DOSE REPONSE EFFECTS OF COTTONSEED OIL CONSUMPTION ON CHRONIC DISEASE RISK FACTORS

By

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(Under the Direction of Jamie A. Cooper)

ABSTRACT

High doses of cottonseed oil (CSO) have exhibited therapeutic effects regarding blood lipids and appetite regulation; however, this resulted in overall dietary fat intake above the recommended levels. The objective of this 4-week outpatient intervention was to determine the impact of enriching diets with 10%, 20%, or 30% of energy from CSO vs. a control (CON) diet on cardiometabolic risk factors including blood lipid metabolism and appetite regulation in individuals at risk of cardiovascular disease (CVD). Participants were randomly assigned into one of three CSO groups: the LOW, MID, or HIGH group, or CON in which participants received 10% energy from an oil mixture. In manuscript #1 (chapter #3), enriching diets with 10%, 20%, and 30% CSO improved fasting blood lipids. In manuscript #2 (chapter #4), 10% and 20% doses improved postprandial subjective appetite. Altogether, 10%, 20%, and 30% doses of CSO improved cardiometabolic health in CVD at-risk adults.

INDEX WORDS: COTTONSEED OIL, BLOOD LIPIDS, CHOLESTEROL, TRIGLYCERIDES, APPETITE, CCK, PYY, GHRELIN

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DEDICATION

Thank you to my family for their endless support throughout my academic career. To my friends, thank you for cheering me on every day, checking in on me, and providing encouragement throughout my time at UGA. Joe, you eased my mind every day I needed it ─ thank you for your undoubted belief in my capabilities as a human being, researcher, and future dietitian. I would like to dedicate the work detailed in this document to the dietetic profession. The passion I have for the importance of dietetics and nutrition interventions has been the driving force behind every step of this journey.

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide, resulting in nearly 18 million deaths annually (1). The primary contributor to CVD is atherosclerosis (2), and known risk factors associated with the pathophysiological development of atherosclerosis are hypercholesterolemia and obesity (3). Currently, the primary clinical intervention for alleviating CVD risk is lowering blood lipids, specifically total cholesterol (TC), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-c) (4-6). Therefore, it is critical to develop interventions that target modifiable risk factors of CVD, such as diet, to mitigate the impact of this chronic condition.

Diet composition is known to impact the progression of CVD. Moreover, the fatty acid composition of the diet is an important modulator of health outcomes in individuals. Diets rich in saturated fatty acids (SFA) are associated with an increased risk of developing CVD (7-10), while higher unsaturated fatty acid consumption suggests the opposite (10-12). For example, consuming foods rich in polyunsaturated fatty acids (PUFAs), specifically linoleic acid (LA), have been shown to improve blood lipid markers (11, 12). Cottonseed oil (CSO), which is rich in PUFAs, and LA in particular, has exhibited therapeutic effects on lowering blood lipids in both healthy young adults (13) and those at risk for CVD based on elevated body mass index (BMI) or hypercholesterolemia (14). However, these findings have been observed with relatively high doses of CSO resulting in high total dietary fat intake. Specifically, consumption of ~30% of

energy as CSO has improved cholesterol profiles without worsening markers of inflammation or coagulation potential (15). Furthermore, CSO consumption has led to improvements in appetite regulation (16, 17), which may encourage energy balance regulation and weight maintenance, and by extension reduced CVD risk. However, the effect of CSO on markers of health, such as blood lipids, appetite measures, and inflammation in lower doses have not yet been evaluated.

The following literature review (Chapter 2) discusses current research related to chronic disease, cholesterol metabolism, influence of dietary fat on cardiometabolic risk factors, and dose-response studies. The aims of this study were to determine the impact of enriching diets with 10%, 20%, or 30% of CSO vs. a control (CON) diet in individuals at risk of CVD on blood lipids/lipid metabolism and appetite (Chapter #3 & #4, respectively). Our central hypothesis was that inclusion of daily consumption of CSO would improve health outcomes and cardiometabolic disease risk in those at-risk for CVD. For both manuscripts, the intervention was a 4-week partial outpatient feeding trial in which participants were randomized into one of four groups. The four groups included three CSO enriched diet groups consisting of either a LOW dose (10% of their energy needs from CSO), MID dose (20% of their energy needs from CSO), or a HIGH dose (30% of their energy needs from CSO). Lastly, the fourth group was a CON group that received 10% of their energy needs from a mixture of oils that matches the fatty acid composition of the U.S. diet.

Specific Aims and Hypotheses

- Aim 1: Compare the change in blood lipids and lipid metabolism from pre- to post-diet intervention between CSO dose groups and CON.
    - Hypothesis: In a dose-dependent manner, the CSO-enriched diets would improve postprandial (triglycerides and free fatty acids) and fasting blood lipid profiles

from pre- to post-intervention (decreases in TC, LDL-c, TGs, apolipoprotein B, and increases in high-density lipoprotein), and all will be different than the CON group.

- Aim 2: Assess the effects of different doses of CSO and a CON diet on markers of appetite regulation and energy balance.
    - Hypothesis: In a dose-dependent manner, the CSO-enriched diets would result in improved fasting and postprandial appetite regulation as evidenced by visual analog scales and greater satiety hormone responses (cholecystokinin (CCK) and peptide YY (PYY) and ghrelin suppression) from pre- to post-diet intervention, and all 3 groups will differ from control.

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CHAPTER 2

REVIEW OF THE LITERATURE

Chronic Disease – Cardiovascular Disease

Chronic disease describes long-term conditions that inhibit activities of daily living and require persistent medical attention (1). The conditions that encompass 80% of deaths from chronic disease are cardiovascular disease (CVD), cancers, chronic respiratory disease, and type 2 diabetes (2). In the United States, chronic disease is a significant factor in the 4.1 trillion-dollar annual healthcare costs that economically burden the country (1). Globally, the World Health Organization (WHO) reports that chronic disease is the leading cause of death, killing nearly 41 million people per year and contributing to 74% of deaths (2). Of these deaths, CVD is the primary contributor with 17.9 million deaths annually (2). Thus, chronic disease continues to impact the quality of life of many, with trends to only increase (3).

The risk factors that increase the prevalence of major chronic diseases, such as CVD, are classified as modifiable or non-modifiable risk factors (2). Modifiable risk factors include tobacco use, physical inactivity, unhealthy diet, and alcohol abuse (2). Metabolic risk factors known to increase the development of chronic disease are hypertension, obesity, hyperglycemia, and hyperlipidemia (2). The interplay of modifiable risk factors influencing metabolic risk factors is an integral element to consider when identifying behavioral modifications that will lead to therapeutic effects among those inflicted with chronic disease.

Cardiovascular disease encapsulates a series of conditions including heart disease, heart failure, stroke, and/or myocardial infarction (4). The predominant contributor to the pathophysiological development of CVD is atherosclerosis (4). Atherosclerosis is the accumulation of fatty materials in the innermost layer of the arteries that develops from hyperlipidemia and lipid oxidation (5, 6). Atherosclerosis develops in a series of physiological processes including fatty streak formation, atheroma formation, and finally atherosclerotic plaque formation (6). The fatty streak phase is initiated by endothelium dysfunction (7). The endothelium is a layer within the lining of the artery that provides a barrier to the blood and the arterial intima (5). In endothelium dysfunction, known vasodilators such as nitric oxide are reduced and, conversely, vasoconstrictors like angiotensin II increase (8). Moreover, oxidative stress and hypercholesterolemia are key drivers of endothelial dysfunction (8, 9). Thus, cholesterol is a key physiological component in the formation of atherosclerotic plaques, and consequently, the development of CVD (7). Therefore, it is important to address modifiable risk factors that will prevent the atherogenic process from further exacerbating CVD occurrence nationally and globally. Moreover, to understand the association between hyperlipidemia and CVD progression, an analysis of dietary fats and lipid metabolism will be necessary.

Dietary Fats

Fat is an abundant energy source within the diet, and it is essential in metabolism, gene expression, responsiveness of hormones, and regulators of production of certain biologically active substances (10, 11). These important functions contradict the general demonization of fat-rich foods in the diet that has persisted since the 1980s and spurred an influx of refined carbohydrate intake and avoidance of fat-rich foods to decrease chronic disease risk (10). Yet, it is well documented in the literature that dietary fat intake combined with health-promoting

factors, such as physical activity, nonsmoking, and weight management, are primary preventative factors in CVD (12, 13). Moreover, health implications are largely driven by the complexity of each dietary fat's composition which translates to diverse physiological effects. Dietary fats are classified based on their degree of saturation and length of the carbon chain (12, 14).

Structurally, fatty acids are differentiated by the length of their carbon chain. Short chain fatty acids (SCFA) are fatty acids with less than 6 carbons, medium chain fatty acids (MCFA) are fatty acids with 6-12 carbons, long chain fatty acids (LCFA) are fatty acids with more than 12 carbons (15). Furthermore, the presence of methyl branches will also influence the physicochemical behaviors of fatty acids. For example, non-branched SFA like palmitic acid and stearic acid will behave differently in the body than branched chain SFA like isopentadecanoic acid found in dairy and beef (16). These distinctions amongst fatty acids will lead to variances in absorption, transport, and terminus in the body (17). To explain, MCFA are more efficiently absorbed into the gastrointestinal (GI) tract and taken directly to the liver to be oxidized, whereas LCFAs are encased in chylomicrons that are circulated through the lymphatic system leading to greater adipose tissue uptake (17).

Saturated Fat

Biochemically, fatty acids are long, aliphatic, hydrocarbon chains, commonly 4-28 carbons in length ending in carboxyl acid groups (15). The saturation status of a fatty acid is dependent on the double bonds within the carbon chain (18). Thus, saturated fatty acids (SFA) are single-bonded hydrocarbon chains that have no interactions that result in double bonds or the addition of functional groups. Moreover, saturated fat is a hypernym of several saturated fatty acids; the most common in the Western diet being palmitic acid, myristic acid, and stearic acid

(17, 19). Exogenous saturated fat sources consist of plant sources such as palm oil and coconut oil and animal sources like butter and lard. Typically, saturated fats are solid at room temperature (11) because of their saturation of hydrogen molecules, which allows fatty acids to be packed very tightly together. The 2020 Dietary Guidelines for Americans suggest saturated fat should encompass less than 10% of total daily calories, some agencies like the National Lipid Association Expert Panel recommend less than 7% of daily from SFA (14, 17). Despite this, the average American consumes 10.7% of their energy from SFA, exceeding recommendations (17).

The differences in metabolic processes among SFA serve as important modulators of CVD risk. Furthermore, the role of SFA in gene expression and, consequently, lipid metabolism works to explain their significance in CVD progression. Saturated fatty acids regulate genes involved in the metabolism and synthesis of cholesterol, fatty acids, triglycerides (TG), low-density lipoprotein (LDL) receptors, and fatty acid synthase (11). An explanation of these mechanisms will be discussed further in the lipid metabolism portion.

Despite their role in CVD progression, SFAs are essential for significant structural contribution to cell membranes and cell signaling domains and molecules such as lipid rafts and ceramides, respectively (11). Additionally, myristic acid and palmitic acid have the ability to covalently modify proteins required for cell signaling (11). Thus, SFA are modulators of normal cell functioning and metabolism, yet the literature suggests that there is a relationship between higher SFA intake and CVD risk (12, 17, 19).

Unsaturated Fat

While saturated fats are typically solid at room temperature, exogenous sources of unsaturated fats are liquid at room temperature and are found in foods such as vegetable and seed oils, seafood, nuts, and seeds (12). Unsaturated fatty acids are distinct from SFA in their

interactions among carbon atoms resulting in one or more double bonds between carbon atoms that allow the addition of an alkenyl functional group to the fatty acid (15, 18). Moreover, unsaturated fatty acids vary by configuration as either cis or trans. A cis conformation is described as neighboring hydrogen atoms on the same side of the double bond, while trans configuration describes hydrogen atoms being on the opposing side of the double bond (15). Therefore, cis unsaturated fatty acids are generally more bent and kinked while trans fatty acids are similar in shape to the linear SFA as previously described (15). Cis conformations are more naturally occurring in fatty acids while trans configurations are most commonly a result of hydrogenation by human processing (15). Unsaturated fats are further classified by the number of double bonds within them; monounsaturated fatty acids (MUFAs) contain one double bond and polyunsaturated fatty acids (PUFAs) contain more than one double bond (18). Currently, the Institute of Medicine suggests a macronutrient range of 20-35% of energy needs coming from fat with a majority of intake from PUFAs and MUFAs and less than 10% from saturated fat (20).

