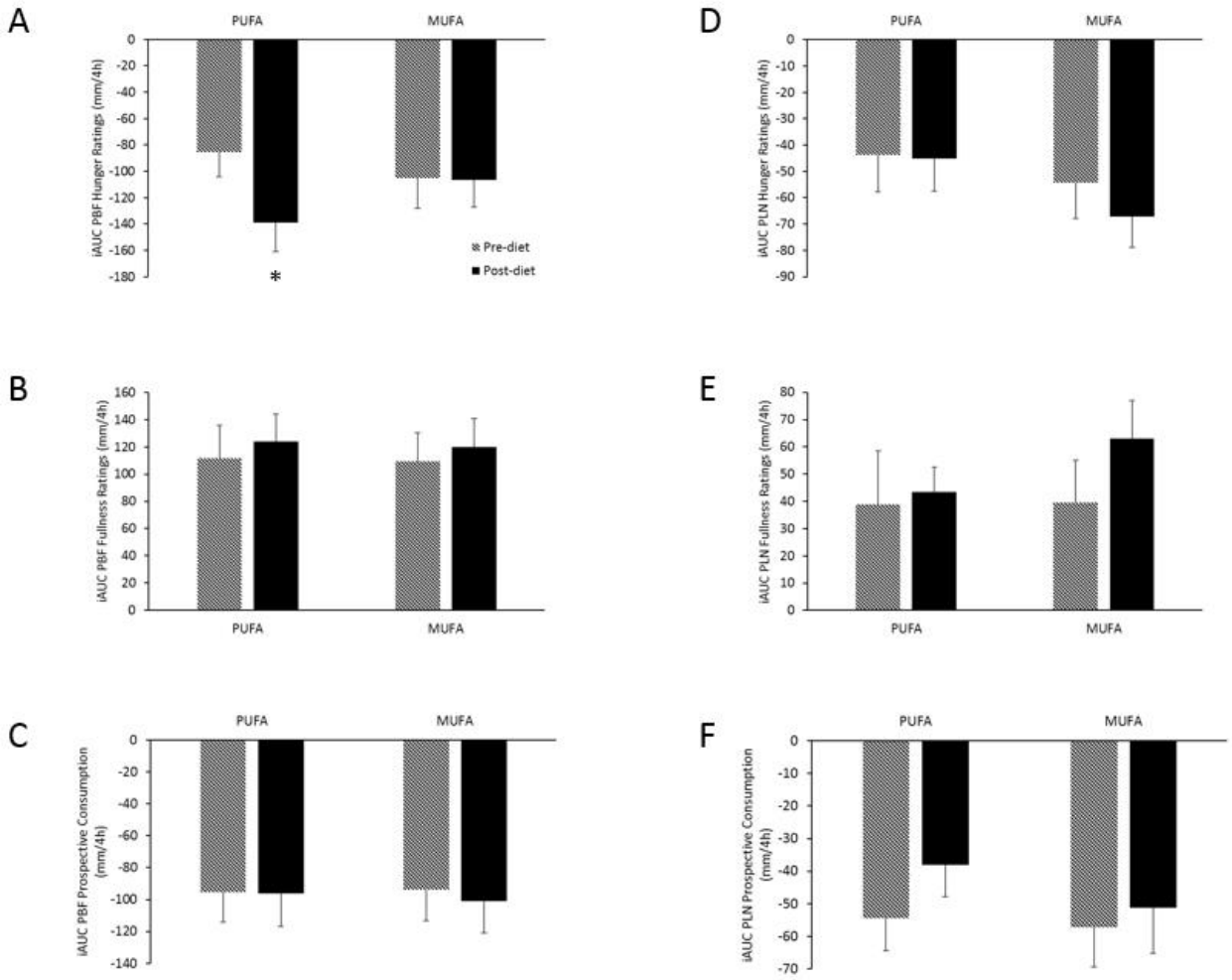

Figure 5.3 Mean ±SE, (**A-C**) Incremental area under the curve (iAUC) for postprandial breakfast (PBF) meal responses for each of the diets, pre- and post-diet interventions. (**D-F**) iAUC for postprandial lunch (PLN) meal responses for each of the diets, pre- and post-diet interventions. (**A, D**) Hunger ratings. (**B, E**) Fullness ratings. (**C, F**) Prospective consumption ratings. *Significant increase in hunger ratings from PUFA pre- to post-diet visits, ($p<0.05$). MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

