A 7-DAY PUFA-RICH DIET REDUCES POSTPRANDIAL TRIGLYCERIDE LEVELS IN HEALTHY FEMALES

by

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(Under the Direction of Chad Paton)

ABSTRACT

Polyunsaturated fatty acids (PUFAs) protect against cardiovascular disease (CVD). Saturated fatty acids (SFA) increase the risk of CVD. The objective of this study was to determine the ability of high-PUFA diet to protect against high-SFA meals. Sixteen subjects (n=8 males, n=8 females) were divided into the PUFA diet and 10 subjects (n=5 males, n=5 females) into the control diet group. Pre-diet triglycerides (TG) were collected for 8 hours after ingestion of 2 SFA-rich meals. After a 7-day PUFA-rich or control diet, SFA-rich meals were repeated. Changes in plasma TG, glucose, free fatty acids (FFA), fat oxidation and insulin were determined. PUFA diet decreased TG and glucose, and increased FFA and fat oxidation levels. This suggests that high-PUFA diet mitigates the effects of high-SFA meals, and may be useful in decreasing blood TG concentrations in males and females. PUFAs may be more protective against atherosclerosis risk in females, compared to males.

INDEX WORDS: Polyunsaturated fatty acid, saturated fatty acid, high fat, triglyceride

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS iv

LIST OF TABLES vii

LIST OF FIGURES viii

CHAPTER

1 INTRODUCTION 1

2 LITERATURE REVIEW 4

Functional Foods 4

PUFA Metabolism and Regulation of Gene Expression 7

Current and Future Direction of the Functional Food Industry 10

Dietary Fatty Acids 15

Recommendations 16

Hypertriglyceridemia 17

Disease Risk Associated with Hypertriglyceridemia 18

Hypertriglyceridemia Treatment 19

3 MATERIALS AND METHODS 20

Study Design and Participants 20

Protocol 21

Biochemical Assays 24

Statistical Analyses 26

4 RESULTS 27

Participants 27

Metabolic Meal Responses 28

5 DISCUSSION 30

REFERENCES 34

LIST OF TABLES

Page

Table 1: Serum Triglyceride Classification 45

Table 2: Participant Characteristics 46

LIST OF FIGURES

Page

Figure 1: Study design outline. 47

Figure 2: Meal and diet macronutrient breakdown.....48

Figure 3: Time course of meal responses for TG in the PUFA-rich diet and the control diet from pre- to post-diet. 49

Figure 4: Mean AUC difference for TG, glucose, FFA, fat oxidation, and insulin in the PUFA-rich diet and the control diet groups between males and females...……...……50

CHAPTER 1

INTRODUCTION

Hypertriglyceridemia is a form of dyslipidemia that is categorized by elevated blood triglyceride (TG) concentrations and affects nearly 30% of the US population [1-3]. According to the National Cholesterol Education Program, normal TG levels are considered to be anything less than 150 mg/dL and elevated postprandial blood TG's have shown to be an independent risk factor for cardiovascular disease and atherosclerosis [4, 5]. Additional data suggests there is a causal relationship between elevated TG levels, type 2 diabetes mellitus and obesity as a result of induced insulin resistance, glucose intolerance, increased very low density lipoprotein production and high fat diets [1, 2, 4, 5]. Due to the numerous negative effects of elevated cholesterol and TG on health and well-being, methods to reduce them are needed.

Typical treatment options for individuals with elevated TG include both pharmacological and non-pharmacological approaches [2, 3]. Initial treatment steps for hypertriglyceridemia include the implementation of therapeutic lifestyle changes (TLC) in which diet and physical activity are the primary focus [2]. Data suggests that weight reduction and engaging in 90-150 minutes of physical activity per week is sufficient to decrease the prevalence of hypertriglyceridemia [6, 7]. Pharmacological interventions are additional options when TLC approaches are unsuccessful in reducing TG [6]. Statins and fibrates, are two of the most common drugs used to treat dyslipidemia and both have been shown to be effective in reducing TG [3, 8, 9]. However, numerous side effects including gastrointestinal discomfort, muscle inflammation and pain, and abnormal liver transaminases are somewhat common side effects of

statin use [8] while long term use of fibrate therapy has been shown to increase the risk of cholelithiasis [10]. Niacin is another dyslipidemic agent, but has shown to increase the risk of insulin resistance and induce diabetes or pre-diabetes in some patients [10]. While these lifestyle and drug therapies can be effective in reducing TG levels, compliance along with undesirable side effects limit their use in the general population.

However, there is a growing interest in the use of dietary modification to manage hypertriglyceridemia and to protect against atherosclerosis. A diet high in long chain omega-3 polyunsaturated fatty acids (PUFA), specifically α-linolenic acid (18:3n3), eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (22:6n3), reduces blood TG levels, increases high density lipoprotein cholesterol, and decreases LDL cholesterol concentrations [11-14]. Contrary to the beneficial role of PUFAs on plasma TG, previous studies suggest that consuming saturated fatty acids (SFAs) can increase low-density lipoprotein (LDL) cholesterol, decrease insulin sensitivity and promote inflammation, therefore increasing the risk of cardiovascular disease [15, 16]. Therefore, it would be reasonable to assume that replacing dietary SFAs for PUFAs may aid in reducing plasma TG levels, especially in response to acute consumption of SFAs, however no data exits to support this idea.

Therefore, we designed a study to analyze the effect of a high PUFA diet on postprandial TG concentrations after the consumption of a high SFA meal. Despite the detailed information on the effect of a high PUFA diet on fasting TG, very little is known about whether a longer-term PUFA-enriched diet can protect against the rise in postprandial TG after the consumption of a high SFA meal. The purpose of this study was to determine whether a high PUFA diet could mitigate the effects of a high SFA meal, and reduce postprandial TG levels. We studied the postprandial TG responses to high SFA meals in both the fasted and fed state of individuals who

completed either a 7-day high PUFA diet or a control diet to analyze the differences in postprandial TG responses in male and female subjects.

CHAPTER 2

LITERATURE REVIEW

Functional Foods

Healthy eating patterns that promote a diverse and balanced diet are the foundations of dietary recommendations. Food has traditionally been viewed as a necessity for optimal growth and development, and deficiency diseases were once a primary focus in nutrition research. However, an aging population, the obesity epidemic, and the increasing prevalence of other lifestyle diseases, such as diabetes and cardiovascular disease (CVD), have led researchers and consumers to incorporate food-based bioactive compounds in the treatment and prevention of chronic diseases [17]. As a result, food companies have capitalized on developing functional foods that target specific health problems.

There is no legal definition for the term ‘functional food’ among regulatory agencies. However, there are many similar working definitions typically used as marketing techniques that promote functional foods as whole-foods or food components that impart health benefits beyond that of their basic nutrition in order to reduce the risk of chronic diseases and improve overall health and well-being [18, 19]. Functional and bioactive food compounds are recognized as both primary and secondary metabolites, or nutrients and non-nutrients, such as proteins, lipids, carbohydrates, vitamins, and phenolic phytochemical compounds [18, 20, 21]. While vitamins with antioxidant capabilities found in fruits and vegetables and omega-3 fatty acids found in some fish and nuts have been heavily researched and promoted in the nutrition community [22],

carotenoids, flavonoids and other phenolic compounds are increasing in popularity and are being incorporated into more processed foods as a method of food preservation or fortification [23-25].

The health benefits of dietary polyunsaturated fatty acids (PUFAs) have been extensively researched for their role in protecting against CVD. Comprehensive supporting evidence for this claim led the Food and Drug Administration (FDA) to issue a qualified health claim for foods containing omega-3 (n-3) PUFAs, stating that the consumption of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) n-3 PUFAs may reduce the risk of coronary heart disease (CHD). However, this qualified health claim was not extended to α-linolenic acid (ALA, 18:3n3), another n-3 PUFA, or omega-6 (n-6) PUFAs due to less conclusive evidence supporting their role in reducing the risk of CHD [26-28]. Although evidence suggests that ALA exhibits many of the same cardioprotective effects as EPA and DHA, methods for delivering ALA in the diet, as well as other limitations observed in clinical study designs have made definitive conclusions difficult to determine [28]. Additionally, while some research suggests that omega-6 PUFA metabolites, called eicosanoids, are pro-inflammatory and aid in the onset of CVD, other studies indicate that n-6 PUFAs may actually have anti-inflammatory properties [26, 27]. As a result of these findings, sources of n-3 PUFAs have been used to enrich a range of food products in order to increase their marketability and help increase consumption of ALA, EPA and DHA. Certain brands of cereal, pasta, eggs, granola and nutrition bars, salad dressings and butter-like spreads are common food products available in the United States that utilize different forms of fish oil, flaxseed, direct sources of EPA and DHA, as well as microalgae to enrich the products with omega-3 PUFAs [29, 30].