Monounsaturated Fat

The most prevalent MUFAs in exogenous sources are oleic acid (18:1n-3, OA), palmitoleic acid (16:1n-7, PO), and vaccenic acid (21). Specifically, OA represents almost 90% of MUFA intake from dietary sources as it is abundant in commonly used plant oils like olive oil (11, 22). Additional foods with high MUFA content are canola oil, peanut oil, safflower oil, nuts, avocados, animal fats, and seeds (22). Consuming MUFAs has gained popularity in recent years due to their predominance in the Mediterranean dietary pattern which has been noted to decrease chronic disease risk (22). Moreover, OA and PO can be synthesized endogenously, and they are key components of cell membrane phospholipids (11). Although OA is often promoted as "anti-inflammatory," the research illustrates limited evidence for profound anti-inflammatory effects

(11). For example, the professed anti-inflammatory properties of olive oil have been proposed to be largely exhibited through its polyphenol content rather than its OA content (11). Furthermore, the role of MUFA on reducing CVD has been debated in the literature. While several meta-analyses and prospective studies observed no significant correlation between MUFA intake and reduced CVD prevalence (11, 23), Schwingshckl et al observed that a higher intake of OA was associated with lower risk of CVD in a systematic review of cohort studies (21). This conversation expands with the addition of PUFA intake in the diet and its role in disease prevention and progression.

Polyunsaturated Fat

Unsaturated fats are defined not only by the number of their double bonds but also by the location of desaturation on the carbon chain (18). Polyunsaturated fatty acids are further classified by the omega notation which illustrates the number of carbons from the methyl end of the aliphatic chain where the double bond is located (18). Therefore, omega-3 (n-3) and omega-6 (n-6) fatty acids implement this nomenclature system. The precursors of n-6 and n-3 PUFAs are linoleic acid (18:2n-6, LA) and alpha-linolenic acid (ALA), respectively (15, 18). These fatty acids are deemed essential because humans lack the ability to synthesize them de novo by the inability to introduce a double bond at the n-3 and n-6 positions of the fatty acid (15, 18). Thus, humans must consume ALA and LA exogenously. The adequate intake (AI) intake for ALA is 1.6g and 1.1g for adult men and women, respectively (24), and with insufficient levels, dermatitis and cutaneous inflammation can occur (25). The LA intake recommendations are represented by a range of 11-12g a day for men and 14-17g a day for women (26). It is important to note that an insufficient amount of dose-response data related to LA's intake recommendations makes it difficult to detail if the specified amounts are physiologically

beneficial or if they need to be modified (26). Dietary sources of n-6 PUFAs are cottonseed oil, safflower oil, and other vegetable oils while exogenous sources rich in n-3 PUFA are some tree nuts, seeds, and fatty fish (11). Many western countries, such as the United States, have made a shift to replace more SFAs, such as butter, with vegetable oils for culinary purposes which in turn leads to greater intake of LA in this population (11). With this shift in vegetable oil use, American women consume 12.6g of LA a day and men consume 16.0g of LA per day on average, this equates to 5.5% and 6.0% of energy, respectively (27).

The essential n-3 fatty acid, ALA, can be converted to eicosatetraenoic acid (EPA; 20:5n-3) and then to docosapentaenoic acid (DPA; 22:5n-3) and docosahexanoic acid (DHA; 22:6n-3) (11). Linoleic acid serves as a precursor for gamma-linolenic acid (GLA), arachidonic acid (AA, 20:4n-60), and dihomo-gamma-linolenic acid (DGLA, 20:3n-6). These fatty acids serve as instrumental constituents of cellular membranes and ceramides in the skin (11). Specifically, AA functions in brain function and development, cell signaling, and has pro-inflammatory components from its action in the nuclear factor-kB (NF-kB) pathway, a transcriptional regulator of inflammatory gene activation (28). When stimuli activate toll-like receptor 4 (TLR4), this allows cLPA2 enzyme to cleave AA from membrane phospholipids (28). This free AA can then serve as a substrate for eicosanoids like prostaglandins enzymes, COX-1 and COX-2 (11, 28). From COX-1 and COX-2 activity, AA is reduced to PGH2 which is converted to various prostaglandins (28). Furthermore, from the cLPA2 cleavage, AA can be converted to 5-hydroperoxyeicosatetraemoic acid (5-HPETE) with arachidonate-5-lipoxygenase (5LOX) (28). Then, 5-HPETE is an intermediate that is acted on by leukotriene C4 synthase and LTA4 hydrolase to produce leukotriene C4 and leukotriene B4, respectively (28). Thus, leukotriene (LT) and prostaglandin (PG) levels in the blood are dictated by the availability of AA (28).

These various eicosanoids, with the addition of thromboxane, are instrumental in biological processes like inflammation, immune response, platelet aggregation, renal function, and tenor cell proliferation (11, 28). These mediators also act in a homeostatic way often having opposing effects to one another (11). For example, PG E2 has many proinflammatory properties, but through its induction of 15-lipoxygenase to produce lipoxin A4, this acts to resolve inflammation (11). Interestingly, n-3 PUFAs exhibit anti-inflammatory effects in animal models and cultures by inhibiting NF-kB binding to TNF-α-specific consensus sequence; this consequently inhibits the NF-kB signal cascade (28).

Blood Lipid Metabolism

Exogenous Lipid Metabolism

The primary goal of exogenous lipid metabolism is to deliver fatty acids to adipocytes and muscle cells, and other tissues to be used for energy or storage (29). The majority of exogenous lipids in the diet are in the form of triglycerides (TG), in which three different fatty acids, PUFA, MUFA, and/or SFA are esterified to a glycerol backbone (30). Other forms of dietary lipids include phospholipids and cholesterol esters (31). Metabolism of exogenous lipids begins in the small intestine where intestinal lipases hydrolyze dietary TGs into free fatty acids (FFAs) and monoacylglycerol (MAG) (29). In the same way, phospholipids are hydrolyzed into FFA and lysophospholipids, and cholesterol esters are hydrolyzed into free cholesterol and a FFA (31). Protein mediated transport of cholesterol and plant sterols into the intestinal cell occurs via the Niemann-Pick C1-like 1 (NPC1L1) transporter on the brush border of the enterocyte (29, 31).

Bile acids, plant sterols, cholesterol, and fat-soluble vitamins emulsify FFA and MAG to form micelles (29). Passive diffusion of FFA into the enterocyte occurs when FFA concentrations in the lumen exceed that of the enterocyte (31). Fatty acid transporters, CD36 and fatty acid transport protein 4 (FATP4), are available for protein-mediated transport of FFA into the enterocyte (29). Once in the enterocyte, FFA and MAG go to the endoplasmic reticulum (ER) where they are acted upon by monoacylglycerol acyltransferase (MGAT) and diacylglycerol transferase (DGAT) in the biosynthesis of TG (29). With MGAT, FFAs are esterified to form diglycerols (DAG) which are in turn acted upon by DGAT to convert DAG to TG (29, 31). Similarly, free cholesterol brought to the ER is esterified by membrane-bound acyl-CoA: cholesterol acyltransferases (31).

The synthesized lipids in the ER can be stored in enterocytes as cytosolic lipid droplets (31). Cytosolic lipid droplets are large spherical particles with a core rich in TG and cholesterol esters surrounded by a monolayer of phospholipids, cholesterol, and proteins (31, 32). During the fasting state, cytosolic lipid droplets are mobilized and secreted into the enterocyte (31). This mobilization of fat may account for the state of hypertriglyceridemia observed just before meal ingestion (31). Additionally, lipid droplet's vital role in fat storage during consumption, and their subsequent mobilization suggests that they are key in lipid absorption and the provision of sustained lipid levels while fasting (31).

In the ER, the newly synthesized cholesterol esters and TG can also be packaged into large lipoproteins called chylomicrons (31). Thus, the core of the chylomicron is rich in TG and cholesterol esters covered by a phospholipid monolayer and free cholesterol, surrounded by a large apolipoprotein B48 (apoB48) (31). Microsomal triglyceride transfer protein (MTP) is the regulator of apoB28 synthesis that is necessary for chylomicron formation (29, 31). MTP is

essential in transferring TGs to apoB48 to expand its core with newly synthesized TG (29, 31). Chylomicrons are delivered to the Golgi where further modifications are made including the addition of different apolipoproteins (29, 31). The apolipoproteins associated with chylomicrons include Apo A-I, Apo A-II, Apo A-IV, Apo A-V, Apo B-48, Apo C-I, Apo C-II, Apo C-III, and Apo E (29).

Apolipoproteins are essential contributors to lipoprotein metabolism in their key roles in the structure and synthesis of lipoproteins, acting as ligands for lipoprotein receptors, and activating or inhibiting enzymes required in lipoprotein metabolism (29). Overall, apolipoproteins enable the transport and distribution of lipids among cells and tissues throughout the body (33). After modification in the Golgi, chylomicrons are sent out of the intestinal cell into the lymph and enter the circulatory system via the thoracic duct (29, 31).

Lipid Distribution to Tissues

Lipoprotein lipase (LPL) is produced in the myocytes and adipocytes and is transferred to the luminal surface of the capillary endothelial cell by lipase maturation factor 1 (29). LPL is then bound and anchored to the capillary endothelium by the action of glycosylphosphatidylinositol anchored high-density lipoprotein (HDL) binding protein 1 (44). Apo C-II on the surface of chylomicrons activates LPL which initiates hydrolysis of TG within the chylomicron that results in FFAs that are available for uptake by the myocytes and adipocytes (44). This FFA transport into myocytes and adipocytes is enabled by FATPs and CD36 (44). Some FFA released from the hydrolysis of TG bind to albumin to be delivered to other tissues (44). Additionally, other important regulators of this process include Apo AV which activates LPL, and Apo C-II which inhibits LPL activity along with angiopoietin-like protein 3 and 4 (44). With the activity of LPL, the chylomicron size is reduced from the hydrolysis of TG

within its core, the result is chylomicron remnants (44). The lost Apo C-II and phospholipids from chylomicrons are transferred to HDL (44). Consequentially, this transfer of Apo C-II downregulates LPL thus decreasing TG hydrolysis (44).

The resulting chylomicron remnants are abundant in cholesterol esters with an affixed Apo E on their surface (44). Apo E is important in recognition by hepatocytes that absorb chylomicron remnants via LDL receptors, LRP-1, and syndecan-4 (29, 34). If mutations exist in Apo E, the rate of chylomicron clearance decreases resulting in elevations in cholesterol and TG levels (44). In the liver, cholesterol and TG are transferred in the formation of very low-density lipoprotein (VLDL) which will initiate the lipoprotein pathway for distribution of lipids and cholesterol to tissues (44).

The ER in the liver synthesizes Apo B-100 to aid in the formation of VLDL (44). An abundance of TG will promote the protection of newly synthesized Apo B-100 and impact the size of VLDL (44). The greater the supply of TG in the liver, the greater the size of the VLDL particles (44). Therefore, the hepatic lipoprotein pathway is largely regulated by the availability of fatty acids from de novo fatty acid synthesis, uptake of lipoproteins ample in TG, and fatty acids from adipose tissue sent to the liver (44).

Similar to the chylomicron, VLDL enters the circulatory system and delivers fatty acids to tissues through LPL activity that hydrolyzes TG which results in competition between VLDL and chylomicrons for LPL (44). From TG removal from VLDL, a remnant abundant in cholesterol esters remains, called intermediate density lipoprotein IDL (44). Like VLDL, IDL contains Apo B-100, but they differ in greater abundance of cholesterol and their procurement of Apo E from HDL (29, 35). In a similar pathway to chylomicrons, IDL is drawn out of the circulation through the binding of Apo E to LDL and LRP receptors (35). Next, hepatic lipase

hydrolyzes the lingering TG in IDL and VLDL, formulating low-density lipoproteins (LDL) predominantly made of cholesterol and Apo B-100 (29, 35).

LDL particles vary by size which will influence their atherogenic impact (35). For example, small dense LDL particles exhibit a stronger association with hypertriglyceridemia, low HDL levels, and inflammation (35). These effects are attributed to their decreased affinity for LDL receptors which increases their time in the bloodstream and their susceptibility to being caught in the arterial wall and oxidized (35). Therefore, small dense LDLs exhibit far more pro-atherogenic properties than large LDL (35). Bowden et al. suggest that LDL size might play more of a contributing role in predicting CVD than LDL number alone (36). Conversely, a prospective nested case-control study evaluated the usefulness of LDL number to size and found LDL concentration is the stronger indicator of managing CVD risk evaluation (37). Other perspectives maintain that more research will need to be put into these predictive tools to determine their relevance in a clinical setting (38).

Another LDL particle is Lipoprotein (a) (Lp(a)). This is an LDL particle with an apolipoprotein (a) bonded to Apo B-100 on the surface (35). Although less understood, an association between elevated Lp (a) and increased CVD pervasiveness exists (39). Independently, Lp (a) stands as a risk factor for CVD, but the physiological mechanism is not clear from animal or human studies (40). Research has indicated that a general reduction in LDL levels will result in decreased Lp (a) as well (39, 40).

Regulation of LDL Production

LDL formation is primarily regulated by the availability and quantity of hepatic LDL receptors; therefore, LDL receptors are the largest determinant of plasma LDL concentrations

(29). Moreover, with ample LDL receptor activity and quantity, hepatic clearance of LDL increases thus, decreasing plasma LDL levels (29). Furthermore, hepatic cholesterol levels are the key regulatory factor in the number of hepatic LDL receptors by modulating sterol regulatory element-binding proteins (SREBPs) (29). Specifically, SREBPs are transcription factors involved in the stimulation of LDL receptor production and other important lipid homeostatic processes (29, 41). Additionally, SREBP is found in 3 isoforms: SREBP-1a, SREBP-1c, and SREBP-2, which will impact various enzymes involved in lipid metabolism (41).