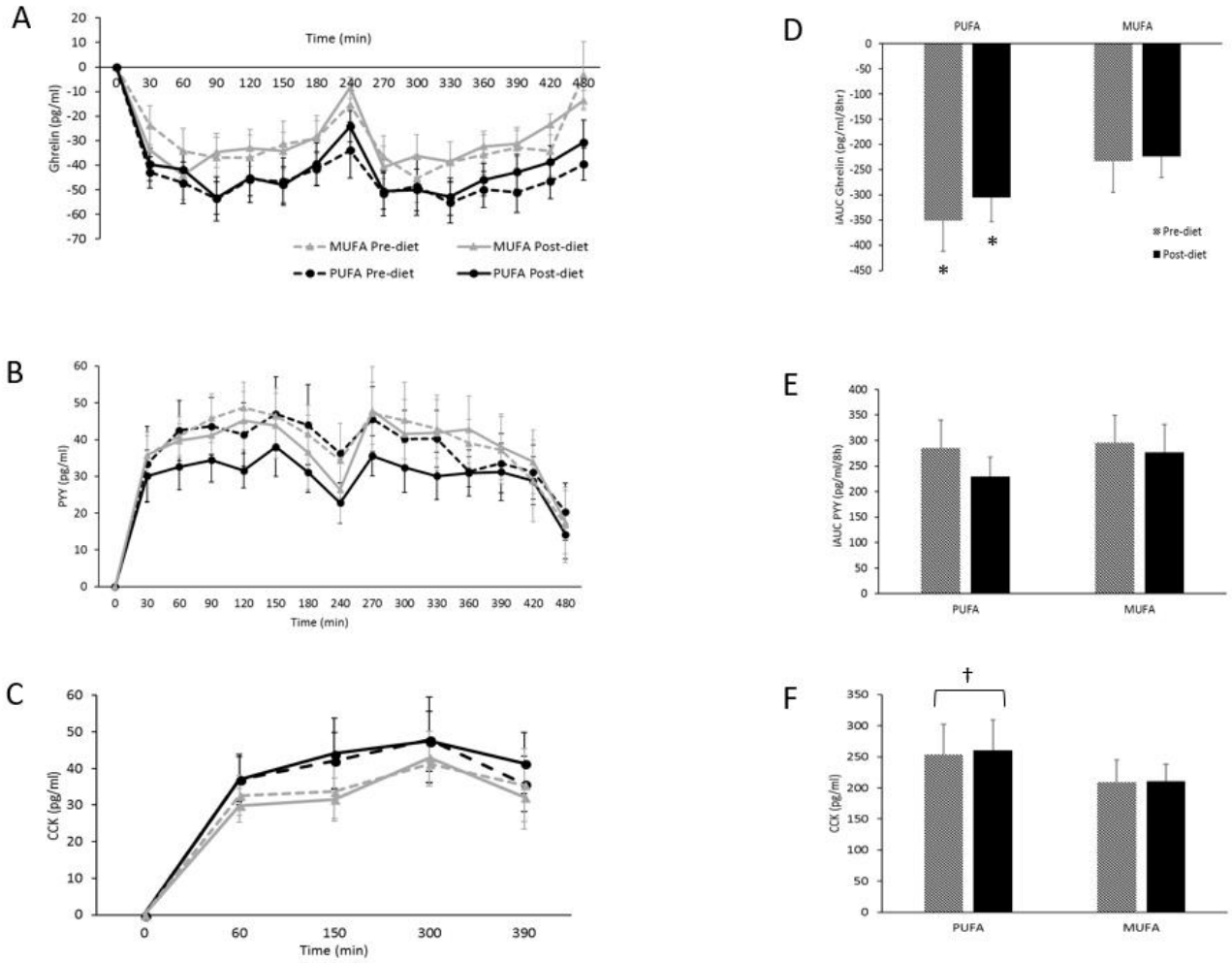

Figure 5.4 Mean ±SE, (**A-C**) Change from baseline for each of the diets, pre- and post-diet intervention. Subjects consumed the HF meal immediately after time 0 and time 240. (**D-F**) Incremental area under the curve (iAUC) for each of the diets, pre- and post-diet interventions. (**A, D**) Ghrelin concentrations. (**B, E**) PYY concentrations. (**C, F**) CCK concentrations. *Significantly lower ghrelin concentrations for PUFA pre vs. MUFA pre-diet visits as well as PUFA post vs. MUFA post-diet visits, ($p<0.05$). †Significant treatment effect with higher CCK for PUFA vs. MUFA ($p<0.05$). MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