Bio-delivery, the process of feeding livestock and hens with foods enriched with omega-3 PUFAs to incorporate these lipids into their tissues, is another method of enriching food sources

with n-3 PUFAs [29]. Although many consumers recognize the health benefits associated with these lipids, drastic changes in dietary choices often deter consumers from buying and consuming more n-3 PUFAs. However, some studies have shown that n-3 PUFA, EPA and DHA consumption rates are significantly increased when conventional-commonly consumed foods are replaced with equivalent omega-3 PUFA-enriched products [31-33].

Although omega-3 enriched food products are becoming more popular in the marketplace, many consumers look to fish oil supplements as a means for increasing their EPA and DHA intake [34]. According to FDA recommendations, no more than 2 g per day of omega-3's should come from supplements to limit the occurrence of any adverse side effects [29]. Nonetheless, many studies have shown the benefits of fish oil supplementation in improving serum lipoprotein concentrations, reducing inflammation, improving insulin sensitivity in type 2 diabetics, and reducing CVD events [14, 35-38]. However, the characteristic fishy smell and taste as well as the concern of environmental pollutants in fish oils and their capsules are some disadvantages when it comes to their use in food products and supplementation use. Nonetheless, fish oils are the primary source of n-3 PUFAs and are in demand, posing a threat to existing fish populations. As a result, the need for alternative sources of omega-3 PUFAs has been identified. In addition to certain plant oils, microalgae sources are novel alternatives to fish oil use and have been found to exhibit many of the same cardioprotective mechanisms observed in fish oil supplementation [35, 39, 40]. Not only are microalgae primary producers of both EPA and DHA, but they also provide PUFAs in the form of both phospholipids and TGs [41, 42]. Research suggests that microalgae oils could also provide additional nutritive value in the form of carotenoids and phytosterols, making them a promising alternative to fish oils [41].

PUFA Metabolism and Regulation of Gene Expression

PUFA intake can be measured either directly in different biological samples, such as plasma, cell membranes and adipose tissue, or indirectly through gene expression. Different FA pools of triglycerides (TG), phospholipids, cholesterol esters and non-esterified FAs (NEFA) found in biological samples are appropriate biomarkers of PUFA analysis and intake [43]. Dietary PUFAs are absorbed through the intestinal lining where they are then packed with cholesterol and lipoproteins into chylomicrons. Chylomicrons then travel through the lymphatic vessels within each intestinal villus to the lymphatic system which subsequently enters into systematic circulation [44]. Endogenous fat sources from the liver are incorporated into complex lipoproteins, such as very low-density lipoproteins (VLDL), intermediate-density lipoproteins, (IDL) and low-density lipoproteins (LDL) that are released into the bloodstream. Once chylomicrons from the lymphatic system and lipoproteins from the liver enter into systematic circulation, they are transported to muscle or adipose tissue and utilized throughout the body. These lipoproteins as well as NEFAs from adipose tissue can be incorporated into cells where they undergo elongation/desaturation, β-oxidation or are incorporated into TG, phospholipids, or cholesterol esters [45]. Once PUFAs are incorporated into the cell, they are then capable of exerting their effects on gene transcription for certain enzymes involved in lipogenesis, cholesterol synthesis, as well as β-oxidation, exerting an overall lipid-lowering effect. There are four known transcription factor families that are regulated by either the abundance or the activity of PUFAs. These transcription factor families include: peroxisome proliferator-activated receptors (PPAR), liver x receptor (LXR), hepatic nuclear factor-4α (HNF-4α), and sterol regulatory element binding protein (SREBP) [45, 46].

Peroxisome Proliferator-Activated Receptors

PPARs are a group of nuclear receptor transcription factors that are activated through the binding of ligands in their ligand binding domain. Fatty acids, particularly PUFAs are one of the suspected primary ligands for PPAR activation [47, 48]. There are three isotypes of PPARs (PPARα, PPARδ, and PPARγ), each of which activates the expression of different target genes associated with lipid metabolism. PPARα is primarily associated with the increase in fatty acid oxidation, particularly in skeletal muscle, liver and adipose tissue where both peroxisomal α-oxidation and mitochondrial β-oxidation occurs [46, 47, 49]. PPARα is notably expressed in the liver where it is associated both with increased hepatic fatty acid oxidation and lipogenesis to prevent the accumulation of free fatty acids in the liver. PPARδ is ubiquitously expressed and shares many of the target genes with PPARα, whereas PPARγ is highly expressed in adipose tissue as an adipogenesis inducer [48, 49].

Liver x Receptors

LXRs are another member of the nuclear receptor family that are responsible for regulating the expression of genes involved primarily in cholesterol metabolism [46]. While oxysterols have been identified as primary ligands inducing activation of LXRs, certain long-chain fatty acids (LCFA) act as antagonists and inhibit the activation of LXRs by blocking its DNA binding ability. The activation of LXRs through the binding of oxysterols up-regulates genes responsible for cholesterol transport, cholesterol absorption in the intestines, and the conversion of cholesterol to bile acids. These genes include the ATP-binding cassette transporters A1 and G1 and the phospholipid transport protein [46]. Additionally, LXRs have been found to indirectly regulate the expression of genes associated with lipogenesis, such as

fatty acid synthase and stearoyl-CoA desaturase-1, by regulating the gene transcription of SREBP-1c [45, 50].

Hepatic Nuclear Factor-4α

HNF-4α is ligand activated by LCFAs, and has been found to both directly and indirectly regulate the expression of multiple hepatic genes involved in fatty acid metabolism [46, 51]. Research suggests that both Hes6 (Hairy Enhancer of Split 6) and PPARα are direct target genes for HNF-4α. When HNF-4α binds with Hes6, it forms a complex that is essential for negatively modulating PPARγ transcription, thereby reducing accumulation of lipids in the liver. Additionally, HNF-4α also been found to repress genes (CD36 and ACOT1) involved in converting long-chain acyl CoA to free fatty acids and transporting fatty acids to the mitochondria for β-oxidation. As a result, more free fatty acids and TG are entered into the β-oxidation pathway and removed from the liver [52].

Sterol Regulatory Element Binding Protein

Unlike the previously mentioned nuclear receptor transcription factors, PUFAs do not act as ligands for SREBP proteins to induce the transcription of genes. However, PUFAs do affect the abundance of the SREBP transcription factor, which has three identified isoforms [46]. SREBP-1c is the most predominant isoform in mammalian cells and is selective to regulating genes involved in fatty acid and TG synthesis, such as acetyl-CoA carboxylase, fatty acid synthase, glycerol-3-phosphate acyltransferase, stearoyl-CoA desaturase-1, and ATP-citrate lysate [53, 54]. However, SREBP-2 regulates the transcription of genes utilized to maintain cholesterol homeostasis, and SREBP-1a is a pre-natal variant that is thought to maintain basal fatty acid and cholesterol homeostasis during gestational development [54]. Genes responsible for maintaining cholesterol homeostasis that have been found to be directly regulated by SREBP-

2 include: LDL receptor, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, and HMG-CoA reductase [53].

In its precursor form, SREBP is located and bound in the endoplasmic reticulum. However, when cellular fatty acids or cholesterol concentrations are low, SREBP-1c or SREBP-2, respectively, undergoes proteolytic cleavage and the active, or mature form of SREBP is released and travels to the nucleus (by way of the golgi) of the cell. Here, the SREBP binds to the sterol regulatory element (SRE) binding site of gene promoters and enhancers to induce gene transcription [55]. PUFAs have been found to inhibit SREBP-dependent gene expression by reducing the active form of SREBP [56, 57]. One possible pathway PUFAs reduce the abundance of the active form of SREBP is by hydrolyzing the sphingomyelin found within cell membranes. This action releases cholesterol from the membrane which is incorporated intracellularly and induces a negative feedback loop for SREBP activation [57]. Therefore, PUFAs repress both lipogenic and cholesterol synthesis gene transcription, exerting a hypolipidemic affect.

Current and Future Direction of the Functional Food Industry

The discovery of bioactive food compounds and the rise of technological advancements have markedly improved their availability to consumers. Food processors have taken advantage of the process of food fortification by adding specific nutrients to a product for added health benefit, regardless of whether or not the nutrient was available in the product prior to processing [58]. In this way, the nutritional quality of the product is improved. Such products include margarines or other food spreads that have phytosterols or omega-3 PUFAs added, as well as orange juice with added calcium. Food enrichment, although often times used interchangeably with fortification, adds back nutrients lost during processing, often times in increased amounts.

Breads enriched with folic acid and low fat milks enriched with vitamins A and D are some examples. Another class of functional food includes altered food products. These products have had some undesirable food component replaced with a different, more beneficial food component. This form of functional food is often seen in reduced or fat-free processed foods where the fat in the original product is replaced with a high-fiber fat replacer [59].