Primarily, SREBP-1c and SREBP-2 function in the liver to impact fatty acid synthesis and cholesterol synthesis, respectively (41). Moreover, SREBP-2 is the isoform largely responsible for the activation of the LDL receptor gene (42). These transcription factors are formulated as membrane-bound proteins in the endoplasmic reticulum (43). When hepatic cholesterol levels deplete, this drives the transport of SREBPs to the Golgi which processes them into soluble fragments that move to the nucleus (58). In the nucleus, transcription is activated and encodes for regulatory enzymes such as HMG-CoA and the LDL receptor (58). However, when hepatic LDL concentrations are high, this consequentially blocks the transport of SREBPs to the Golgi, inhibiting the synthesis of LDL receptors (29, 43) This acts as a compensatory mechanism to prevent excess cholesterol accumulation in the liver (43). Therefore, SREBPs combatant effects on lipoprotein levels represents their homeostatic function in relation to lipid metabolism.

Lastly, PCSK9 is a protein responsible for the degradation of LDL receptor activity in the lysosomes (29). Therefore, reduced PCSK9 activity inhibits LDL receptor degradation, thus increasing LDL receptor availability (29). Accordingly, inhibition of PCSK9 will decrease LDL concentration (29).

Reverse Cholesterol Transport and HDL Synthesis

Reverse cholesterol transport (RCT) is the movement of excess cholesterol from the peripheral tissues back to the liver for excretion of redistribution. The central lipoprotein involved in this process is HDL (44). HDL's integral structural component is Apo A-1, so its synthesis begins the cascade of HDL formation (29). The other apolipoproteins associated with HDL are apo A-II, Apo CI-III, and Apo E (35). The secreted cholesterol and phospholipid from the hepatocytes and enterocytes are picked up from Apo A-1 which forms pre-beta-HDL (29). ATP-binging cassette protein A1 (ABCA1) is the primary facilitator of cholesterol and phospholipid efflux to lipid-poor HDL through Apo A-1 acceptance (29, 45). Therefore, mutations in ABCA1 can lead to low HDL levels since there is an inability to add cholesterol to Apo A-1 (29). In addition, another pathway exists by ABCG1 that mediates the transfer of lipids to mature HDL (45). A subset of HDL exists that is composed of about 20% Apo A-II, a structural protein in about two-thirds of HDL in the body secreted by the liver (45).

The metabolism of chylomicrons and VLDL initiates the transfer of cholesterol and apolipoproteins to HDL which is mediated by phospholipid transfer protein (PLTP) (44). This mechanism explains the theory that low TG metabolism results in low HDL levels; low HDL levels (<40mg/dL) are observed as a risk factor for CVD (29, 45). Additionally, high HDL levels (>50 mg/dL) are regarded as cardioprotective and aid in the prevention of atherosclerosis by promoting RCT (45).

When HDL obtains cholesterol and phospholipids, they reside merely on the surface of the lipoprotein (29). To be moved into the core of HDL, free cholesterol must be esterified, and lecithin: cholesterol acyltransferase (LCAT) is the catalyst of this reaction (29). In this, Apo A-1

stimulates LCAT's transfer of a fatty acid from a phospholipid to free cholesterol (29, 45). The resulting cholesterol esters are translocated into the core of the now-mature HDL (29).

The key regulator of RCT is an increase in the cholesterol efflux pathway (46). The main cholesterol transporters to HDL, ABCA1 and ABCG1, regulate intracellular cholesterol levels by efflux to maintain homeostasis (46). When intracellular cholesterol concentration is high, liver X receptors (LXRs) will drive the expression of ABCA1 and ABCG1 to efflux cholesterol (46). Additionally, SREBP activity will decrease leading to reduced cholesterol synthesis and absorption by inhibiting the expression of low-density lipoprotein receptor (LDLR) and HMG-CoA reductase (46). Small microRNA, specifically, miR-33, is located within SREBP-2 and mediates fatty acid metabolism by targeting SREBP-2 activation (47). The SREBP-2 gene selects for ABCA1 And ABCG1 mRNA for degradation (29). Moreover, an increase in cellular cholesterol decreases miR-33 level, inhibits SREBP-2 action, and results in increased expression of ABCA1 and apoA1 receptors (29, 47).

The pathways described above drive RCT initiation and regulate HDL metabolism. Once HDL uptakes cholesterol from peripheral tissues, it can be brought back to the liver via one of two routes: direct or indirect (29, 45, 46). Scavenger receptor class BI (SR-BI) is the promoter of direct RCT; a process in which it selectively uptakes both free and esterified cholesterol into the liver without apolipoprotein degradation (45). In this process, SR-BI allows for absorption of the whole HDL particle, removes cholesterol, and excretes lipid-poor HDL (45). The indirect pathway is modulated by cholesterol ester transfer protein (CETP) which mediates the pathway for TG transfer for ApoB-containing lipoproteins and HDL cholesterol esters (29, 45). Those with lessened CETP activity have high HDL levels, large HDL, and decreased LDL cholesterol

(29). Therefore, a therapeutic target for CVD has been CETP inhibition, but has not been proven to improve outcomes consistently in clinical studies (46).

From this process, the cholesterol-rich apoB-containing lipoproteins (VLDL/LDL) are taken up via the LDLR on the hepatocytes (29, 45). The TGs relocated to HDL are hydrolyzed by hepatic lipase to produce small HDL, release of Apo A-I, and subsequent increased Apo A-1 degradation (29). Moreover, endothelial lipase (EL) has been observed to have preference for phospholipase activity in association with HDL compared to TG-rich lipoproteins (45). Therefore, those with increased plasma EL exhibit low HDL levels and symptoms of metabolic syndrome (45). This mechanism accounts for the low HDL concentration noticed in insulin resistance (45).

ApoA-I catabolism mechanisms are important for determining apoA-1 and HDL levels in the plasma (45). Apo A-I has two catabolic pathways through the kidney or liver (29, 45). In the kidney, cubilin, a protein in the proximal renal tubular cells, binds Apo A-I and HDL. This complex binds with megalin to facilitate uptake and degradation of Apo A-I in the renal tubular cell (29, 45). Hepatic catabolism of Apo A-I is less understood but suggested to involve Apo E (29, 45). The subset of HDL with Apo E binds to LDLR for degradation; this mechanism also contributes to the hepatic uptake of HDL (29, 45).

The cholesterol delivered to the liver is eliminated from the body through excretion into the bile, which travels to the intestinal cells and can then be excreted in feces (29, 45, 46). There are multiple routes for secretion into the bile, in the first, ABCG5 and ABCG8 mediate the transfer of cholesterol into the bile for excretion from the body (44, 46). Stimulation of LXR increases the expression of ABCG5 and ABCG8, therefore, an influx of hepatic cholesterol

levels will consequently activate LXR, and ultimately enhance secretion of bile acid (29). Secondly, cholesterol can be directly converted into bile acids and released into bile (29).

Endogenous Cholesterol Synthesis

To maintain cholesterol homeostasis, the body can synthesize cholesterol endogenously in a series of enzymatic reactions mainly in the liver, followed by the intestines and skin (48). Cholesterol synthesis occurs in two phases, the first is the reaction of isoprenoid to squalene, and second, is the cyclization of squalene to yield lanosterol (48, 49). To begin this pathway, acetyl coenzyme A (acetyl-CoA), a metabolite of pyruvic acid decarboxylation of beta-oxidation of fatty acids, condenses with acetoacetyl-CoA (48, 49). This reaction is catalyzed by enzyme 3-hydrxy-3-methylglutaryl-coenzyme A synthase (HMG CoA synthase) to form 3-hydroxyl-3-methylglutaryl CoA (HMG-CoA) (48-50). Through a reduction reaction, HMG-CoA is converted to mevalonate with the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (48-50). This step is often referred to as the rate-limiting, governing step in cholesterol synthesis (48-50). Therefore, this is the target for various lipid-lowering medications such as statins (48, 49). Next, mevalonate is phosphorylated into isopentenyl pyrophosphate (IPP); IPP is polymerized into farnesyl pyrophosphate (FPP) (48, 50). From the condensation of two FPP molecules, squalene is produced (50). In another rate-limiting step, squalene monooxygenase (SM) acts on squalene to form lanosterol (50, 51). Lanosterol will be converted into cholesterol in a series of reactions on the ER membrane (50, 51). The newly formed cholesterol will be rapidly distributed to cellular membranes and organelles (50). Furthermore, cholesterol will be utilized throughout the body as it is the precursor of steroid hormones, bile acids, and oxysterols in specialized tissues (50).

Regulation of Cholesterol Synthesis

To sustain cholesterol homeostasis, feedback regulation is utilized to target the expression of key proteins (49, 50). Among these proteins is the SREBP-2 cleavage activating protein (SCAP), part of an important complex: the SCAP-SREBP-2 complex (49, 50). An increase in the intracellular concentration of cholesterol stimulates the SCAP-SREBP-2 complex in the ER (49, 50). When cholesterol concentrations are low within the cell, SCAP will act as a transporter of SREBP-2 from the ER to the Golgi (49). In the Golgi, the complex is split, allowing SREBP-2 to translocate to the nucleus and activate transcription factors to enhance cholesterol synthesis (49, 50). Other factors that influence endogenous cholesterol synthesis include genetics, body weight, hormones, diet, gut microbiome, circadian rhythm, as well as external factors like environment and drug treatments (49).

There are a multitude of dietary components and behaviors that will influence cholesterol biosynthesis (49). For example, increased meal frequency has been observed to reduce cholesterol synthesis (49). Importantly, dietary fat intake has been a primary contributor to cholesterol homeostasis (49). For instance, a diet enriched in SFA will impact cholesterol synthesis differently than a PUFA or MUFA-enriched diet (49). Nevertheless, it will be important to understand that an imbalance of cholesterol regulation will impact disease risk.

Atherosclerosis

Alterations in cholesterol metabolism and homeostasis influence atherosclerosis. Initially, atherosclerotic lesion formation occurs due to endothelial dysfunction where lesions occur in areas with disturbed blood flow (7). At a lesion site, small LDLs are readily retained by proteoglycans within the arterial wall to form aggregates (5, 7, 35). Other lipoproteins, including

VLDL and apoE remnants, are trapped in the subendothelial space (7). The trapped LDL is oxidized which subsequently promotes endothelial cell activation (6, 7). Moreover, the entrapment of apoB-containing lipoproteins in the arterial wall is considered the critical initial step in the atherogenic process (52).

Endothelial activation drives monocyte interaction with adhesion molecules and chemoattractant cytokines (MCP-1), which increases the translocation of monocytes into the vascular lesion (6, 7). The internalized monocytes evolve into macrophages that have receptors to enable the incorporation of VLDL, apoE remnants, and oxidized LDL to form foam cells and secrete pro-inflammatory mediators (5-7, 44). Furthermore, leukocytes and T cells enter the arterial intima which influences smooth muscle cells and endothelial function (5-7). The accumulation of leukocytes regulates modulators that guide smooth muscle cells into the intima (5-7). The resulting atherosclerotic lesion progresses into an atherosclerotic plaque as more macrophages, smooth muscle cells, and LDL particles are recruited to the intima, and cellular apoptosis occurs, thus resulting in a necrotic core (5-7). Smooth muscle cells work to provide a barrier to other aggregates, but unresolved inflammation impairs collagen synthesis leading to a thinning of the fibrous cap (5-7). Moreover, atherogenic plaques held under thin fibrous caps lend themselves to rupture (5-7). Thrombosis, which is blood clot formation, results in cardiovascular events, such as stroke, because of its ability to occlude the blood vessel (5-7).

Currently, LDL is the primary targeted atherogenic lipoprotein in CVD management (53). This is due to the correlation of high serum LDL, specifically small dense LDL, with an increased risk of CVD (53). Moreover, modified LDL (oxidized LDL) is the predominant facilitator of endothelial dysfunction, macrophage acquisition, thromboxane release, and death of

smooth muscle and endothelial cells (52). Therefore, LDL is a significant contributory factor in the pathophysiological atherogenic process and key therapeutic target (52).

Conversely, HDL, in the process of RCT, is regarded as protective against atherosclerosis (45). Specifically, the efflux of cholesterol from foam cells in atherosclerotic plaques is accepted by HDL in RCT to be ultimately eliminated through the feces (44). Therefore, HDL's antiatherogenic effects are evident and remain an important factor in the prevention of CVD (44, 45).

Impact of Dietary Fats on Blood Lipids and Cholesterol

Saturated Fat

Despite their structural and physiological function, SFA has been a target in combating CVD because of SFA's ability to negatively impact lipid profiles (17). Generally, SFA increases LDL when compared to other nutrients (17). Moreover, the variances in structure with respect to chain length (SCFA, MCFA, and LCFA), will also influence the mechanisms of absorption, transport, and cellular destination which affects blood lipid outcomes(17). Studies have shown that saturated MCFA has more metabolically favorable outcomes than saturated LCFA including myristic and palmitic acid (17). Conversely, the LCFA stearic acid has not elicited the same negative lipid response due to its ability to desaturate into oleate acid which has not been shown to raise LDL cholesterol and has little effect on HDL cholesterol (17).