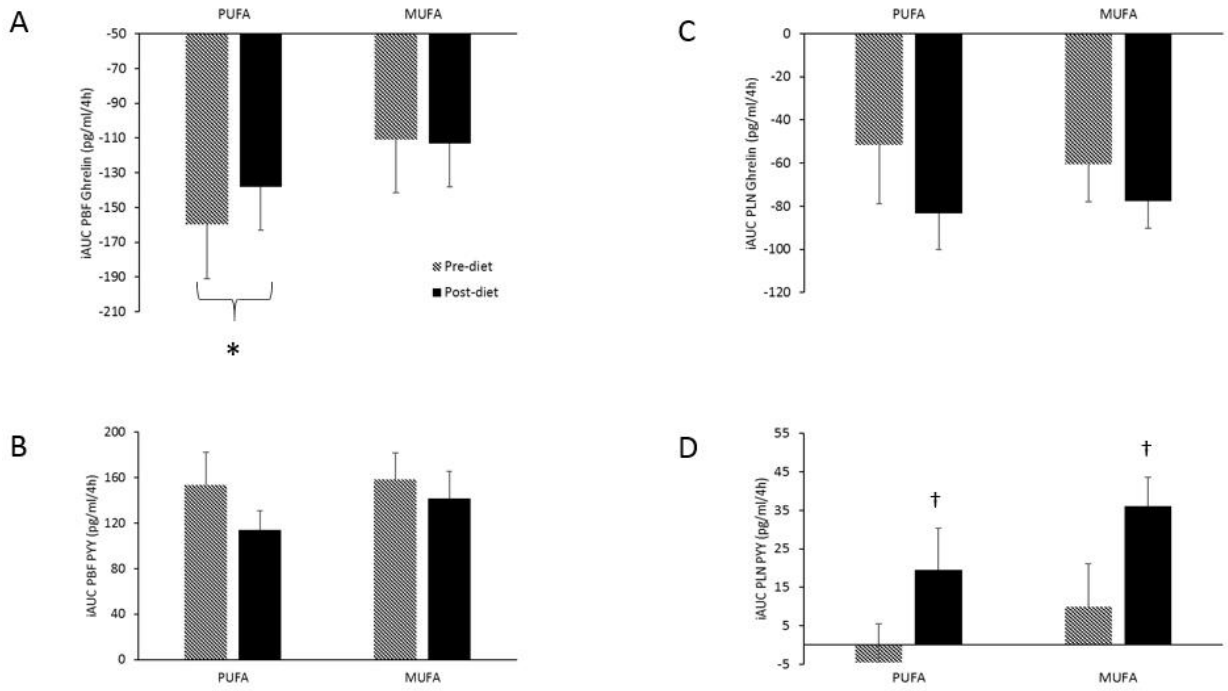

Figure 5.5 Mean ±SE, (**A-B**) Incremental area under the curve (iAUC) for postprandial breakfast (PBF) meal responses for each of the diets, pre- and post-diet interventions. (**C-D**) iAUC for postprandial lunch (PLN) meal responses for each of the diets, pre- and post-diet interventions. (**A, C**) Ghrelin concentrations. (**B, D**) PYY concentrations. *Significantly lower ghrelin concentrations for PUFA vs. MUFA, ($p<0.05$). †Significant visit effect for PYY levels (higher post- vs. pre-diet PYY in both MUFA and PUFA), ($p<0.05$). MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