Enhanced commodities, an additional classification of functional foods, is a leading area of research and technology for the development of foods and food products with enhanced nutritional value and composition [59]. The practice of biodelivery, as mentioned previously, is one such method of producing an enhanced product by selectively feeding livestock and hens feed that contains added-value nutrients (PUFAs, vitamins, minerals) with the intent of increasing these nutrients in their tissue or milk products [29]. Extensive research has been conducted in the field of biofortification, where plant breeders are able to develop micronutrient-rich staple crops through the use of conventional breeding or genetic modification [60, 61]. Conventional breeding practices aim to identify desired nutrient characteristics of crop varieties and traditionally breeds them to create an improved crop. This method of breeding typically focuses on improving staple crops, such as corn, wheat, rice, beans and potatoes, with the key micronutrients, iron, zinc and provitamin A. Additionally, staple crops are also enhanced with micronutrients through the addition of micronutrient-rich fertilizers, crop rotation strategies and plant growth-promoting rhizobacteria. Orange flesh sweet potatoes and quality protein maize are prominent examples of this type of biofortification [60].

When traditional breeding methods are not enough to increase nutrient yields, genetic modifications (GM) through the use of genetic engineering can be applied to add nutrients that may not be present in a plant at all or in insufficient amounts [60]. While conventional breeding

allows for the incorporation of micronutrients, genetic engineering allows for the incorporation of both macronutrients and micronutrients, as well as the elimination of antinutrients and allergens [62]. GM involves the insertion of genes from a variety of sources into the plant of interest to alter gene transcription and produce the desired nutrient improvement. Such biotechnological methods have been used to improve protein balance in maize, introduce new polysaccharides into potatoes and remove saturated fats from canola oil, among other improvements [62].

Additionally, the genetic engineering of rice to produce carotenoids in the endosperm of the granule is an iconic example of a genetically modified crop that is representative of the potential for GM foods in the incorporation of added-value nutrients that can help improve the health of its consumers [60, 61]. This form of GM is more frequently used in underdeveloped countries where micronutrient deficiencies are more prevalent. In these countries, crops have been GM to improve the micronutrient stores of staple crop seeds, which has not only made them more resilient and capable of producing higher crop yields in mineral-deficient soils, but has also improved the bioavailability of micronutrients as well. Increased consumption of these crops could potentially provide sufficient levels of micronutrients to consumers who may have micronutrient deficiencies, therefore improving their overall health and well-being [63]. Although GM crops have great potential for improving micronutrient deficiencies and crop yields, safety concerns over their long-term consumption does exists. However, based on animal toxicity studies, there is no conclusive evidence to suggest that the consumption of micronutrient improved GM crops have any negative side effects, but the need for further studies is acknowledged [64].

Nanotechnology Utilization in the Functional Food Industry

Beyond the field of crop biofortification, advances in nanotechnology have become a vastly emerging area of food science. Nanotechnology has been used to improve processing and packing techniques, as well as to expand delivery methods of bioactive compounds. Research has focused on the incorporation of nanoencapsulation of food components, such as flavors, vitamins, minerals, antioxidants, and other bioactive compounds to control odors and tastes and protect active ingredients from moisture, heat and other environmental factors [65-68]. As a result, an array of delivery systems has been developed to improve bioavailability of the compounds. Nanoemulsions are one type of delivery system that consist of a lipid phase dispersed in an aqueous phase with emulsifier agents surrounding the oil droplet. This delivery system is capable of increasing the bioactivity and palatability of lipophilic compounds such as bioactive lipids, antioxidants and flavors [65, 67]. Nanoemulsions are also capable of increasing intestinal absorption due to permeability changes induced by the composition of the emulsifier agents [66]. Other delivery systems include dendrimers, hydrogels, and microspheres, such as micelles [69]. Liposome or lipid vesicles are also a popular form of nanoemulsion that incorporate an aqueous core surrounded by a phospholipid bilayer structure. Their ability to incorporate both hydrophilic and hydrophobic components within its structure, deliver bioactive compounds to a targeted site, and be altered to increase their stability in response to environmental changes (pH, temperature), have made these nanoparticles of particular interest for drug and bioactive carriers [66, 69]. The use of nano-liposomes for the encapsulation of fish oil to be incorporated into yogurt is one successful application for this type of nanotechnology, resulting in increased stability of both EPA and DHA with reduced oxidation [70].

Although many of the previously mentioned nanoencapsulations are considered stable compounds, additional secondary methods are combined with a drying technique to improve stability. Two forms of drying typically used in the food industry include spray drying and freeze drying. Spray drying involves spraying the nanoencapsulated product into a hot drying medium. In doing this, fine, uniform, dried particles are formed that are more protected from their surrounding environment during processing. Freeze drying, on the other hand, is a multistep process that involves the freezing, sublimation, desorption and storage, resulting in a stable, dehydrated product. This technique is mostly used to remove water from an nanoencapsulated product without changing its shape or structure [68].

Probiotics and Prebiotics

The functional food industry has traditionally focused on improving the nutritional quality of foods through the incorporation of bioactive compounds, such as antioxidants, PUFAs, and other vitamins and minerals, in food products to impart an added benefit to health. However, recent studies have focused on the association between diet composition and changes in gastrointestinal (GI) microflora, and how their interaction affects overall health and wellbeing. As a result, probiotics and prebiotics have become a therapeutic means for re-establishing homeostasis within the gut microbiome [71]. Probiotics are living micro-organisms, and prebiotics are fermented, non-digestible, food ingredients, that both promote changes in the composition or the activity of microflora present in the GI tract [72, 73]. Probiotics are primarily incorporated into fermented dairy products and have been associated with improved immune responses, prevention of allergic responses and other metabolic diseases [71, 73].

Dietary Fatty Acids

Dietary fatty acids are typically consumed through the diet in the form of phospholipids or TGs that are hydrolyzed into their functional fatty acid units. Fatty acids (FA) are carboxylic acids with a methyl group attached to the end of its hydrocarbon chain. Dietary FAs can be classified according to their chain length, degree of saturation, number and placement of double bonds and conformation. Hydrocarbon chains with fewer than 6 carbons are classified as short-chained FAs, while medium-chained FAs have between 6 and 12 carbons, and long-chained FAs have 14 or more carbons in their hydrocarbon chain. Dietary FAs with 16- or 18- carbon fatty acid chains are the most common FAs found in the diet.

A hydrocarbon chain that contains the maximum number of hydrogens attached to each carbon, and therefore contains no double bonds, is classified as a saturated fatty acid (SFA). If, however, a fatty acid chain contains one or more double bonds, resulting in one or more missing pairs of hydrogens, the fatty acid is classified as an unsaturated fatty acid. Unsaturated fatty acids are further classified as either mono- (MUFA) or polyunsaturated fatty acids (PUFA) depending on if there is only one or more than one double bond present in the carbon backbone. PUFAs can be further distinguished based on the placement of the first double bond from the terminal methyl (omega) group. A PUFA with its first double bond at the third carbon from the methyl group is an n-3 or omega-3 PUFA, while a PUFA with its first double bond at the sixth carbon from the methyl group is an n-6 or omega-6 PUFA.

The presence of a double bond in MUFAs or PUFAs can exist as either the more common *cis*, or the less common *trans* configuration. When the two hydrogen atoms are on the same side of the carbon-carbon double bond, the configuration is said to be *cis*. When the two hydrogen atoms are on opposite sides of the carbon-carbon double bond, the configuration is said

to be *trans*. FAs having a *cis* configuration, compared to a *trans* configuration, have greater degrees of bend within the chain. As a result, the FAs are unable to pack as tightly together, and therefore have lower melting points, are typically liquid at room temperature and are most commonly of plant origin. FAs having a *trans* configuration, however, are straighter lending them the ability to pack more tightly together, be firmer at room temperature, and have higher melting points. Trans-FAs are not as common in nature, but can be found in ruminant meats, milk fats and more commonly in commercially produced products, such as partially hydrogenated vegetable oils and snack foods. Differences in FA chain length and the presence, number, placement and configuration of double bond(s) dictates their function in food as well as their metabolism in the body and how they affect human health.

Recommendations

The acceptable macronutrient distribution range for dietary fats supported by the Food and Agriculture Organization (FAO) of the United Nations recommends that only 20 – 35% of a person's total daily caloric intake come from dietary fat, while the National Cholesterol Education Program recommends that 25-35% of calories come from fat [74, 75]. These fat recommendations are developed based on evidence that suggests these ranges have not been found to be associated with CHD events, but will provide adequate amounts of energy, fat soluble vitamins and essential fatty acids [74]. However, more recent research emphasizes the quality of the fat within the diet. The American Dietary Guidelines and the American Heart Association have also supported FAO recommendations to decrease SFA intake to no more than 10% of total fat calories in order to help reduce total and low-density lipoprotein (LDL) cholesterol levels and therefore reduce the risk of CVD [22, 76]. Additionally, it is recommended that *trans* fats be severely limited (<1% of calories coming from fat be from *trans* fats) or even

eliminated from the diet, as they have been found to increase total and LDL cholesterol and decrease high-density lipoprotein (HDL) cholesterol levels [77, 78]. Although there is no current recommendation for MUFA consumption, studies have shown that replacing SFAs with MUFA reduce total and LDL cholesterol as well as total cholesterol:HDL cholesterol ratio [75, 79].