After high-fat meal intake, postprandial lipemia occurs, a state in which blood TG concentration increases. Systematic reviews have observed that acute high SFA intake (>32g SFA per meal) will exacerbate the postprandial lipemic response which increases risk of CVD (54, 55). Multiple mechanisms interplay to elicit this hyperlipemia response, including activation

of transcription factors to promote lipogenic gene expression (56). In animal models, high SFA feeding upregulates expression of PGC-1β which coactivates the SREBP-1a, SREBP-1c, and LXR transcription factors; thus, inducing cholesterol synthesis and lipid mobilization in the form of VLDL (56). Because of apparent contributions of SFA consumption on CVD risk, scientists and clinicians have suggested SFA intake be replaced by unsaturated fat for cardioprotective benefits (12, 17, 54, 57).

Monounsaturated Fat

Unsaturated fat has been regarded for its beneficial effects on blood lipids through their inhibition of cholesterol synthesis, reductions in LDL and total cholesterol to HDL (TC:HDL) ratio, and increasing hepatic LDL receptor expression (12, 54, 55). Monounsaturated fat has gained recent popularity with its connection to the Mediterranean diet (54, 57). Specifically, oleic acid, found in olive oil is a primary component of the Mediterranean diet which is associated with low risk of CVD (54). Moreover, studies have observed meals high in MUFAs elicit a similar postprandial TG response to SFA, but a quicker clearance of TG to return to homeostatic conditions (54, 55).

Monounsaturated fat also exhibits preventative mechanisms against LDL oxidation, a trademark in the atherosclerotic process (58). Due to their low tendency for lipid peroxidation, their stability lends itself to higher clearance since LDLRs have a low affinity for oxidized LDL (58). Furthermore, in studies where SFA were replaced by MUFAs a shift from small, dense LDL to large, buoyant chylomicrons, VLDL, and LDL was noticed (54). Despite these benefits, meta-analyses have suggested that MUFAs display modest TC and LDL lowering effects compared to PUFAs, and their effect on HDL is inconsistent between studies (11, 12, 57).

Polyunsaturated Fat

The targeted intervention for the prevention of CVD is LDL reduction, and PUFAs, specifically LA, have been recommended in replacement of SFA in the diet compared to MUFAs from randomized control trials which exemplified greater reductions in LDL (11, 57, 59). Evidence suggests that MUFAs exhibit less lipid- lowering effects than PUFAs (17, 57). For example, PUFA-rich meals elicit a lesser TG response than MUFA rich meals (55). Linoleic acid has been largely observed to lower LDL concentrations, decreasing atherogenesis (11). Moreover, LA reacts with SREBPs to favor hepatic clearance of circulating LDL by upregulating LDL receptor gene and protein expression (11, 60). Therefore, it was recognized that in LA-treated hepatic cells, an influx of receptor activity was noticed which led to an increased degradation of LDL (60). Additionally, PUFAs are recognized as SREBP-1c repressors that suppress lipogenic activity by affecting the LXR-RXR activation pathway (11, 61). LXRs and RXRs form a heterodimer that connects with LXR response elements (LXREs) in specific regions of target genes (62). Regardless of n-3 or n-6 PUFA, their ability to inhibit LXR-RXR promotion of SREBP-1c shifts consequentially enables peroxisome proliferator-activator receptors to enhance the lipid degradation pathway (11, 61).

Another proposed theory for LA's reduction of blood cholesterol concentrations is by increasing gene expression of cholesterol 7α-hydroxylase, the rate-limiting enzyme that synthesizes bile acids from cholesterol (11, 63). Systematically, this effectively increases biliary excretion, increases LXRα activity, and lowers hepatic cholesterol (11, 27, 63). In a study comparing OA, LA, and coconut oil (SFA) in their effectiveness in this process, LA proved most beneficial and efficient (63).

Compared to LA, n-3 PUFAs such as EPA and DHA are recognized to lower TG blood concentrations because of their ability to increase expression of LPL, thus promoting TG clearance (11, 27, 64). Moreover, EPA and DHA facilitate VLDL production by inhibiting Apo-B100 synthesis but inducing ApoC-III that promotes LPL activity (27, 64). When investigating other biochemical markers, research is inconclusive on LA's impact on HDL and ApoA and fairly conclusive that LA reduces ApoB (28). Nevertheless, it is well documented in the literature that PUFA intake is instrumental in the prevention of CVD (10-12, 21, 27, 57-59, 61-66).

Plant Oils and Blood Lipids

Oils are a primary source of PUFA intake, both LA and ALA, in the Western diet (12, 22, 27). The replacement of SFA with PUFAs from vegetable oils has been illustrated to reduce cholesterol levels (57, 67). In a study of healthy adult men, LA in soybean oil was more effective in lowering serum LDL and TC than olive oil (OA) and cocoa butter (SFA) (67). In addition, another study on adults with dyslipidemia examined the impact of high PUFA corn oil (50-55% PUFA) compared to coconut oil (85% SFA) on blood lipids (68). Their findings corroborate the trend that dietary consumption of PUFA, as opposed to SFA, will exemplify more cardioprotective effects by reductions in TC and LDL (68). In studies comparing n-3 and n-6 oils in hypertensive adults, the n-3 PUFA oil exhibited greater cardioprotective effects (69), but in other studies in normolipidemic men, no difference was observed (70). Since there are so few studies comparing n-3 vs. n-6 PUFAs on blood lipids, more research in this area is needed.

Cottonseed Oil and Blood Lipids

Cottonseed oil (CSO) is a regularly used vegetable oil in the United States with over 160,000 tons consumed per year (71). When compared to all of the United States population, that

equates to 31 tablespoons of CSO consumed by every American each year. However, other oils still surpass CSO intake in the United States such as olive oil, corn oil, soybean oil, and canola oil (72). CSO is utilized in the production of baked goods, margarine, salad dressings, and potato chips (72). Additionally, CSO's stability from an abundance of tocopherols, especially gamma tocopherol, and high smoke point makes it ideal for frying (73, 74). CSO's mild, buttery, nutty flavor, and appetizing mouthfeel are also positively reported by consumers (73) . Despite its prominence in the United States food system, it is largely under-researched on its health effects in humans (72). (81). CSO is a PUFA-rich oil with a fatty acid profile of 27% SFA, 19% MUFA, and 54% PUFA (**Table 5**; 81). Specifically, 53% of the fatty acid profile is LA, 18% is OA, and 22% is palmitic acid (72, 75).

The initial studies on the impact of CSO on CVD risk factors were done in animal models. Radcliffe et al. determined in rat models that replacing corn oil with CSO proved more beneficial in the prevention of hypercholesterolemia (76). Furthermore, there were significant reductions in LDL in the rats fed solely a CSO high-fat diet compared to a corn oil high-fat diet (74). Another animal study in hens that were fed CSO had improved lipid outcomes including reduced LDL and TC (77). Scientists hypothesized that this effect could be attributed to the oil's fatty acid composition (76). This evidence was corroborated by Paton et al. who examined specific bioactive properties of CSO, including dihydrosterculic acid (DHSA), a cyclopropanol intermediate in the formation of sterculic acid (78). Sterculic acid is a cyclopropyl fatty acid that will inhibit stearoyl-CoA desaturase-1 (SCD1), a rate-limiting step in de novo lipid synthesis (75). Specifically, SCD1 catalyzes the synthesis of MUFAs from SFA to serve as mediators for TG and cholesterol esters (79). Therefore, in the presence of SFA, SCD1 inhibition results in a shift from TG esterification to FFA oxidation (78). To illustrate, DHSA effectively inhibits the

desaturation of palmitic acid, found in CSO, to palmitoleic acid; the physiological response shifts to block storage and, by default, to promote FFA oxidation. Moreover, CSO's unique composition with DHSA in combination with LA inhibited SCD1 in mouse models and suppressed lipogenic livers (78).

Even with promising animal data showing improvements in cardiometabolic outcomes following CSO consumption, the body of human research with CSO remains low. There are few studies evaluating the potential cardioprotective effects of CSO intake on humans. In one, healthy adults were given 5 CSO-enriched muffins (7g each) and a lunch given in the laboratory (60g CSO) for 5 days (72). They found significant reductions in TC in women and a trend in men (72). Furthermore, there was a trend for lowered LDL in both males and females, but no change in HDL or TG (72). In a different outpatient feeding crossover design, young, healthy men were placed on a 5-day high-fat diet (44% of energy needs) of CSO or olive oil (80). In that study, Polley et al recorded significant reductions in fasting and postprandial TG and fasting LDL and TC in the CSO group, whereas no significant changes were reported in the olive oil group (80). Additionally, Polley et al provided evidence of DHSA activity by performing a desaturation index on participants (13). Palmitoleic acid, a known product of desaturase activity, was significantly reduced at fasting and postprandially following the CSO diet, therefore, exemplifying the action of DHSA inhibiting SCD1.

A recent 8-week clinical trial was completed in hypercholesteremic adults following an outpatient feeding method in which 30% of their energy needs were met by either olive oil or CSO (81). The CSO group had significant improvements in their lipid panel including fasting TC, LDL, HDL, and TC:HDL ratio, non-HDL cholesterol, and ApoB (14). Moreover, the olive oil group had only improvements in their HDL and TC:HDL ratio (14). When presented with a

SFA meal challenge, the olive oil group had a worsening postprandial TG and glucose response while CSO had a beneficial effect by suppressing non-esterified fatty acids (NEFAs) and glucose response (14). Therefore, these notable improvements to blood lipid profiles, despite the presence of a HF diet, are hypothesized to be due to CSO's composition. CSO has a unique cardioprotective makeup in both its high PUFA content's ability to affect lipogenic transcription factors and the presence of a bioactive lipid DHSA that channels SFA utilization into FFA oxidation. These properties, coupled with its prevalence in the food system, make CSO a warranted oil of study in the human model. In order to articulate the therapeutic effects of CSO on CVD risk, more studies in the human model in various populations, durations, and doses need to be completed.

Inflammation and Coagulation

Inflammation is a defense system within the body that contributes to the pathogenesis of a variety of chronic diseases (82). It has been hypothesized that a variety of foods, such as fatty acids, influence inflammatory processes, either by inducing or inhibiting chronic inflammation that can promote CVD (11, 82, 83). The inflammatory response is characterized by the recognition of stimuli like tissue damage, fatty acids, or alcohol which activates inflammatory pathways that induce the release of inflammatory markers, and finally recruitment of inflammatory cells (82). Furthermore, there are two classifications of inflammation: acute and chronic (84). Acute inflammation is defined as a short-term response identified by the recruitment of leukocytes whereas chronic inflammation is prolonged lymphocyte and macrophage occupancy with proliferation of connective tissue and blood vessels (84). Moreover, obesity can intensify the inflammatory response thus promoting metabolic dysfunction (84).

Obesity is indicative of an excess of nutrients, and adipose tissue responds to this stimulus by hyperplasia and hypertrophy of adipocytes (84). Additionally, excessive growth of adipocytes has been shown to reduce blood flow to them resulting in hypoxia (84). Hypoxia promotes a systemic inflammatory response promoting the release of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF-α), interleukin (IL)-6, IL-1β, and secretion of c-reactive protein (CRP) (83, 84). This will assist in endothelial dysfunction which, as previously detailed, is a trademark of atherosclerosis (84). This system can be further aggravated by the coagulation potential of pro-inflammatory cytokines (85).

The coagulation pathway contributes to hemostatic conditions, but an imbalance in this system can result in either excessive bleeding or clot formation (85). The coagulation cascade has been categorized into two pathways: intrinsic and extrinsic (85, 86). These two pathways are a series of activation cascades that will ultimately promote the common pathway to produce thrombin (85, 86). This pathway is carefully balanced through a series of clotting factors labelled factor I-XX (85, 86). The first four clotting factors, I, II, II, and IX, are commonly referred to by the name fibrinogen, prothrombin, tissue factor (TF), and calcium, respectively [84]. Further, many of these factors act as precursors for zymogens, and an "a" is added to the factor numeral when the corresponding zymogen is activated (85, 86).

Endothelial damage will expose endothelial collagen which promotes the intrinsic pathway, so factor XII which consequently activates factor XI, then factor IX, which interacts with its cofactor factor VIII which catalyzes the activation of factor X (85, 86). The extrinsic pathway is activated by vascular damage to the subendothelial tissue which stimulates TF which binds to factor VII to promote factor X activation (85, 86). Therefore, the extrinsic and intrinsic pathways both work to amplify the activation of factor X, then the common pathway can occur

(85, 86). In this cascade, factor X and its cofactor V form a complex to cleave prothrombin to thrombin (85, 86). Then, thrombin induces fibrinogen activation to fibrin which combines with other fibrin subunits to form fibrin strands (85, 86). When fibrin strands come together, this forms a network that will stabilize a platelet plug (85, 86). Thrombin activation also incites a negative feedback response that will trigger the cleavage of plasminogen to plasmin and stimulation of antithrombin to prevent over coagulation (85, 86). Additionally, thrombin activity will enhance protein C activity which inhibits factors V and VIII to promote anticoagulation (87). Moreover, disruption in this system by inflammation can alter hemostatic conditions (86).