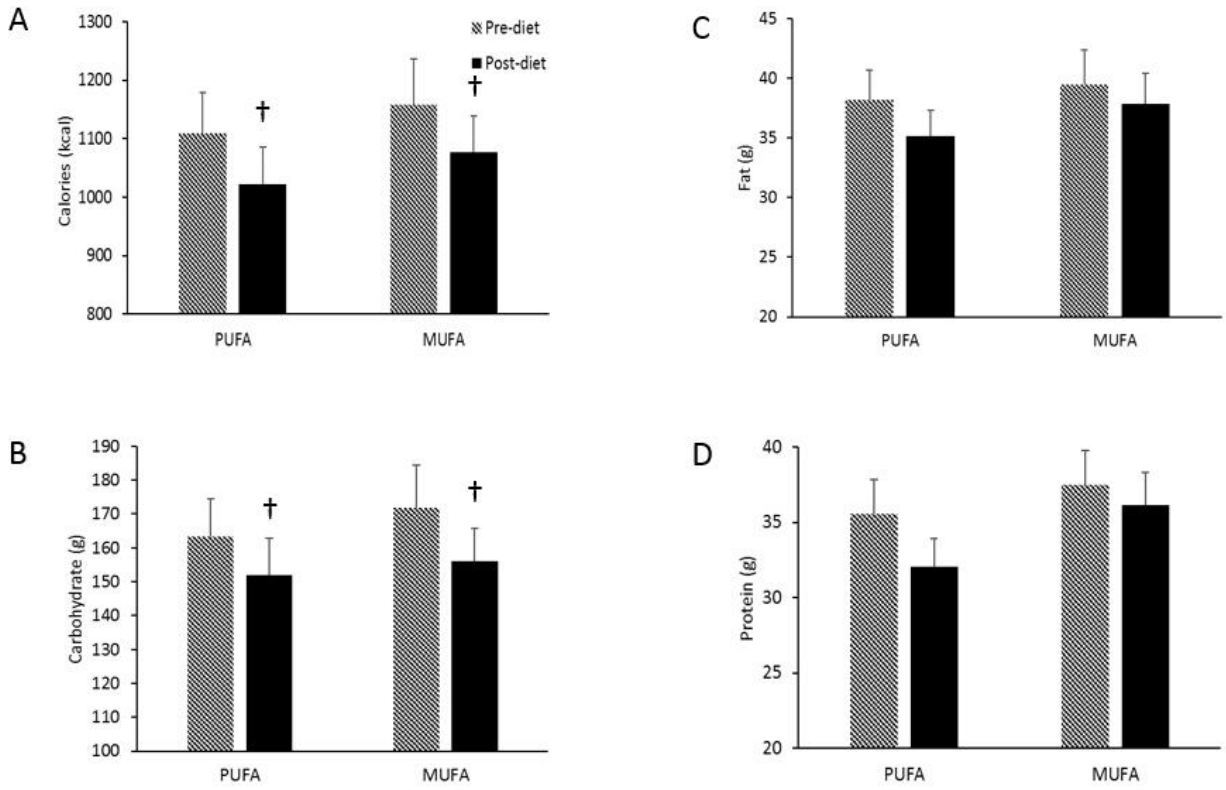

Figure 5.6 Mean ±SE, (**A**) Average energy intake, (**B**) Average carbohydrate intake, (**C**) Average fat intake, and (**D**) Average protein intake for each of the diets, pre- and post-diet interventions. †Significant visit effects (lower energy intake and carbohydrate intake post-diet for both PUFA and MUFA) ($p<0.05$). MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, kcal = kilocalorie