Omega-3 and omega-6 PUFAs are the most common dietary PUFA. However, humans are unable to synthesis these FAs and therefore must be consumed through the diet. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most common long-chained n-3 PUFAs found primarily in fatty fish, while ALA is the most common short-chained n-3 PUFA primarily found in English walnuts, seeds and vegetable oils. Current dietary recommendations for these essential fatty acids advises a daily consumption of 0.6 to 1.2% of calories from fat come from ALA, and .5-1.0% of total fat calories come from EPA and DHA [28, 30, 80]. While evidence isn't as convincing for n-6 PUFAs, an adequate intake of 3 to 10% of caloric intake from linoleic acid (LA; 18:2n6) is recommended. Research suggests that replacing SFAs with PUFAs and consuming the recommended amounts of PUFAs is cardioprotective [74, 79].

Hypertriglyceridemia

Hypertriglyceridemia is a form of dyslipidemia characterized by elevated blood TG concentrations of greater than 150 mg/dL [75]. Nearly 30% of the US population is affected by this form of dyslipidemia and varying TG concentrations may pose an increased risk to health (Table 1) [2, 81]. Two types of hypertriglyceridemia exist. Primary hypertriglyceridemia is a genetic disorder in which TG metabolism is altered resulting in elevated blood TG concentrations. However, secondary hypertriglyceridemia is typically the result of lifestyle factors and metabolic disorders [2].

While the liver is responsible for esterifying fatty acids into TGs from adipose tissue and packing them into VLDL during fasting and reforming fatty acids into TG after having passed through muscle tissue, insulin resistance can cause the liver to make an excess of TG from glucose and fructose. Diets typical of a Westernized Diet that are high in fat, particularly saturated fat, and carbohydrates have been shown to elevate both fasting and postprandial blood TG concentrations [4, 82, 83]. Research suggests that postprandial hypertriglyceridemia could also be an indicator of type 2 diabetes as a result of increased insulin resistance and glucose intolerance [1, 4]. Additionally, increased intake of dietary fats, in relation to total energy intake, has a direct correlation to increases in body weight and adiposity, leading to obesity [84, 85]. Further obesity research has shown that increases in visceral fat and waist circumference are often accompanied by both elevated TGs and lowered HDL cholesterol levels [86]. Hypertriglyceridemia is also one of the possible metabolic abnormalities associated with the metabolic syndrome, which affects nearly 34% of the US adult population [87]. The metabolic syndrome is characterized by a clustering of three or more risk factors, including many of the previously discussed metabolic abnormalities (abdominal obesity, hypertriglyceridemia, hyperglycemia, low HDL cholesterol levels and increased blood pressure) that ultimately result in an increased risk for CVD [88, 89].

Disease Risk Associated with Hypertriglyceridemia

CVD is the leading cause of death in the US, accounting for nearly 32% of all deaths, or 800,000 deaths per year in the US alone [90, 91]. Hypertriglyceridemia is an independent risk factor for atherosclerosis and CVD [92-95], and is associated with a greater risk for cardiovascular and all-cause mortality [96, 97]. Additionally, severe hypertriglyceridemia, as defined by TG levels $\geq$ 500 mg/dl, affects nearly 3.4 million Americans and is also associated

with increased risk of mortality caused by cardiovascular related events [3, 97]. Research also suggests that the presence of TG-rich chylomicrons in individuals with severe hypertriglyceridemia promote pancreatic inflammation, ultimately leading to acute pancreatitis [3, 5, 98, 99].

Hypertriglyceridemia Treatment

Therapeutic lifestyles changes (TLC) are often the first form of treatment for individuals with hypertriglyceridemia. Considering many of these individuals are also obese, diabetic, hypertensive, and/or insulin-resistant, of which all are risk factors for CVD, reducing the risk of CHD through the reduction of LDL cholesterol levels is the primary goal for treatment. Treatment focuses on increasing physical activity, weight reductions and dietary modifications. Dietary modifications focus first on lowering LDL cholesterol levels by reducing saturated fat intake to less than 7% of total calories consumed and reducing cholesterol intake to less than 200 mg/day [75, 100]. Individualized diet therapy aimed at reducing total fat and calorie intake in individuals with severe hypertriglyceridemia has also been found to be an effective means of treatment in reducing TG levels [13]. Long-chain omega-3 PUFA supplementation, especially EPA and DHA, has also been shown to reduce both TG and LDL cholesterol concentrations and increase HDL cholesterol levels [11, 40, 101, 102].

When TLC is not adequate to lower TG concentrations to <200 mg/dL, pharmacological agents are required [5, 6, 75]. Statins, fibrates, and niacin therapies are the most common dyslipidemic agents and have been proven to reduce TG concentrations, as well as CVD risk and the risk for developing acute pancreatitis [98, 103, 104]. However, these drugs have notable undesirable side effects that limit their use and compliance [8-10].

CHAPTER 3

MATERIALS AND METHODS

Study Design and Participants

The study was a randomized, single-blinded, placebo-controlled, parallel trial for a 10-day feeding study designed to test TG responses to two SFA-rich high fat (HF) meals both before and after a 7-day diet. The study consisted of a screening visit, a 3-day lead-in diet, a pre-diet visit (baseline), a 7-day feeding protocol (either PUFA-rich diet or control diet), and a post-diet visit (final), which was completed by all participants (Figure 1). All testing procedures were completed at the Human Nutrition Lab (HNL) after an overnight fast and an abstinence from exercise for at least 12 hours. The University's Institutional Review Board approved all procedures and written consent was obtained prior to the start of the study.

Thirty-two (n=16 males and n=16 females) apparently healthy, normal weight, as determined by a body mass index of 18-24.9kg/m^2, and sedentary (performed <3h/wk of structured exercise) men and women between the age of 18 and 35 years were recruited and randomized to either the PUFA diet group or the control diet group. Individuals with a history of chronic, metabolic or endocrine disease, dyslipidemia (based on fasting blood lipids at screening visit), gastrointestinal disorders, or had a previous surgery that could affect digestion or hormone signaling were excluded from this study. Exclusion criteria also extended to individuals who were pregnant, lactating or planning to become pregnant, as well as individuals who had experienced a recent change in body weight within the past 3 months or were planning to alter their current physical activity level. Lastly, persons with dietary restrictions, on a medically

prescribed diet, taking supplements or medications that alter metabolic rate, body weight or composition, appetite, as well as individuals who use tobacco were also excluded from the study. Additional study design details are previously discussed [105].

Protocol

Screening visit and 3-day lead-in diet

All participants reported to the HNL and completed a screening visit where anthropometrics (height, weight, blood pressure) were measured and a fasting blood sample was obtained to assess blood lipids and their qualification for participating in the study. A 10ml fasting blood sample was collected into vacutainers and centrifuged at 3000 rpm for 15 min at 4^{o} C, and then sent to the Covenant Health Laboratory (Lubbock, TX) to run a lipid panel (total, high-density lipoprotein, and low-density lipoprotein cholesterol, and triglycerides). The participant's resting metabolic rate was measured using a metabolic cart and was calculated using the modified Weir equation. Each participant's resting metabolic rate multiplied by 1.65 (an average United States physical activity value) was used to determine their estimated daily energy needs for the 3-day lead in diet, 7-day PUFA diet, 7-day control diet and the HF SFA-rich meals. The diets were designed to maintain energy balance throughout the duration of the study. After participants qualified for the study based on a normal lipid panel (total cholesterol < 200 g/dL, HDL > 40 mg/dL, LDL < 100 mg/dL, TG > 150 mg/dL) they were randomly assigned to either the PUFA-rich diet group or the control diet group and were scheduled for their pre-diet visit.

Each participant was provided a lead-in diet for 3 days prior to their pre-diet visit. This diet was representative of a standard American diet that provided 50% of calories from carbohydrates, 35% of calories from fat and 15% of calories from protein. Participants came to

the HNL to consume their breakfast meals, and were directed to consume all the food that was provided to them for the remainder of their meals. However, no additional foods or caloric beverages were permitted throughout the course of the study. Lastly, participants were asked to keep a food and physical activity log to promote compliance.

Pre-diet visit

At the conclusion of the 3-day lead-in diet, participants arrived at the HNL in a fasted state and without having exercised for 12 hours and completed the pre-diet visit. Baseline anthropometrics including, height, weight, blood pressure, waist and hip circumference and body fat percentage were measured. Body fat percentage was measured using air displacement, and resting metabolic rate was measured as described in the screening visit.