When discussing the contribution of inflammation in the prevention and progression of chronic disease, the relationship between coagulation and inflammation is important to consider. Moreover, there is a constant interplay between inflammation and coagulation; inflammation initiates coagulation, and coagulation activity further induces inflammation (87). Importantly, this interaction will influence thrombosis progression by regulating coagulation potential that directly contributes to thrombin generation (87). For instance, it has been displayed through several studies that IL-6 is instrumental in the initiation of coagulation (87). The determinants of coagulation potential are assessed by observing TF and plasminogen activator inhibitor-1 (PAI-1) (83, 87). Specifically, IL-6 modulates the synthesis and release of fibrinogen, TF, factor VIII, and platelets (88). Furthermore, TNF-α and IL-1 are mediators in modulating anticoagulation (87). TNF- α promotes PAI-1 and IL-1 stimulates the protein C pathway (87, 88). Nevertheless, a modifiable factor that contributes to inflammation and coagulation potential is diet, specifically fatty acids (82).

Dietary fatty acid composition has been a focus within the literature for their role in inflammation (11). SFAs, specifically lauric and palmitic acid, are regarded as pro-inflammatory

from their interaction with the transcription factor nuclear factor – kB (NF-kB), and consequentially, toll-like receptors (TLRs) [11, 88]. Activation of TLRs is essential for induction of the NF-kB pathway that releases pro-inflammatory cytokines (82, 89). Therefore, it has been hypothesized that SFA acts through the formation of lipid rafts to directly induce TLR-4 signaling leading to a release of pro-inflammatory cytokines(11, 89). Conversely, it has been suggested that MUFAs and n-3 PUFAs are anti-inflammatory. Moreover, n-3 PUFAS inhibit NF-kB binding to TNF-α-specific consensus sequence; this consequently inhibits the NF-kB signal cascade (25). MUFAs, specifically OA, have exemplified anti-inflammatory gene expression and evidence to reduce CRP serum concentrations (90, 91). Moreover, OA has been recognized to protect against SFA-induced inflammation through activation of peroxisome proliferator-activated (PPAR-α) and protein kinase A mechanisms (92). Additionally, MUFAs are anti-inflammatory by stimulating β-oxidation and TG accumulation (92). Less understood, though, is the impact of n-6 PUFAs on inflammation.

There has been controversy within the literature regarding the influence of n-6 PUFAs on inflammation. Scientists have hypothesized that since LA is a precursor to AA, which can give rise to pro-inflammatory mediators from its production of eicosanoids, LA promotes inflammation (11, 58, 83). Because of this, LA-rich diets have been a disputed therapeutic intervention for CVD risk despite their association with positive blood lipid reductions (11, 83). Importantly, it has been determined that very little dietary LA (0.2%) actually gets converted into AA (93). Furthermore, epidemiological evidence has combatted the notion that LA is prothrombotic and inflammatory showing that higher concentrations of LA are actually associated with reduced prevalence of CVD and Coronary Artery Disease (94). However, there remains a lack of human model research in this area (11, 83). One clinical trial assessed pro-

inflammatory markers and coagulation potential in an OA-rich versus LA-rich diet and recognized no significant difference in their biomarkers (83). Additionally, in a crossover trial of normal-weight males in which they were fed a 5-day high fat diet of CSO (LA-rich) and olive oil (OA-rich) reductions in TNF- α and TF were observed in the CSO group compared to the olive oil group (95). Moreover, CSO has other constituents that may contribute to its lack of pro-inflammatory response, and potentially an anti-inflammatory response. CSO is high in vitamin E, specifically alpha and gamma tocopherols, which have been noted to suppress NF-kB and JAK-STAT pathways that can then minimize TNF-α and IL-6 expression (83, 95). Altogether, this emphasizes the importance of analyzing the whole food source of fat when considering its inflammatory and coagulation potential.

The role of Appetite in Obesity and Weight Maintenance

Energy Balance and Obesity

Obesity is a primary contributor in the development of CVD; thus, weight management remains a key component in the prevention of chronic disease. Obesity results from homeostatic dysregulation of energy balance (96). Energy balance encompasses two elements: energy intake (EI; caloric intake) and energy expenditure (EE) (97). Total EE has is made up of the thermal effect of food (TEF), which is the energy needed to metabolize nutrients consumed, resting metabolic rate (RMR), which is the energy needed to maintain homeostasis and normal physiological conditions in an individual, and physical activity (97). Moreover, it could be assumed that a positive energy balance in which EI exceeds EE can result in weight gain (96, 97). However, the body elicits compensatory mechanisms that work to combat body mass changes. For example, if EI decreases the body will compensate by reducing RMR and stimulating feeding signals (97, 98). Nevertheless, the body's compensatory mechanisms to

positive energy balance seem to be poorer than to negative energy balance (97). High-fat (HF) diets have been observed to be obesogenic due to their energy density therefore contributing to EI and positive energy balances (96, 99, 100). This has been disputed within the literature, however, because of the observance of foods such as nuts and oils eliciting no weight gain when added to the diet (99, 100). This could be due to energy compensation, changes in appetite, or other factors that may influence the obesogenic potential of foods (99). Moreover, weight maintenance is multifaceted and requires a multidisciplinary approach that evaluates environmental, behavioral, physiological, and dietary factors.

Fatty Acid Composition and Metabolism

As previously mentioned, the TEF or diet-induced thermogenesis (DIT) plays a key role in EE (97, 101). Additionally, fatty acid utilization has been an area of study to understand the effect of different FA on metabolism. Moreover, it has been suggested that an individual's ability to compensate for HF meals through increased fat oxidation aids in the mitigation of chronic disease (102, 103). To understand this relationship, HF meal challenges and feeding trials are used to determine substrate oxidation and EE. To assess these differences in humans, indirect calorimetry, breath tests, and isotope labeling are utilized.

There have been several studies examining the differences in metabolic effects of SFA and MUFAs. For example, in a 4-week clinical trial in which patients were on either a high MUFA or high SFA diet, researchers concluded that subjects following the MUFA-rich diet had greater rates of fat oxidation compared to those following the high SFA diet (101). Other clinical trials have corroborated this finding stating that high SFA intake lowered FA oxidation as compared to high MUFA intake (104-107). These findings mimic the research comparing PUFA intake and SFA regarding fat oxidation. Feeding trials found that subjects who consumed

a high PUFA to SFA ratio had increased fat utilization and oxidation (108-110). In a crossover study with healthy adult males, EE was investigated utilizing indirect calorimetry to determine RMR after following either a high PUFA, low SFA diet or high SFA, low PUFA diet (111). The participants following the high PUFA diet were analyzed to have a significantly higher RMR and DIT following ingestion of a standardized HF meal (46% fat) (111). Furthermore, there have been studies that evaluate SFAs, MUFAs, and PUFAs in regard to their postprandial metabolism.

There is controversy within the literature regarding the differences PUFAs, MUFAs, and SFA exhibit in fat oxidation and EE. In premenopausal women, a HF meal challenge enriched with either PUFAs, MUFAs, or SFAs were examined for DIT and fat oxidation (101). This study reported a significantly greater DIT of PUFAs compared to MUFAs and SFAs, but no difference in oxidation (101). Conversely, a study examining the EE potential of PUFA, MUFA, and SFA in overweight adults observed no significant differences between their EE measured by a respiration chamber (112). Although research comparing SFA, PUFA, and MUFA remains fairly inconclusive, some researchers have compared MUFAs and PUFAs due to the theory that their differences in degree of saturation will influence fat oxidation and EE. Multiple crossover studies have been conducted to investigate the differences PUFAs and MUFAs have on postprandial metabolism. Logan et al. examined the relationship between fat oxidation and EE utilizing fish oil supplementation and comparing its effects to olive oil (113). The 12-week fish oil supplementation trial in older female adults noticed increased EE and fat oxidation in the fish oil intervention group compared to an olive oil intervention group (113). Additionally, Jones et al. researched differing fatty acid compositions in sunflower oil (LA rich), flaxseed oil (alpha-linolenic acid rich), or olive oil (oleic acid rich) rich meals (60% energy) in connection with fat oxidation and EE (114). In 15 adult, normal weight men, a significant increase in EE was

observed in olive oil feeding compared to flaxseed oil and a trend in increased EE compared to sunflower oil, but no differences in fat oxidation were reported (114). Lastly, a trial of 15 normal weight men evaluated DIT and substrate oxidation in PUFAs (CSO) versus MUFAs (olive oil) by utilizing both a 5-day feeding trial and meal challenges (115). Acutely, MUFA-rich HF meals were noted to elicit greater fat oxidation but both PUFA and MUFA exemplified metabolically favorable outcomes after the feeding trial by increasing fat oxidation (115). Importantly, this marked increase in fat oxidation could be due to CSO's specific composition; DHSA promotes an increase of oxidation by prevention of TG formation (79, 115). In sum, it is evident that FA composition contributes to metabolic responses within the body, but the conflicting evidence makes it difficult to translate a clear relationship between FA composition and metabolism.

Appetite Regulation

One of the predominant factors influencing EI is appetite; thus, it is an important consideration when formulating therapeutic interventions for weight management [98]. Appetite is governed by satiation and satiety which induce the feeling of fullness which will inhibit continuous eating (115). Specifically, satiation occurs within the prandial state while satiety occurs during the postprandial state. There are multiple properties of a meal that influence satiety including sensory and cognitive components of the food, palatability, chemical properties of the food, and the physiological effects of the food (116). Moreover, the palatability of food drives food selection and preference (117). Therefore, HF foods are generally recognized as highly palatable lending them to favor overconsumption and impacting energy balance (105, 116-118). However, the FA composition might impact appetite regulation by modifying the response of hunger and satiety hormones (100).

To modulate hunger and satiety signals, the central nervous system (CNS) is the primary regulator of energy homeostasis (119). Specifically, the hypothalamus is the center for deploying neuronal mechanisms for adapting to food intake (119). Within the hypothalamus, bordering the third ventricle above the hypophyseal system, lies the arcuate nucleus (ARC) (120). In the ARC exists neurons with opposing effects on appetite and energy homeostasis including the anorexic pro-opiomelanocortin/cocaine and amphetamine-related transcript (POMC/CART) neurons and orexigenic neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons (119-121). Furthermore, it has been stated that the lateral hypothalamus (LH) is involved in the termination of intake while the paraventricular nucleus of the hypothalamus (PVN) is involved in the initiation of intake (121). To illustrate this system, in a fasted state, ghrelin is released from the stomach, activating the NPY/AgRP neurons (121, 122). Consequentially, NPY activity in the PVN initiates feeding behaviors and reduction of EE (122). Conversely, satiety can be promoted by circulating leptin that acts on the POMC/CART neurons (121, 122). POMC activation induces expression of α-melanocyte stimulating hormone (α-MSH) to be delivered to the PVN; the system is also mediated by mealmocortin-4 receptors (MC4Rs) (121, 122). Thus, the enhanced signaling of the MC4rs in the PVN lessens food intake (122). Furthermore, these signals are communicated through hormones within the blood stream or, predominantly, through the vagal nerve afferent connection (119-122). Therefore, hormones are distinct in their role of control of appetite signaling and regulation.

Accepted assessment of appetite includes evaluation of hunger and satiety hormones including ghrelin, cholecystokinin (CCK), and peptide YY (PYY). Ghrelin is an orexigenic peptide known as the “hunger hormone” for its responsibility in stimulating food consumption (123). This hormone prepares the body for meal initiation rising pre-prandially and falling

quickly post-prandially (123). In low energy states ghrelin is produced in the P/D1 cells in the stomach (122, 124). Once ghrelin is released, it must be acetylation for optimal targetization of the ghrelin receptor Growth Hormone Secretagogue Receptor (GHSR-1a) (123, 124). Then, as previously described, ghrelin will submit this periphery information from the stomach cells to the hypothalamus via the NPY pathway (122). Importantly, ghrelin is responsive to energy dense and high-volume foods (116). However, the other hormones that will be explained oppose ghrelin and act as satiety hormones.

CCK is released by result of nutrient consumption, specifically NEFA and protein, in the I-cells of the duodenum and jejunum (122). When NEFAs and proteins interact with the g-coupled protein receptor GPR40 lining the I-cells of the intestines, vagal innervation is stimulated which delays gastric emptying and initiates feelings of satiation (122, 125). Moreover, the release of CCK is directly associated with CART release from the vagal nerve, promoting an anorexigenic response (126). Interestingly, leptin and insulin have been noted to interact with CCK and enhance its satiating effects (119). In addition, obesity dampens the effects of CCK by desensitizing its vagal nerve response (125). Importantly, HF diet consumption coupled with reduced CCK receptors in the gut effectively suppress CART expression by increasing circulating ghrelin levels (125).

PYY is secreted from the L-cells in the distal gut including the ileum, colon, and rectum (116, 127) and aids in satiation. PYY can be triggered by macronutrient contact and neurohormonal stimulus which are hormones secreted from neuroendocrine cells that are initiated by nerve stimulation (127). Although PYY exists in two forms (PYY 1-36 and PYY 3-36), PYY 3-36 is thought to be the most potent regulator of food intake due to its high affinity for the Y2 receptor that inhibits the NPY/AgRP system, thus increasing POMC activity, inducing

satiety (122, 127, 128). Since PYY is a satiety hormone, it rises postprandially within 15 minutes after nutrient consumption with a peak at about 60-90 minutes postprandially and remains elevated levels for up to 6 hours (127). Moreover, PYY functions to delay gastric emptying, slowing GI movement, as well as impede gastric acid and pancreatic exocrine enzyme production (121, 132). Nevertheless, it remains widely inconclusive on the direct association of appetite and circulating PYY levels despite its importance in nutrient intake (127).