CHAPTER 6

SUMMARY AND CONCLUSIONS

The main purpose of this dissertation was to investigate the metabolic and appetite responses to 5-day HF diets varying in FA composition. Both metabolism and appetite play important roles in weight management and energy balance, and through these roles can further impact disease risk. The results presented in Chapter 3 highlight the metabolic effects of consuming both an acute HF, PUFA- vs. MUFA- rich meal, as well as the 5-day diet effects of consuming a HF, PUFA- vs. a HF, MUFA-rich diet. We showed that acutely, a MUFA-rich meal resulted in greater fat oxidation and DIT compared to a PUFA-rich meal. However, after 5-days, changes in the HF, PUFA-rich diet were significantly greater than the HF, MUFA-rich diet. Therefore, the metabolic differences that were found acutely were negligible after the diet interventions. This demonstrates the metabolic adaptability of the PUFA-rich diet. Further, the results described in Chapter 4 indicate that the HF, PUFA-rich diet had a greater impact on fasting cholesterol profiles and TG levels than the HF, MUFA-rich diet. Specifically, the HF, PUFA-rich diet significantly improved fasting cholesterol profiles (lower total cholesterol, lower LDL cholesterol, and higher HDL cholesterol) compared to the HF, MUFA-rich diet and also significantly lowered fasting and postprandial TG levels, whereas the MUFA-rich diet only had a trend towards lower fasting and postprandial TG levels. Lastly, the results presented in Chapter 5 focus on the differences in appetite responses between consuming a HF, PUFA-rich diet vs. a HF, MUFA-rich diet. We showed that following the 5-day diet intervention, the HF, PUFA-rich diet suppressed subjective ratings of hunger, and the hunger hormone ghrelin, to a greater extent

than the HF, MUFA-rich diet. However, this did not translate to a difference in energy intake at a buffet meal between the two diets. Subjective ratings of fullness and the satiety hormone PYY also were not different between the two diets.

The data represented in Chapters 3, 4, and 5 demonstrate potential benefits of consuming a PUFA-rich diet compared to a MUFA-rich diet. However, when interpreting these results, it is also important to consider the whole food that was used to enrich these diets, and not just the FA composition of the diets. For the HF, PUFA-rich diet, CSO was used to enrich the diets, and in the HF, MUFA-rich diet, OO was used to enrich the diets. A strength of the study design was that all other components of the diets were identical except for the oil used to enrich the diets, so differences found between the two diets can be attributed to these two oils. A majority of the oil used was made up of the particular FA of interest, however other bioactive components present in the oils could have contributed to driving differences seen between the diets. Therefore, it is possible that some of the metabolic, blood lipid, and/or appetite differences that were found in this study could be attributed to some other component of those oils in addition to the differences in their fatty acid profile. In Chapter 4 we were able to elude to a mechanism that may be contributing to the PUFA-rich diets effect on lipid metabolism by looking outside of just the PUFA content of the diet and further into other components present in the oil used.

Overall, the most robust effect found with our 5-day HF diets was that the PUFA-rich diet (enriched with CSO) significantly improved cholesterol profiles and TG levels. This is especially interesting since our participants were healthy and normocholesterolemic. More research is needed to investigate whether a diet intervention longer than 5 days utilizing CSO would result in further improvements and if these results would benefit patients with dyslipidemia. It may also be necessary that studies emphasize the oil used to enrich diets with,

rather than generalizing FA composition of diets/meals. The more understanding we have of foods and nutrition, the more complex food and nutrient interactions become. Therefore, controlled and well thought out study designs are becoming essential to interpretation and translation of results.

Altogether, this study is the first to compare the health benefits of consuming a HF PUFA-rich diet high in CSO to a HF MUFA-rich diet high in OO on metabolism, cholesterol and lipid levels, and appetite measures in healthy adults. The positive outcomes seen with CSO indicate that this oil may be particularly beneficial in weight management strategies and lowering disease risk.