Following the completion of baseline measurements, a 15 ml fasting blood sample was taken to assess blood lipids. The blood sample was collected in a vacutainer and centrifuged at 3000 rpm for 15 min at 4^{o} C. 5ml of the sample was transported to the Covenant Health Laboratory to complete a lipid panel analysis. Participants then completed one of two identical high-fat saturated fat-rich liquid meals. The first meal was given at breakfast (0800 hours) and the second meal was given at lunch (1200 hours). The participants were given 5 min to consume each liquid meal in its entirety, and the liquid meals were designed to provide 35% of the participants estimated daily energy needs (Figure 2a).

Following ingestion of both high-fat saturated fat-rich meals (breakfast and lunch), 10 ml blood samples were taken every 30 min for 4 hours post-breakfast and 4 hours post-lunch. Whole blood samples were centrifuged at 3000 rpm for 15 min at 4^{o} C to separate out the plasma. Plasma samples were stored in a -80^{o} C freezer until used for biochemical analysis (plasma TG, glucose, free fatty acid concentrations). Postprandial macronutrient oxidation and energy

expenditure was also calculated from respiratory gas measurements for 4 hours post-breakfast and 4 hours post-lunch as determined by indirect calorimetry.

7-day diet

At the conclusion of the pre-diet visit, participants began the 7-day diet (either the PUFA-rich diet or the control diet). Participants came to the HNL to consume breakfast, and received the remainder of their food and beverages for the rest of the day. They were instructed not to consume any other foods or caloric beverages outside of what was provided to them, and the diets were designed to keep participants in energy balance.

The percentage of macronutrients for both the PUFA-rich diet and the control diet were the same, providing 50% of calories from carbohydrates, 35% of calories from fat and 15% of calories from protein (Figure 2b and c). The control diet had the same macronutrient and fatty acid breakdown as the standard American diet used as the lead-in diet. Specifically, the fatty acid composition in the control diet was 7% of total energy from polyunsaturated fatty acids, 15% from monounsaturated fatty acids, and 13% from saturated fatty acids. However, the fatty acid composition of the PUFA-rich diet was 21% polyunsaturated fatty acids, 9% monounsaturated fatty acids and 5% saturated fatty acids. The majority of the PUFAs were omega-6 PUFAs, followed by omega-3 PUFAs, and a small amount from EPA and DHA. A whole foods approach was taken to achieve the intended PUFA levels by consuming salmon, tuna, walnuts, flax seed oil, canola oil, and fish oil supplements that provided ~3g/day of EPA and DHA, combined.

Post-diet visit

After the 7-day diet, participants reported to the HNL and completed the post-diet visit. The exact same procedures and measurements from the pre-diet visit were followed and obtained at the post-diet visit.

Biochemical Assays

Triglyceride Assay

The Wako Diagnostics L-Type Triglyceride M Assay kit (Wako Chemicals USA, Inc., Richmond, VA) was used to quantify plasma TG concentrations. Ten time points between pre- and post-diet visits (baseline, 120, 240, 360, 480 min post-meal) were measured for each subject, and were run on the same assay plate. Plasma samples were thawed over ice and vortexed prior to the initiation of the TG assay protocol. The assay kit includes Color A reagent that contains glycerol kinase(GK), glycerol-3-phosphate oxidase (GPO), N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS), a catalase and adenosine 5'-triphosphate disodium salt (ATP). When this reagent is added to the plasma samples, the free glycerol is decomposed. The free glycerol, in the presence of ATP, is converted to glycerol-3-phosphate by GK. GPO then oxidizes the glycerol-3-phosphate to produce hydrogen peroxide. Then the Color B reagent, which contains lipoprotein lipase (LPL), horseradish peroxidase (POD), and 4-aminoantipyrine, is added to the samples. This reagent hydrolyzes the TG in the sample to free fatty acids and glycerol using the LPL. The free glycerol undergoes the same reaction as previously described, and the hydrogen peroxide that is produced causes a quantitative oxidation of 4-aminoantipyrine and HMMPS that results in the generation of a blue pigment. The measured absorbance of the blue color is proportional to the original TG concentration.

Glucose Assay

Plasma glucose concentrations were measured, utilizing enzymatic methods, at 30 different time points between the pre- and post-diet visits for each subject (baseline, 30, 60, 90, 120, 150, 180, 240, 270, 300, 330, 360, 390, 420, 480 min post-meal). Plasma samples were thawed over ice and vortexed prior to the initiation of the glucose assay protocol. The assay buffer included a 0.1 M sodium phosphate buffer, 0.2% dimethylaniline, 20 mM 4-aminoantipyrine, water, peroxidase, and glucose oxidase. A 300 mg/dL glucose standard solution was used to develop a standard curve. The addition of the enzymatic assay buffer to the plasma samples resulted in a color change proportional to the plasma glucose concentration. Glucose, in the presence of glucose oxidase, is converted to gluconic acid and hydrogen peroxide. Hydrogen peroxide then reacts with 4-aminoantipyrine in the presence of horseradish peroxidase to produce oxidized 4-aminoantipyrine. This step is a color generating reaction, resulting a purple colored product. The original glucose concentration was determined by measuring the intensity of the purple color at 550 nm, which is proportional to the original concentration.

Free Fatty Acid Assay

Plasma free fatty acid (FFA) concentrations were measured using the Wako Diagnostics HR Series NEFA-HR (2) assay kit (Wako Chemicals USA, Inc., Richmond, VA). Sixteen time points between the pre- and post- diet visits (baseline, 30, 60, 90, 120, 150, 180, 240 min post-meal) were measured for each subject. Plasma samples were thawed over ice and vortexed prior to the initiation of the FFA assay protocol. The kit contains Color Reagent A, which contains acyl-coenzyme A synthetase (ACS), coenzyme A (CoA), adenosine triphosphate (ATP), 4-aminoantipyrine, and ascorbate oxidase. When this reagent is added to the plasma samples, the non-esterified FFA in the serum form acyl-CoA in the presence of CoA and ATP. Then the

Color B reagent, which contains acyl-CoA oxidase (ACOD), peroxidase, and 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA), is added to the samples. This reagent oxidizes acyl-CoA and produces hydrogen peroxide, which in the presence of peroxidase, forms a purple-colored product. The original free fatty acid concentration was determined by measuring the intensity of the purple color at 550 nm, which is proportional to the original concentration.

Statistical analyses

Statistical analyses were performed using the SAS version 9.4 statistical software package. Descriptive statistics were calculated for all outcome variables, including mean, range and standard deviation. A paired samples t-test was used to determine significant differences between pre- and post-diet for both males and females in both the PUFA group and the control group for anthropometrics, blood pressure, and fasting blood lipids. A three-way repeated measures ANOVA was used to test for differences in TG concentrations for the two treatment conditions (PUFA vs. control diet) based on time and visit. Additionally, mean area under the curve (AUC) difference was calculated for TG, glucose, FFA, insulin and fat oxidation for both diet groups (PUFA vs. control diet) and compared using a two-sample t-test. Statistical significance was set at $p < 0.05$, and data are presented as mean ± SE, unless otherwise specified.

CHAPTER 4

RESULTS

Participants

Twenty-six sedentary normal weight men and women completed the entire study protocol and were included in the final study analysis (PUFA-diet: n= 8 women and n= 8 men; control diet: n=5 women and n=5 men). Six participants either dropped out or were not included in the final analysis due to poor diet compliance. A greater dropout rate in the control group resulted in an uneven number of participants in the PUFA diet and control diet group at the end of the study. Participants were between the ages of 18 and 35 years of age and subject characteristics and blood lipids from pre- to post-diet are detailed in Table 2. In the PUFA-rich diet group, there were no significant changes in height, body weight, BMI, percent body fat, waist circumference, hip circumference, WHR, systolic blood pressure, or diastolic blood pressure between pre- and post-diet visits in either the male or female participants. A significant decrease in weight (males: 73.2 ± 9.8 – 72.8 ± 9.9 kg, $p < 0.05$; females: 60.1 ± 7.5 – 59.5 ± 7.7 kg, $p < 0.05$) and subsequently BMI (males: 21.9 ± 2.2 – 21.8 ± 2.2, $p < 0.01$; females: 21.8 ± 2.3 – 21.6 ± 2.3 kg/m^2, $p < 0.05$) was seen for both males and females, respectively, in the control diet group from pre- to post-diet. There was also a significant decrease in HC (100.1 ± 7.7 – 99.1 ± 8.3 cm, $p < 0.5$) in the female control subjects between study visits. There were significant decreases in total cholesterol, triglycerides, non-HDL, LDL cholesterol, VLDL cholesterol, and cholesterol/HDL ratio from pre- to post- diet visits in both the male ($p < 0.05$) and female ($p < 0.01$) participants in the PUFA-rich diet group. There were significant decreases in total

cholesterol, non-HDL cholesterol, and LDL cholesterol in the male ($p < 0.5$) control subjects from pre- to post-diet visit.