Appetite Regulation and Fatty Acid Composition

As suggested earlier, FA composition has been hypothesized as a potential factor in appetite regulation and control. Appetite can be assessed through the hormones previously described, but it can also be measured through subjective and applied measures (100). Visual analog scales (VAS) evaluate individual's subjective desire for food, hunger, and fullness, but this is complicated by other emotions or environmental factors that play into individual's intake (100, 129). Applied measures of appetite are usually measured by estimated EI and can utilize the *ad libitum* (eating without restriction) approach in which EI is measured in a laboratory setting (100, 130). Furthermore, participants can self-report EI by keeping food records (130). In the studies completed comparing FA saturation on appetite measures, very few have been completed with PUFAS and even less on CSO specifically.

The following crossover studies utilized an acute meal challenge in which participants were given varying FA compositions and subjective (VAS) and applied (EI) measures were analyzed. Four studies revealed no differences with differing degrees of fat saturation regarding applied or subjective measures (96, 112, 131-135). Casas-s-Agustench et al. hypothesized that the mode of delivery of the FA might be more important than the FA composition alone (132). For example, participants have experienced different satiety when eating dietary fat in whole

food form versus an oil (132). In a different study in which healthy men and women were presented with a meal challenge of a muffin with either canola oil (PUFA rich), peanut oil (MUFA-rich), or butter (SFA-rich), their results were inconclusive on the effectiveness of appetite regulation utilizing VAS and EI measured by food record post-lab visit (134). Notably, the researchers stated that when participants were given fat-free muffins hunger and satiety scores dropped and EI increased (134). Similarly, in a study with normal weight women given either a HF liquid shake of either PUFAs, MUFAs, or SFAs encompassing 33% of their EN no differences in subjective appetite or EI were reported (135). These studies elucidate no relationships between SFA, MUFA, and PUFA intake on appetite measures when considering applied and subjective measures, other studies, however, evaluate physiological responses to acute meal challenges.

There have been other acute, crossover studies that highlight differences in appetite responses regarding FA composition. In one study comparing MUFAs (olive oil) to SFA (coconut oil), SFA was found to increase satiety, fullness, and hunger (136). This suggests that the composition of the fatty acid may influence satiety, specifically, Valente et al hypothesized that the availability of the coconut oil's MCFAs to be readily absorbed enhances satiety (136). Another study carried out among overweight adults found that MUFAs, specifically oleic acid, had a weak control over appetite compared to PUFAs (137). Lawton et al. suggested that this finding may be due to the high affinity for adiposity storage of oleic acid (137). In other studies, however, appetite hormones are measured in conjunction with applied measures that illustrate differences between FA composition and appetite.

A study in which obese women were given three HF meals (70% of EN) high in MUFAs, PUFAs, and SFAs and provided an *ad libitum* buffet, no subjective appetite differences were

stated (96). However, reductions in ghrelin were significantly greater for PUFAs and MUFAs versus SFA. Additionally, a greater PYY response for PUFA was reported as compared to MUFA and SFA (96). This data was corroborated by Kozimor et al. that reported meals rich in PUFAs and SFAs elicited a greater PYY response than the MUFA rich meal, while the SFA-rich meal was shown to increase feelings of fullness, but no significant differences in hunger were found (138). Further, another study utilized ileal injections of either shea oil (SFA), canola oil (MUFA), or safflower oil (PUFA) to compare fat saturation to satiety (139). The unsaturated FAs reduced hunger compared to the control saline infusion and shea oil and all oils induced an increased PYY response compared to the control (139). Moreover, since PUFAs have exhibited beneficial appetite outcomes, studies have directly compared PUFAs to SFA and/or MUFAs.

Few longer-term studies exist examining an HF PUFA-rich diet on appetite outcomes. A 7-day randomized trial in which healthy adults were allocated to a PUFA-rich diet or control diet showed a decrease in fasting ghrelin and increase in fasting PYY after the PUFA diet intervention (140). Moreover, the postprandial PYY response post PUFA-rich diet increased compared to baseline (140). Additionally, the subjective measures of hunger and fullness were not significant despite the physiological changes (140). Another randomized controlled trial sought to analyze fat-specific satiety after giving overweight men and women GLA, LA, and OA (141). Through this treatment, they observed that those given LA did not eat as much fat at dinner compared to OA (141). Despite the limited evidence, there is a trend for beneficial appetite responses from high PUFA diets.

With respect to CSO-rich diets, limited studies have evaluated their appetite regulation potential. Polley et al. utilized a crossover study with normal weight adults in a 5-day HF diet PUFA-rich (CSO) or MUFA-rich (olive oil) to analyze appetite responses (142). Although acute,

post PUFA-rich diet intervention showed lower ghrelin and an increase in postprandial CCK compared to the MUFA-rich diet (142). However, there were no differences in PYY response, but post PUFA diet postprandial hunger was lessened (142). Lastly, the most recent study analyzing PUFA (CSO) versus MUFA (OO)-rich diet was an 8-week randomized parallel trial with sedentary adults utilizing an outpatient feeding intervention to meet 30% energy needs (100). Prater et al. reported that the CSO group had superior fasting CCK responses and decreased postprandial ghrelin compared to the olive oil group as well as increased fullness and decreased EI (100). The literature suggests that a diet enriched with CSO could benefit the appetite physiological response through suppressed ghrelin and increased postprandial CCK (100, 142). Furthermore, subjective measures of fullness and hunger were improved by a CSO-enriched diet (100, 142). Although the studies done with PUFAs and CSO demonstrate beneficial appetite regulation, the lack of homogeneity between studies makes it difficult to establish clear causal relationships between subjective, applied, or hormonal appetite regulation with respect to FA composition. Moreover, less research has been done with a whole foods approach to evaluate FA saturation in relation to appetite.

Dose-Response Studies

Dose-response studies are critical for establishing relationships between the most effective dose for the effect of concern (143). Specifically, there is a need to identify dose-dependent effects in different populations to improve therapeutic recommendations and clinical outcomes. For example, in this study, we will be evaluating which dose of CSO leads to the most beneficial cardiometabolic outcomes in hyperlipidemic and/or overweight adults. Conversely, it is important to consider the amount of CSO or PUFAs that elicit adverse effects, if any (144). There have been few dose-response studies with oils and/or HF foods. One meta-analysis

concluded consuming 15% of EEN from canola oil elicited the most beneficial blood lipid responses (145). While a randomized control trial utilizing 20% EN from corn oil was effective in reducing LDL-c (146), dose-response studies with n-6 fatty acids on metabolic health are scarce.

Despite the overall lack of research associated with dose responses of fatty acids regarding CVD risk, the majority has been done with n-3 FA. Similar to LA, marine-derived EPA and DHA have been proven to be beneficial for cardiovascular outcomes (147). The amount of EPA and DHA to obtain reductions in TG and improved cardiovascular health has been determined to be > 3.0g per day (147, 148). This has been established through a series of dose-response studies with n-3 supplementation in which multiple concentrations of fish oil were utilized. In a study with healthy volunteers, supplementation of 1.5, 3, and 6g/day were given to participants, and the most beneficial lipid-profile changes were noted with 3g/day of supplementation (148). Another dose-response trial tested low doses of n-3 PUFAS (0.5-2.0g/day) in healthy volunteers and noticed no beneficial effects (149). Moreover, participants had adverse effects including increased TC and LDL concentrations (149). Finally, a crossover trial in adults with hypertriglyceridemia corroborated these studies' findings and stated participants had no improvements in blood lipids given a low dose of n-3 PUFAs and, differentially, reductions in apoB, apoC-III, and VLDL with a high dose (150). Therefore, these clinical trials aided in establishing a therapeutic n-3 dose for individuals at risk of developing chronic diseases such as CVD.

Even with the n-6 PUFA, LA, exemplifying beneficial effects on cardiometabolic outcomes (11, 60, 63), there is still a lack of research on the dose-response effects of differing amounts of LA in the diet. Specifically, there is a lack of dose-response research regarding whole

food sources rich in LA, such as CSO. However, HF foods have been evaluated for their dose-response effects. There have been two dose-response almond consumption clinical trials concerning cardiometabolic outcomes. In a study evaluating a "full dose" (73g/day) and "half dose" (37g/day) of almond intake, researchers were able to discern greater reductions in LDL cholesterol with a full dose and a dose-response of 7g of almonds per day equating to 1% reduction in LDL (151). These differences were also attributed to the composition of almonds; in addition to unsaturated fat, are rich in protein and fiber (151). This was supported by a secondary analysis of this data which outlined the improvements in the fatty acid profile of its participants, specifically, higher OA and MUFA concentrations in TG and NEFA fractions (152). The second study was done by Josse et al. where analyzed the postprandial glycemic response following doses of either 30, 60, or 90g/day of almonds (153). In a dose-dependent way, almonds lessened the glycemic response following a refined carbohydrate meal (153).

Other tree nuts have also been evaluated for their dose-response effects including pistachios and hazelnuts. Researchers utilized a 4-week randomized crossover isocaloric controlled-feeding trial in which 1 serving or 2 servings of pistachios per day (10 % or 20% of EN, respectively) were given to analyze the effects on blood lipids (154). In a dose-dependent manner, 1 and 2 servings of pistachios reduced LDL by 9% and 12%, respectively (154). In a different study, 30g or 60g/day of hazelnuts were allotted to participants. They were instructed to follow their usual dietary habits and incorporate their dose of hazelnuts (155). Despite researchers lacking significance in blood lipid profiles, endothelial function, and inflammation, they did report participant's negative remarks following the 60g of hazelnuts per day (155). Thus, this informs adherence that high doses of nuts may be difficult for the population to implement into their dietary habits. Nevertheless, n-6 PUFA-rich oils, and CSO in particular,

remains understudied in a dose-response trial despite its evidence for improving markers of chronic disease.

Previously, only high doses of CSO (30% of EN or higher) have been utilized to evaluate its therapeutic effects on cardiometabolic outcomes (72, 80, 81). Therefore, it is essential to understand the threshold or dose of observable benefits, and whether or not it can be achieved while staying within dietary guidelines for recommended total fat intake. To date, no studies have evaluated the lipid profiles of those receiving less than 30% of their energy needs from CSO. Moreover, to articulate clinical recommendations for mitigating chronic disease it is vital to understand the dose-dependent response of CSO that generates therapeutic outcomes such as improved cholesterol profiles, appetite regulation, inflammatory, and coagulation markers.

Summary and Discussion

As chronic disease continues to burden millions of people, it is crucial to determine effective, modifiable actions that individuals can take to lessen their disease risk. Although complex, diet is a primary modifiable factor that can positively or negatively impact chronic disease, specifically CVD and obesity. Therefore, it is critical to identify dietary behaviors that will benefit those at risk of developing or progressing these diseases.

Polyunsaturated fatty acids, specifically LA, have strong evidence for their lipid-lowering effects. The current clinical recommendation in the prevention and management of CVD is LDL reduction (59). Through interactions of SREBPs, LA can increase hepatic clearance of circulating LDL and upregulate the lipid degeneration pathway (60-62). Additionally, LA activates the rate-limiting enzyme, cholesterol 7α-hydroxylase, that synthesizes bile from cholesterol which works to further lower hepatic cholesterol concentrations (11, 63). However,

despite these positive outcomes, a precise recommendation for the intake of LA to observe these beneficial effects has not been determined due to the lack of dose-response studies utilizing LA (26). An oil rich in LA, CSO, has shown evidence to elicit strong lipid-lowering effects.

In three clinical trials, CSO has exemplified therapeutic effects in healthy and hypercholesterolemic adults (72, 80, 81) . These beneficial outcomes can be explained by CSO's fatty acid composition; CSO is rich in LA and DHSA (79). Thus, CSO elicits a cardioprotective effect by inhibition of SCD1, a rate-limiting step in de novo lipid synthesis, therefore increasing FFA oxidation (78, 79). Furthermore, after dietary interventions, CSO exemplified beneficial appetite regulation responses evident by suppressed ghrelin and increased postprandial CCK (100, 142). Currently, the clinical trials completed utilizing CSO have only been done with high doses of CSO (>30% EN). In order to enhance recommendations to those at risk of developing, or inflicted, with CVD, it is essential to further investigate the relationship between CSO, blood lipids, and appetite regulation. This will aid in establishing the most effective dose of CSO for health-promoting outcomes in this population.