Metabolic Meal Responses

TG concentrations over time were analyzed to assess metabolic response to the two SFA-rich HF meals before and after the 7-day diet. The time course of TG concentrations from pre- to post- diet visits is shown in Figure 3. Fasting TG concentrations were not significantly different between pre- and post-diet visits for females or males in either the PUFA-rich or the control diet group (ns). There was a significant diet x visit x time interaction between females in the PUFA-rich diet group at 120 min (p=.0004), 240 min (p<.0001), and 360 min (p<.0001) post SFA-rich meal ingestion from the pre- to post- diet visits, however there were no significant interaction effects observed 0 min post ingestion or at any time point in the females in the control diet group (ns). There were no other significant three-way interactions (ns) between diet groups, genders, or visits. However, there was a significant main effect of time (p<.0001) and a significant visit effect (p=.02) for the male PUFA diet group. Furthermore, Figure 4a shows the mean TG AUC difference is significantly lower for females in the PUFA diet group compared to the control diet group (p=.02), but no significance was found between males in the PUFA and control diet groups (ns) (Figure 4b).

Additionally, mean AUC differences for glucose, FFA, fat oxidation and insulin are found in Figure 4c-j. There were no significant differences in mean AUC between genders and treatment groups (PUFA vs. control diet) for FFA, fat oxidation and insulin (ns). However, mean glucose AUC was significantly lower in females in the PUFA diet group compared to the control diet group (p=.01). No significant difference was found in the males between the PUFA and control diet groups (ns). Additionally, a multiple regression was run to predict glucose, FFA, fat

oxidation and insulin from gender, diet, and visit. The multiple regression model statistically significantly predicted insulin (p=.0002) for females, but did not find any statistical significance for males (ns).

CHAPTER 5

DISCUSSION

The aim of this study was to determine whether a high-PUFA diet could mitigate the effects of a high-fat SFA-rich meal by reducing postprandial TG levels. Research suggests that both the amount and type of dietary fat influences postprandial lipemia, showing that test meals containing up to 50g of fat can substantially increase TG concentrations [106]. Furthermore, previous studies have reported reduced postprandial TG concentrations in response to n-3 PUFA consumption, while SFA consumption leads to increased postprandial TG concentrations [107, 108]. However, to our knowledge no study has utilized a whole-food based, longer-term PUFA diet to measure postprandial TG response to high fat meals rich in SFA. Therefore, for the first time, we are showing that a PUFA-rich diet reduces postprandial TG concentrations in females, following a SFA-rich meal containing ~58 g of fat. While this study design is unique, our results are consistent with previous literature showing that postprandial TG concentrations are lower after consuming either a PUFA or SFA meal while following a PUFA background diet compared to a SFA background diet [109]. These findings indicate that a diet rich in PUFAs may provide metabolic protection from high-fat SFA-rich meals.

Furthermore, the PUFA-rich diet in the current study reduced glucose concentrations in females. Although there were no other significant differences observed in FFA, fat oxidation and insulin, these results suggest that a PUFA-rich diet may improve lipid metabolism and may help explain the reduction seen in TG concentrations postprandially. Metabolic flexibility is the body's ability to adjust fuel oxidation in response to fuel availability [110]. Therefore, in

response to a high-fat diet, the body preferentially utilizes fat for fuel by hydrolyzing FFA from adipose tissue and TG-rich lipoproteins, all while sparing glucose from oxidation [111]. The results from our study suggests that metabolic flexibility may be improved in response to a high PUFA diet, at least with respect to lipid metabolism.

The differences in TG concentrations between the male and female participants were unexpected. Although this was not a mechanistic study, and we do not know the exact mechanism by which PUFAs reduce postprandial TG concentrations differently for males and females, we can speculate on why this occurred. Although there are noticeable physiological differences between males and females, sex has been determined as a biological variable that needs to be accounted for in basic and clinical research [112]. In looking at the differences between the way males and females metabolize and store fat, research is consistent with our results in finding that males and females do not respond to meal ingestions in the same way [113]. It is believed that the gonadal hormones, estrogen and androgen, are responsible for the differences in energy balance and meal response between genders [114]. Specifically, studies suggest that estradiol suppresses free fatty acid and TG synthesis and accumulation in the blood and tissues, and increases fat oxidation in females, compared to males. Some studies have also shown that females are more resistant to metabolic disturbances caused by high-fat diets compared to males due to the presence and abundance of estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) [114-116]. ERα is the main receptor in hepatocytes and in conjunction with the presence of estradiol, they control genes involved in glucose, lipid, protein and cholesterol homeostasis [116].Taken together, these findings might help explain the differences seen in TG concentrations in our study between the male and female participants.

There were some limitations in the study, which are outlined below. Although participants were provided all the meals they were supposed to consume for the duration of the study, they were not confined to the Human Nutrition Lab and could have consumed food or beverages outside of what was provided to them. Additionally, while the macronutrient composition was controlled for in both the PUFA diet and the control diet, participants in the control diet group consumed more SFA, trans fat and cholesterol compared to participants in the PUFA diet group. Participants in the PUFA diet group consumed more fiber compared to the participants in the control diet group. Further, the amount of PUFAs consumed in the PUFA treatment diet (21% of total calories from fat) may not be representative of what is feasible on a daily basis for the average adult. Therefore, using a whole food approach, it was our goal to provide as many long-chain PUFAs without providing a high fat meal. However, the concern of adverse side effects, such as fishy aftertaste, gastrointestinal discomfort, increased LDL concentrations and reduced glycemic control in diabetics with increased PUFA consumption have contradictory or unfounded results. Nonetheless, fish oil capsules are a suitable replacement for whole food forms of PUFAs. Their consumption would decrease the number of calories consumed from other PUFA sources, ensure only EPA and DHA PUFAs are consumed, and are capable of providing equivalent amounts of PUFAs when compared to the amount consumed from whole food sources. Additionally, research indicates from dose-response tests that greater n-3 PUFA consumption is associated with positive clinical results [117], while n-6 PUFA consumption is less conclusive [118]. One final limitation in our study was that even after trying to control for pre-existing dietary patterns and influences by providing a lead-in diet, we found different fasting values between the PUFA diet participants and the control diet participants.

In conclusion, typical American dietary patterns consist of multiple meals or snacks a day that are rich in SFAs and refined carbohydrates, leaving the body in the fed state for prolonged periods of time (up to 18 hours per day) [107, 119]. As a result, the body is at an increased risk for CVD due to the increased level of postprandial TGs. However, based on the results of the current study, a diet rich in n-3 PUFAs may provide protection against the development of CVD and hypertriglyceridemia, and may also promote better utilization of macronutrients.

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Table 1. Serum Triglyceride Classification

Triglycerides (mg/dL)	Triglyceride Classification
<150	Normal
150 – 199	Borderline- High
200 – 499	High
≤ 500	Very High/ Severe

Table 2. Participant Characteristics

	PUFA-rich diet (n=16)				Control diet (n=10)			
	Males (n=8)		Females (n=8)		Males (n=5)		Females (n=5)	
	Pre-diet	Post-diet	Pre-diet	Post-diet	Pre-diet	Post-diet	Pre-diet	Post-diet
Age (year)	23.3 ± 5.3	-	23.4 ± 3.9	-	20.2 ± 1.3	-	22.8 ± 6.4	-
Height (cm)	177.2 ± 9.7	-	166.3 ± 10.7	-	182.6 ± 7.0	-	165.8 ± 4.0	-
Weight (kg)	72.9 ± 7.2	72.5 ± 7.4	59.9 ± 10.9	60.3 ± 10.5	73.2 ± 9.8	72.8 ± 9.9*	60.1 ± 7.5	59.5 ± 7.7*
BMI (kg/m^2)	23.3 ± 2.2	23.1 ± 2.2	21.6 ± 2.7	21.7 ± 2.7	21.9 ± 2.2	21.8 ± 2.2*	21.8 ± 2.3	21.6 ± 2.3*
Body Fat %	17.5 ± 7.0	17.0 ± 7.4	25.7 ± 5.9	26.6 ± 6.5	21.9 ± 6.1	21.3 ± 7.2	30.8 ± 5.5	30.1 ± 6.4
WC (cm)	81.8 ± 5.4	81.4 ± 6.2	71.1 ± 5.1	70.5 ± 5.5	82.7 ± 6.3	82.1 ± 6.9	73.6 ± 3.6	72.6 ± 4.4
HC (cm)	96.5 ± 4.0	96.1 ± 3.8	96.5 ± 5.7	96.9 ± 5.4	100.0 ± 4.2	98.6 ± 7.1	100.1 ± 7.7	99.1 ± 8.3*
WHR	0.8 ± 0.04	0.8 ± 0.04	0.7 ± 0.06	0.7 ± 0.03	0.8 ± 0.05	0.8 ± 0.04	0.7 ± 0.03	0.7 ± 0.02
SBP (mmHg)	116.7 ± 12.9	119.6 ± 12.5	108.6 ± 9.5	102.7 ± 7.0	112.1 ± 9.0	113.6 ± 12.4	107.9 ± 8.8	104.9 ± 8.8
DBP (mmHg)	69.1 ± 8.7	71.5 ± 8.3	68.9 ± 4.1	64.2 ± 5.7	70.9 ± 7.5	72.3 ± 4.7	70.1 ± 3.8	71.9 ± 8.7
Cholesterol (mg/dL)	138.9 ± 23.5	112.3 ± 16.5*	158.1 ± 22.6	124.4 ± 16.4*	149.8 ± 18.4	138.8 ± 15.2*	146 ± 29.1	146.8 ± 27.7
Triglycerides (mg/dL)	66.4 ± 24.9	48.3 ± 28.4*	74.1 ± 10.5	48.8 ± 10.0*	74.4 ± 21.5	60.8 ± 9.8	64 ± 14.5	58.2 ± 13.5
HDL-cholesterol (mg/dL)	43.4 ± 8.4	44 ± 8.3	52.4 ± 14.1	51.9 ± 10.3	40.4 ± 3.9	41.8 ± 9.3	55.6 ± 14.1	55.4 ± 13.9
Non-HDL (mg/dL)	95.5 ± 22.7	68.3 ± 18.6*	105.8 ± 16.7	71.3 ± 14.7*	109.4 ± 18.2	97 ±17.5*	90.4 ± 19.6	91.4 ± 16.8
LDL cholesterol (mg/dL)	79.8 ± 23.8	52.4 ± 21.2*	91.8 ± 17.9	57.9 ± 13.6*	98.2 ± 20.0	85.2 ± 17.4*	78.8 ± 19.5	76.4 ± 17.7
VLDL cholesterol (mg/dL)	13.4 ± 5.0	9.6 ± 5.7*	14.8 ± 2.1	9.7 ± 1.9*	14.8 ± 4.5	12.2 ± 1.6	12.8 ± 3.1	11.4 ± 2.8
Chol/HDL Ratio	3.3 ± 0.81	2.6 ± 0.7*	3.1 ± 0.6	2.5 ±0.4*	3.7 ± 0.6	3.4 ± 0.8	2.7 ± 0.3	2.7 ± 0.4