Methodology

Indirect Calorimetry

When direct calorimetry, a direct measure of heat production is unavailable, indirect Calorimetry (IC) is a valid and reliable procedure to determine RMR by measuring the oxygen consumed (VO_2) and carbon dioxide released (VCO_2) by an individual (156). In humans, energy is sourced from chemical energy released through the oxidation of nutrients in food (157). Instead of directly measuring the amount of heat released from an individual, IC calculates heat indirectly through the assessment of substrate utilization and its byproducts (157). This is

calculated through the Weir equation which evaluates the total heat produced during a given time (158):

$$\text{Total kcal} = (3.9 * VO_2) + (1.1 * VCO_2)$$

This equation assumes that protein is responsible for 12.5% of total energy expenditure at a given time. Moreover, IC can be used to provide information on the type of substrate being oxidized within the body. This assumption is derived from the understanding that the ratio of VCO_2 to VO_2 is defined as the respiratory quotient (RQ) or Respiratory Exchange Ratio (RER) [154, 156]. Thus, RQ informs substrate utilization. Glucose is oxidized according to this equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2$$

According to the equation above, the RQ for carbohydrates is 1.0 under normal conditions. Substrate utilization can be determined through the use of the RQ by the following equations from Frayn (159):

Fat oxidation (g/min) = $1.67VO_2 - 1.67VCO_2 - 1.92$(urinary nitrogen excretion)

Carbohydrate oxidation (g/min) = $4.56VCO_2 - 3.21\ VO_2 - 2.89$(urinary nitrogen excretion)

Blood Lipid Quantitative Measurements

For measurement of standard blood lipid markers, we use Spectrophotometry. This is a commonly used approach in determining the amount of a chemical substance in a sample (160). This method of quantitative analysis measures the intensity of light as it passes through a sample (160). Every chemical compound absorbs, transmits, and reflects light within specific wavelengths. Using Beer's Law, the absorbance of a sample can be used to determine the

concentration of a sample (160). This is done by understanding the path length and molar absorptivity of the substance being analyzed. This method can be used to measure TC, HDL, and non-HDL-c.

Ion mobility lipoprotein fractionation is utilized to measure subclasses of lipoproteins directly. Through this, a gas phase electrophoresis separates unmodified and ionized lipoproteins depending on their electrical field and size. Particles leave a separation chamber and are identified and counted. As the voltage of the electric field of the chamber increases gradually, lipoproteins of specific size and charge can exit the chamber one at a time. Therefore, this system is utilized to quantity small, medium, and total LDL particles as well as LDL peak size and pattern, and large LDL particle number. Quest Diagnostics (Quest Diagnostics, Secaucus, NJ) performs this test.

Enzymatic Assays

Triglycerides and NEFAs are analyzed by the enzymatic method (FUJIFILM Wako Diagnostics, Mountain View, CA) The enzymes glycerol kinase (GK), glycerol-3-phosphate oxidase (GPO), and catalase are utilized in the TG assay. In the sample, catalase works to decompose free glycerol into O_2 and H_2O. Then, LPL, GK, and GPO are utilized to oxidize the glycerol of the remaining TG into hydrogen peroxide. This causes N-(3-sulfopropyl)-3-methoxy-5-methylanine (HMMPS) to condense; thus, producing a blue color that can be measured for absorbance to determine the concentration of TG. Similarly, in the NEFA assay, enzymes are used to oxidize the NEFAs into H_2O_2. This allows for the condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-alinine (MEHA) with 4-aminoantipyrine that produces a shaded product. This product's optical density is then measured to determine the concentration of NEFAs in the sample. Similarly, blood glucose utilizes the same methodology through an in-lab glucose

oxidase colorimetric enzymatic assay. Glucose oxidase produces H_2O_2 from glucose, from this, peroxidase catalyzes the condensation of 4-aminoantipyrine to yield a purple shaded product. Then, the absorbance of the purple pigment is measured to determine the concentration of glucose in the sample.

In these assays, a standard curve of increasing, known amounts of the analyte being measured is used as a reference for determining the concentrations of the unknown analyte. Moreover, the standard curve produces an equation where the unknown value can be calculated. In each assay quality controls are provided with known concentrations of the analyte being measured to ensure the accuracy and precision of the assay. This monitors the performance of the assays; the values determined for the controls should fall within the range set by the manufacturer. Additionally, our lab utilizes pooled plasma as a reference for all assays completed. Through this, the accuracy and measure of consistency within and between assays is evaluated. In every assay performed, a replicate of each pooled plasma sample is utilized to analyze the variance within the assay.

Radioimmunoassay (RIA)

To measure fasting and postprandial insulin, and the appetite hormones Ghrelin and PYY in response to the high SFA liquid meal challenge, RIAs will be utilized. A target molecule (antigen) is measured in RIAs by competitive binding to a specified, primary antibody compared to a radiolabeled antigen. For example, a standard amount of the antigen can be labeled with a radioactive isotope like I^{125} that will compete for limited binding sites on an introduced antibody with the unlabeled antigen in the sample. Therefore, when a sample has greater concentrations of the unlabeled antigen, less of the labeled antigen will be able to bind to the antibody; thus, exhibiting less radioactivity. This can be measured after a secondary is introduced to facilitate

the precipitation of the bound antibody. This works to separate the bound and unbound antigens. The unbound particles are removed, and the remaining bound samples are measured for radioactivity to determine the concentration of the unlabeled antigen in the sample (EDM Millipore St. Louis, MO).

Like an enzymatic assay, a standard curve of increasing concentrations of the antigen to calculate the unknown concentrations. Pooled plasma and quality controls are also used to interpret the accuracy, precision, and variance of the assay's performance. To determine the concentration of the unknown antigen, non-specific binding counts are subtracted from all the counts from the standard and unknown samples. This takes away the background of any binding that would occur regardless of the unknown antigen being added. The percentage of total binding for each standard is calculated using the 100% binding calculated. Thus, the standard curves are produced by plotting the percent binding versus the known concentration of the standard. Then, the unknown samples are calculated through interpolation of the reference curve produced.

Moreover, many factors influence the interaction of the antigen antibody complex in RIAs (161). These include temperature, relative concentration of antigen-specific antibodies, and affinity for antigen to antibody binding (162). These factors may contribute to differences in length of time required to perform an insulin assay (2 days) versus a PYY assay (3 days).

Enzyme-Linked Immunosorbent Assays (ELISAs)

Enzyme-linked immunosorbent assays will be used to quantify CCK. In ELISAs, a primary antibody is bound into the wells being utilized (162). Then, the sample of interest is added. If the antigen of interest is present in the sample, it will bind to the primary antibody. Next, a wash step will remove all unbound antibodies within the well. A secondary antibody is

added to the well binding to a secondary antigen epitope. This will facilitate the detection of the antigen. Following another wash step, a solution is added to the wells, enabling color development proportional to the concentration of antigen present. Then, the intensity of the color is measured to determine the amount of the antigen in the well. A standard curve is utilized to determine the concentrations of the analyte.

Body Composition

*T*o assess fat mass and fat free mass of our participants, air displacement plethysmography (i.e. BodPod) will be utilized. The BodPod comprises two chambers; the test chamber holds the participant, and the reference chamber contains the device that measures the changes in pressure between the two chambers (163). Specifically, this technique determines body composition by relying on a precise electronic scale and a volume measurement from the test chamber (164). Then, once density is determined (density=mass/volume), the BodPod will predict the Thoracic Gas Volume that is utilized in a densitometric equation to calculate the percent fat and fat-free mass of the participant (164).

The volume of the test chamber is calculated through pressure changes between the test chamber and reference chamber through a moving diaphragm mounted on the common wall between the chambers (163). This relies on Boyle's Law: $P_1/P_2 = V_1/V_2$. In this, the pressure and volume of the test chamber are calculated prior to the participant's entry, and P_2 and V_1 are calculated while the participant is in the test chamber (163). Therefore, the volume of the participant is the volume of the test chamber prior to participant entry minus the volume of the test chamber with the participant enclosed (163). To correct for erroneous data from differences in isothermal air that might result in a lower pressure output for a given body volume, the BodPod manufacturers recommend certain controls (163). Isothermal air (i.e. air in hair or loose-

fitting clothing) in the test chamber during a body composition will result in a lower approximation of body volume due to its easily compressibility (163).

Some corrective procedures the manufacturers recommend to ensure the accuracy of the BodPod measurement are to perform tests in a dry place that maintains the same relative temperature and instruct participants where form-fitting clothing and a swim cap (163). Therefore, using predictive equations body fat percentage can be calculated through the body density. Moreover, since fat mass and fat-free mass are dependent on factors such as age, gender, and race, different models are utilized to correct for these factors (165). The Siri model (% body fat = (495 / Body Density) -450) is used for those other than African Americans, and the Ortiz model (% body fat = (483.2/ Body Density) - 436.9) is used for African Americans (166). Body composition measurements performed by the BodPod have been discerned as highly reliable and accurate (167, 168).

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CHAPTER 3

DOSE RESPONSE EFFECTS OF COTTONSEED OIL ON BLOOD LIPID RESPONSES IN ADULTS AT-RISK FOR CARDIOVASCULAR DISEASE: A RANDOMIZED CONTROLLED TRIAL

[1] Hawkins, C. D., Prater M.C., Ward C. A., Paton C. M., Cooper J. A. Submitted to the *American Journal of Clinical Nutrition,* 4/7/2025.

Abstract

Background: Cottonseed oil (CSO) has displayed beneficial effects regarding blood lipids, albeit in relatively high doses.

Objective: The aim was to examine the effects of three doses of CSO versus a control (CON) diet on blood lipids in adults at risk for cardiovascular disease (CVD).

Methods: Ninety-one adults with obese/hypercholesterolemia completed this randomized, parallel clinical trial with a 4-week partial outpatient feeding intervention. Participants were randomized into one of four groups: LOW (n=23), MID (n=23), or HIGH (n=22) providing 10%, 20%, or 30% of estimated energy needs (EEN) from CSO, respectively, or CON (n=23), receiving 10% of EEN from a combination of oils. Fasting blood lipids were obtained at pre- and post-intervention visits, followed by a high-fat meal challenge (35% EEN) with 5-hour postprandial blood draws to evaluate lipid metabolism and glycemia.

Results: Compared to the control diet, total cholesterol (TC) (-10.2 mg/dL; 95% Confidence Interval (CI) -5.1, -15.3; -12.5 mg/dL 95% CI -7.2, -17.8; -13.9 mg/dL; 95% CI -8.5, -19.2) decreased in LOW, MID, and HIGH, respectively ($p < 0.05$ for all). LDL cholesterol (-11.7 mg/dL; 95% CI -16.6, -6.8; -12.9 mg/dL; 95% CI: -17.7, -8.0) reduced in MID and HIGH, respectively, ($p < 0.05$ for both) with a trend in LOW (-6.8 mg/dL; 95% CI: -11.5, -2.2 and -0.2; 95% CI: -0.4, 0.0, respectively) $p = 0.08$ versus no change in CON. Postprandial triglycerides reduced in HIGH (-14.7 mg/dL; 95% CI: -23.3, -6.1; p=0.02) versus LOW, MID, and CON.

Conclusions: Enriching diets with 10%, 20%, and 30% CSO improved fasting blood lipids in CVD at-risk adults with the best results in the 20% and 30% doses. This trial was registered at clinicaltrial.gov as NCT05686954.

Introduction

Cardiovascular disease (CVD) is the leading cause of death globally, accounting for nearly 18 million deaths annually (1). A primary inducing condition in the development of CVD is atherosclerosis. Two commonly identified risk factors in the development of atherosclerosis are elevated blood lipids (2) and obesity (3). In atherosclerosis, elevated blood lipids (hypercholesterolemia) increase circulating levels of cholesterol-rich low-density lipoprotein (LDL) which can penetrate the vascular intima (2). This elicits a series of adaptive immune responses that stimulates the oxidation of LDL, which promotes inflammation within the arterial lining (4). This pro-inflammatory response attracts macrophages that consume oxidized LDL and evolve into foam cells, which, when accumulated, result in a fatty streak. Moreover, the presence of foam cells coupled with ongoing inflammation drives a cycle of lipid accumulation and atherosclerotic plaque growth, progressing to atherosclerosis (5). Furthermore, excess adiposity can cause an influx of inflammatory cytokines that trigger the progression of atherosclerosis, such as endothelial dysfunction (3). Common therapeutic approaches for reducing CVD risk involve targeted modulation of blood lipids through manipulations to dietary nutrient intake and/or overall dietary patterns.

Dietary fatty acid composition has been shown to influence fasting and postprandial blood lipids (6-8). For instance, an effective strategy in mitigating hypercholesterolemia is the replacement of saturated fatty acids (SFA) with unsaturated fatty acids (8, 9) since high intake of some SFAs has been attributed to increase CVD risk (10). Nevertheless, the intake of unsaturated fats, specifically polyunsaturated fatty acids (PUFAs), is known to demonstrate favorable responses concerning blood lipids and overall CVD risk (8, 11). Furthermore, the omega-6 (n-6) PUFA, linoleic acid (LA; 18:2n-6), uniquely influences gene expression in hepatic lipid and

cholesterol mechanisms to lower plasma total cholesterol (TC) and LDL cholesterol concentrations (12).