Values are presented as Mean ± SD

BMI body mass index, *WC* waist circumference, *HC* hip circumference, *WHR* waist-to-hip ratio, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very low-density lipoprotein

* Indicates significant difference from pre- to post- diet, $p < 0.05$

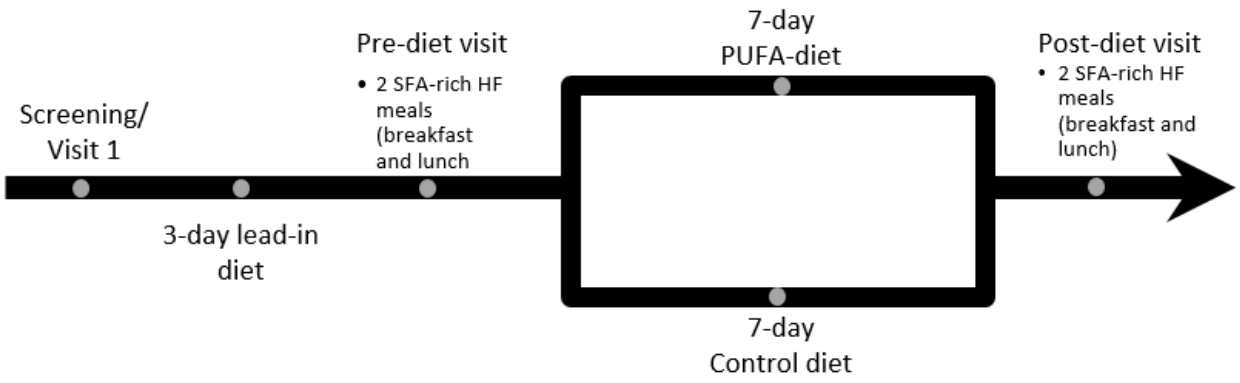


Figure 1. Study design outline

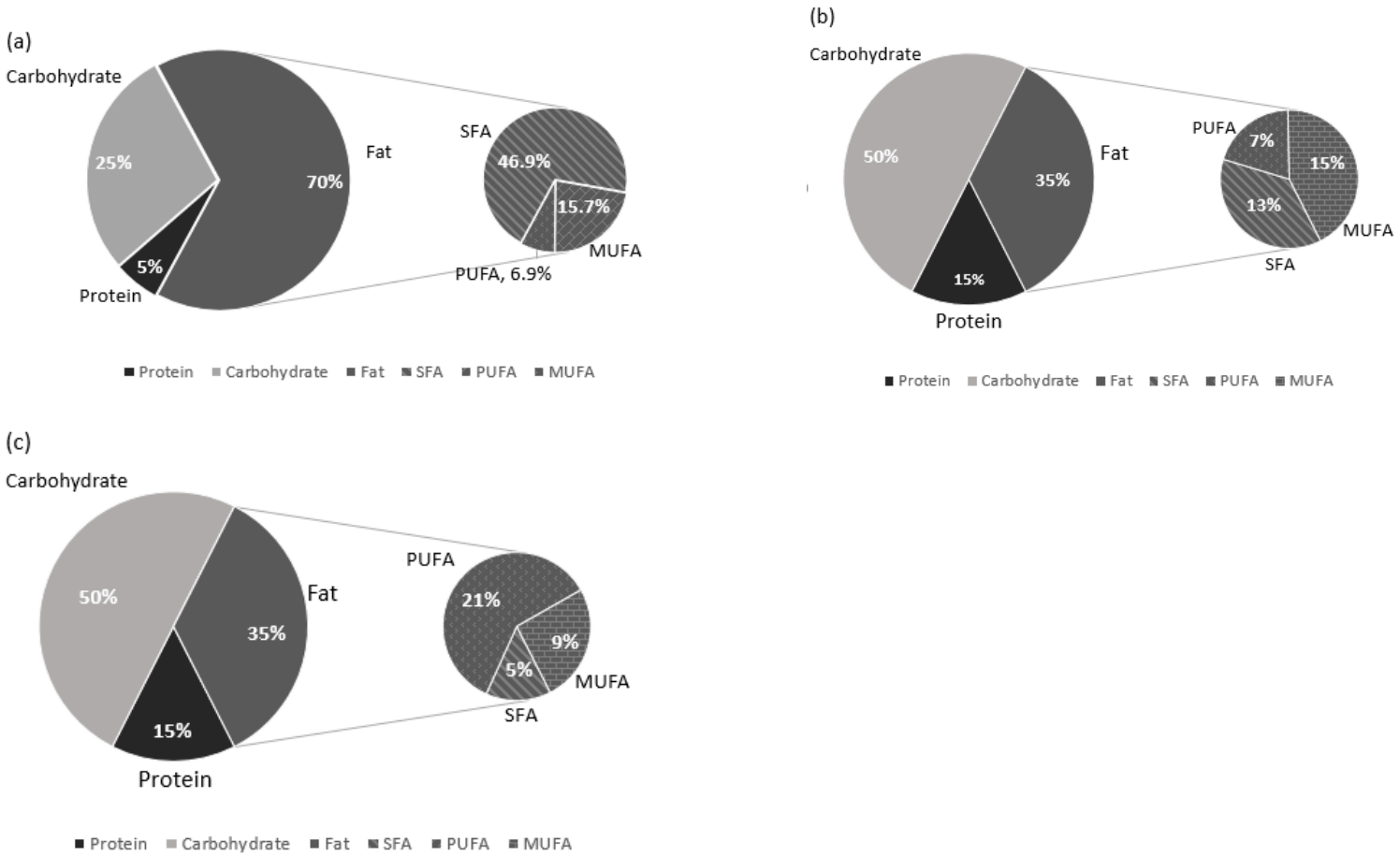


Figure 2. Meal and diet macronutrient breakdown. (a) SFA-rich HF meal composition. (b) Control and 3-day lead-in diet composition. (c) PUFA-rich diet composition

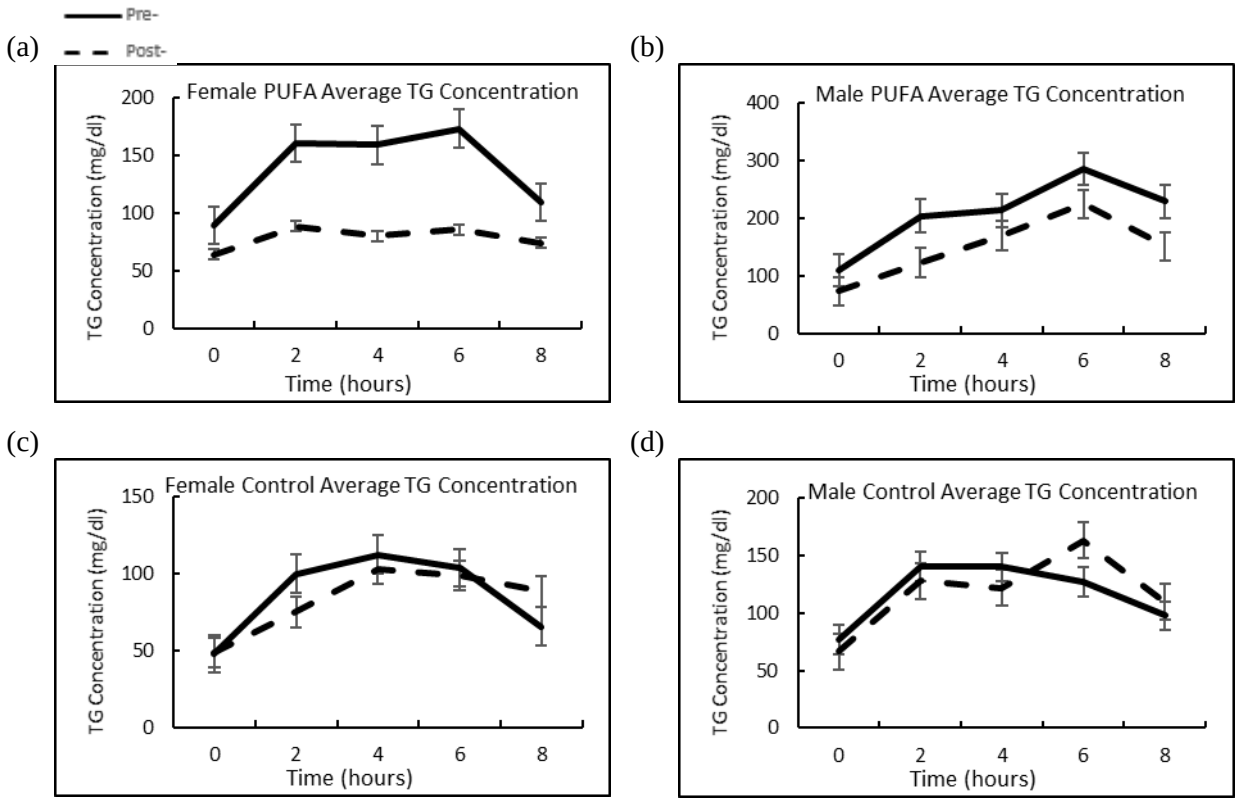
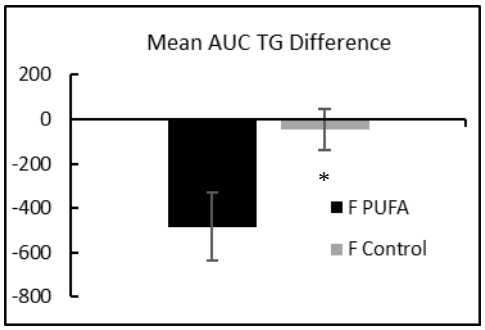
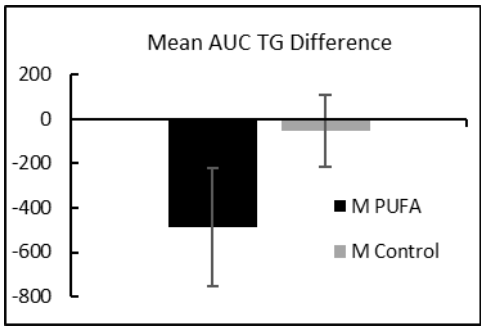


Figure 3. Time course of meal responses for TG in the PUFA-rich diet and the control diet from pre- to post-diet between males and females.

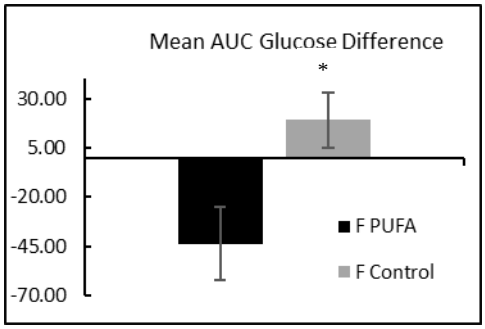
(a)


Mean AUC TG Difference
200
0
-200
-400
-600
-800
*
F PUFA
F Control

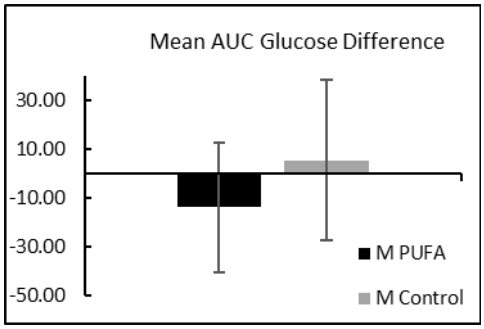
(b)


Mean AUC TG Difference
200
0
-200
-400
-600
-800
M PUFA
M Control

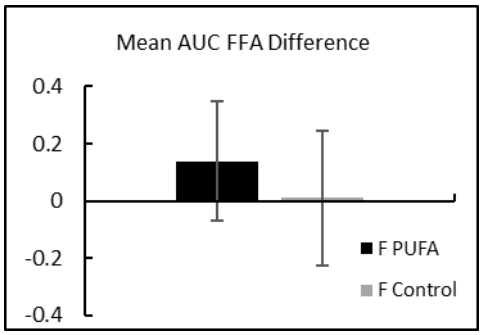
(c)


Mean AUC Glucose Difference
*
30.00
5.00
-20.00
-45.00
-70.00
F PUFA
F Control

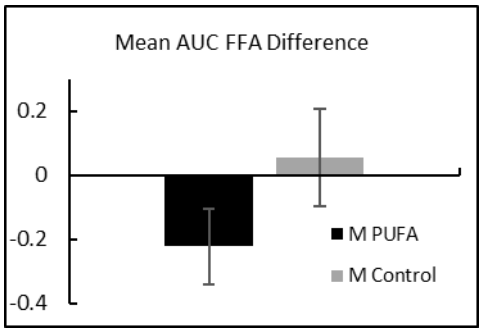
(d)


Mean AUC Glucose Difference
30.00
10.00
-10.00
-30.00
-50.00
M PUFA
M Control

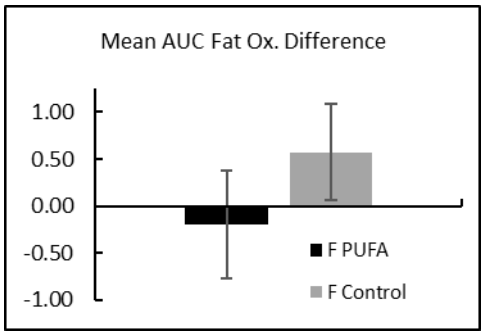
(e)


Mean AUC FFA Difference
0.4
0.2
0
-0.2
-0.4
F PUFA
F Control

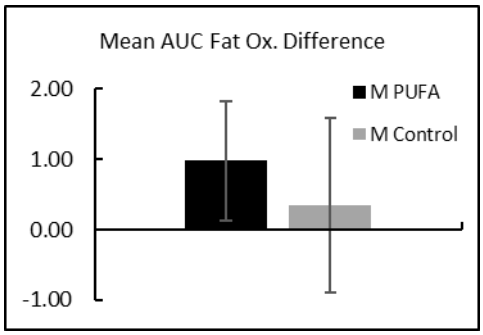
(f)


Mean AUC FFA Difference
0.2
0
-0.2
-0.4
M PUFA
M Control

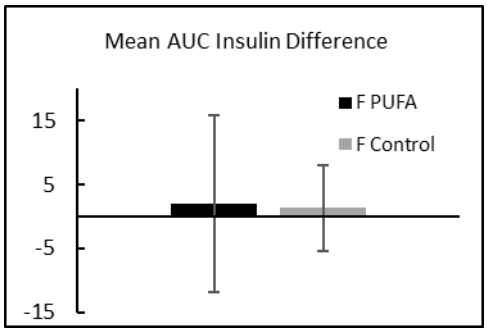
(g)


Mean AUC Fat Ox. Difference
1.00
0.50
0.00
-0.50
-1.00
F PUFA
F Control

(h)


Mean AUC Fat Ox. Difference
2.00
1.00
0.00
-1.00
M PUFA
M Control

(i)



(j)

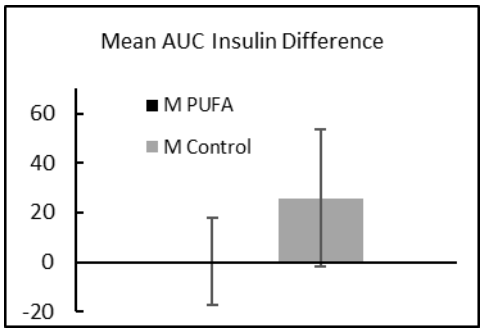


Figure 4. Mean AUC difference for TG, glucose, FFA, fat oxidation, and insulin in the PUFA-rich diet and the control diet groups between males and females. *AUC* area under the curve
*Indicates significant difference, $p<.05$