Cottonseed oil (CSO) is a regularly used vegetable oil in the United States, with 160,000 tons consumed per year (13). Common food sources include mixed oil blends, salad dressings and other condiments, snack foods, and its use as a frying oil. Despite its prevalence in the food system, the body of literature on its effects on human health remains slight. CSO is rich in PUFAs, especially LA, and has exemplified therapeutic effects regarding improvements in blood lipids in both healthy adults (14, 15) and adults with hypercholesterolemia (16). CSO is also unique from other high LA oils as it contains a bioactive lipid, dihydrosterculic acid (DHSA), a known inhibitor of the lipogenic enzyme stearoyl-CoA desaturase-1 (SCD1) (15). Therefore, CSO has a unique cardioprotective makeup in both its high PUFA/LA content's ability to affect lipogenic transcription factors and the presence of DHSA that channels SFA utilization into free fatty acid (FFA) oxidation. Importantly, though, the previously observed benefits were achieved with relatively high doses of CSO which resulted in a high dietary fat intake (>35% estimated energy needs; EEN). Specifically, a high-fat (HF) diet (50% EEN) enriched with 44% of fat from CSO resulted in reductions in fasting TC, LDL cholesterol, and fasting and postprandial triglycerides (TG) in healthy young men (14). These results were complimented by an 8-week trial in hypercholesterolemic adults in which participants had improvements in fasting TC, LDL cholesterol, high density lipoprotein (HDL) cholesterol, TC:HDL ratio, non-HDL cholesterol, and apolipoprotein B (ApoB) following a partial outpatient feeding trial providing a HF diet (49% EEN) that included a high dose of CSO (30% EEN) (16).

Despite evidence demonstrating beneficial effects of n-6 PUFA intake on cardiometabolic outcomes, there is a lack of research on the dose response effects of differing

amounts of PUFAs in the diet. The purpose of this study was to determine the impact of different doses of CSO versus a control (CON) diet over a 4-week period on fasting blood lipids and postprandial lipid metabolism following a high SFA meal challenge in adults at risk of CVD. This was completed by enriching diets with 10%, 20%, or 30% of EEN with CSO; the CON group received 10% of their EEN from a mixture of oils that matched the fatty acid composition of the typical American's diet. We hypothesized that, in a dose-dependent manner, the CSO-enriched diets would improve fasting and postprandial blood lipids (decreases in blood lipid measures except for increases in HDL cholesterol), and all would be different than CON.

Methods

Study Design

This was a single-blind, parallel randomized control trial (clinicaltrials.gov: NCT05686954). There were four groups in which participants were randomized to: the LOW group received 10%, the MID group received 20%, and the HIGH group received 30% of their EEN from CSO. The fourth group served as a CON group in which participants received 10% of their EEN from a mixture of oils designed to match the fatty acid profile of the typical American diet (17). An individual not involved in the data collection or analysis created the randomization. Participants were randomized into 1 of the 4 groups, with an allocation ratio of 1:1:1:1, using a balanced block randomization list stratified by based on the combination of both LDL cholesterol (LDL was categorized into 4 strata: optimal LDL cholesterol < 100mg/dL; above optimal LDL cholesterol 100-129 mg/dL; high LDL cholesterol 130-189 mg/dL; very high LDL cholesterol ≥ 190 mg/dL) and BMI (BMI was categorized into 5 strata: healthy BMI of 18.5-24.9 kg/m^2; overweight BMI of 25-29.9 kg/m^2; obese class I BMI of 30 -34.9 kg/m^2; obese class II BMI of 35-39.9 kg/m^2; obese class III BMI of ≥ 40 kg/m^2). Participants were blinded to their

group assignment. Data collection occurred from March 2023-May 2024, when the goal of at least 20 participants per group was achieved. This study consisted of a 28-day intervention with six visits: one screening visit (v0), a pre-intervention visit (v1), short weekly visits during the intervention (end of weeks 1,2, and 3; v2-4), and a post-intervention visit (end of week 4; v5). The most important primary outcomes included fasting blood lipids (TC, HDL cholesterol, TG, LDL cholesterol, and ApoB). Other primary outcomes of interest included lipid subfractions (LDL particle number (LDL-P), LDL small, LDL medium, LDL peak size, and HDL large) and postprandial measures (TG, non-esterified fatty acids (NEFAs), glucose, and insulin). All primary outcomes were measured at v1 and v5. This study was approved by the University of Georgia Institutional Review Board, and written informed consent was obtained from all participants before any study procedures began.

Participants

Ninety-one (n=23 LOW; n = 23 MID; n = 22 HIGH; n=23 CON) sedentary adults between the ages of 25 and 75y at increased risk of CVD were included in the per-protocol analysis (**Figure 1)**. Increased risk for CVD was defined by either elevated cholesterol profiles or overweight/obesity. Elevated cholesterol profiles were defined as "At risk" in two or more of the variables (TC: 180-239 mg/dL, LDL cholesterol (110-159 mg/dL), TGs (130-199 mg/dL) or “High” in TC (≥240 mg/dL), LDL cholesterol (≥160 mg/dL) or TGs (between 200 - 350 mg/dL). Overweight/obesity was defined by body mass index (BMI ≥ 25 kg/m^2).

To rule out participants with probable familial hypercholesterolemia (18), individuals with TC >290 mg/dL or LDL > 190 mg/dL plus a family history of myocardial infarction (MI) before 50y in a 2nd-degree relative, or below age 60 in a 1st-degree relative were not eligible to

participate in this study. Other exclusion criteria included females on hormone replacement therapy for < 2y, females who are pregnant or nursing, individuals who regularly exercised > 3h/wk, weight gain or loss >5% of body weight in the past 3mo, plans to begin a weight loss/exercise regimen during the trial, history of medical or surgical events that could affect digestion or swallowing, gastrointestinal surgeries, conditions or disorders, any chronic diseases (including moderate to severe asthma, chronic lung disease, and kidney disease), metabolic diseases, atherosclerosis, previous MI or stroke, cancer, fasting blood glucose levels > 126 mg/dL or blood pressure > 180/120 mmHg were also excluded. Individuals with medication use affecting digestion and absorption, metabolism (e.g., thyroid meds), lipid-lowering medications, medications for diabetes, steroid/hormone therapies, or current antibiotic cycles were excluded. Individuals who were on a medically prescribed or special diet or had food allergies (specific to the foods in the study) were not eligible. Additionally, those taking fish oil supplements, excessive alcohol use (> 3 drinks/d for males; > 2 drinks/d for females), tobacco or nicotine use, or underweight BMI (<18.5 kg/m^2) were excluded.

Screening Visit (v0)

Participants completed an online screening form to assess preliminary qualification. After preliminary qualification, participants underwent an initial phone screening, then, participants reported to the Human Nutrition Lab (HNL) following an 8-12h overnight fast and abstaining from exercise and alcohol for at least 24h. Height, weight, and blood pressure were recorded, and BMI inclusion criteria was confirmed. A fasting blood sample was obtained to assess for standard blood lipids and blood glucose to determine eligibility as described above in exclusion criteria (Piedmont Athens Regional Laboratory, Athens, GA). An Alcohol Use Disorders Identification Test (AUDIT) was given to participants to determine alcohol consumption habits

(19). Next, resting metabolic rate (RMR; kilocalories/day) was measured utilizing indirect calorimetry (TrueOne 2400, Parvo Medics, Sandy, UT) following standard procedures (20). Total daily EEN were calculated based on a participant's RMR multiplied by an activity factor of 1.55 (21). This calculation was used for the 28-day diet and meal challenges at the pre- and post-intervention visits. Individuals who qualified for the study were then randomized into one of the four groups with a random number generator.

Pre-Intervention Visit (v1)

Participants arrived at the HNL in a fasted state (8-12h fast) and 24h without alcohol or exercise. Upon arrival, participants turned in a 3-day food record (2 weekdays, 1 weekend day) (22). All participants consumed a standardized meal and snack for dinner the night before v1 comprised of ~35% of total energy from fat, 15% protein, and 50% carbohydrate. In the lab, anthropometrics were measured as described in the screening visit (height and weight). Additionally, at this visit waist & hip circumference were measured in triplicate to calculate waist-to-hip ratio. Body composition was measured using a Bod Pod (Cosmed USA, Inc.). Then, an intravenous (IV) catheter was placed and a fasting blood sample was collected 5 minutes after IV insertion. The line was kept patent with a saline solution. Next, participants consumed a high-fat meal challenge rich in SFAs within a 10-minute period. The liquid meal contained a milk-chocolate, ready-to-drink shake (Ensure, Abbott Laboratories, Inc.), unsalted butter, red palm oil, coconut oil, soy lecithin granules, and powdered chocolate drink mix. The meal provided 35% of the participant's total EEN, and the composition of the meal can be found in Prater et al. (16). A validated sensory questionnaire, using a modified 9-point hedonic scale with '1' indicating 'dislike extremely' and '9' indicating 'like extremely,' was given to assess perception of the study foods (23). Blood draws then occurred at 30, 60, 90, 120, 150, 180, 240, and 300 minutes

(5h) postprandially. Finally, participants completed questionnaires including the perceived stress scale (PSS) (24), the State Trait Anxiety Inventory (STAI) (25), and the international physical activity questionnaire (IPAQ) (26).

28-day Dietary Intervention

Following v1, participants began the 28-day diet intervention. Before leaving the HNL after v1, participants were given a one-week supply of daily shakes and snacks that corresponded to their assigned diet group. Participants were instructed to consume all provided foods and maintain their normal dietary habits for their remaining meals each day. Participants in the MID, LOW, and CON group received all their oil in the form of a breakfast shake with an additional snack item that did not contain oil. The HIGH group received oil through both their breakfast shake and the daily snack **(Table 1).** The ingredients for the breakfast shakes and snacks were identical between groups with the only differences being the amount of the assigned oil incorporated into the foods. Participants were provided the amount of calories they would be receiving from their provided intervention foods. Participants were then counseled to consume the shake as a meal replacement and replace the snack with a food item of a similar caloric density from their diet. To implement this, research personnel worked with participants to understand or identify foods in their usual intake (from their food records) that could be suitable replacements for the intervention foods. In order to provide doses to the groups at 10, 20, and 30% of energy needs from oils, by design, participants received different amounts of total energy from the intervention foods. This was not a complete in-patient or out-patient feeding study (where research personnel could adjust the energy content of other meals to ensure energy balance for each individual), so the self-selection of energy content and amount for remaining meals was left to the participant in this free-living scenario.

During weeks 2 and 4 of the intervention, participants completed 3-day food records (two weekdays and one weekend day). Daily nutrient intakes from the food diaries were assessed using The Food Processor SQL software (version 11.11.32; Salem, OR, USA). Participants were also asked to record the foods consumed daily during the intervention using the Compliance Checklist. Participants that consumed less than 75% of provided foods were deemed non-compliant and removed from the final data analysis. Additionally, participants were asked to return empty food containers at their weekly visits and the post-intervention visit to aid in compliance checks (v2-5).

Visits 2, 3, and 4 (v2-4)

During the 28-day intervention participants returned to the HNL each week for short study visits (v2-v4). At these visits, a fasting blood draw and body weight was collected. Additionally, at v3 the IPAQ was completed. Participants consumed their breakfast shake in the lab, turned in study documents, and collected their foods for the next 7 days. Those documents included (1) compliance checklist, (2) saved food containers, (3) 3-day food records (v3 only), and (4) the sensory evaluation logs (as completed). At v4 participants were additionally provided the lead-in meal to be consumed the night before v5.

Post-Intervention Visit (v5)

The day after the 28-day intervention, participants reported to the HNL for v5 following the same 8-12h fast and 24h abstinence from alcohol and exercise. The evening before v5, participants consumed the dinner meal and snack provided at v4, which was the exact same meal as that consumed prior to v1. All procedures performed at the pre-intervention visit (v1) were

repeated including consumption of the same high SFA meal, anthropometric measurements, questionnaires, and blood draws.

Sample Analysis

All blood samples were drawn into K2 EDTA-coated vacutainers (Becton, Dickinson, and Company), and immediately placed on ice. For the lipid panel at pre-and post-intervention visits (v1 and v5), a portion of the fasting blood sample was drawn into a serum separator clot activator vacutainer and kept at room temperature for 30 minutes before centrifugation. Blood samples were centrifuged for 15 minutes at 3,000 rpm at 4°C. The serum was held at 4°C until the advanced blood lipid panel was performed (Quest Nichols Institute, Chantilly, VA, USA). This lipid panel, which was the primary outcome, included TC, HDL cholesterol, TG, LDL cholesterol, LDL-P, LDL size, HDL size, ApoB, and lipoprotein(a) through spectrophotometry and ion mobility lipoprotein fractionation. With the same serum from v1 and v5, a liver function panel was performed as a secondary outcome (Piedmont Regional, Athens, Georgia, USA). This panel included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), albumin, albumin:globulin ratio, and total protein through spectrophotometry. The remaining fasting sample and all postprandial samples were centrifuged under the same conditions, then aliquoted, and stored at -80°C until analysis. Primary postprandial outcomes of plasma TG (inter-assay coefficient of variation (CV): 7.3%; intra-assay CV: 3.1%) and NEFAs (inter-assay CV: 7.5%; intra-assay CV: 2.0%) were measured by enzyme-based calorimetric assays (Wako Chemicals USA, Inc., Richmond, VA). Additionally, glucose (inter-assay CV: 1.1%; intra-assay CV: 2.2%) was measured using a colorimetric glucose oxidase and peroxidase method (glucose oxidase, G2133; peroxidase, P8250; Sigma Aldrich), and insulin (inter-assay CV: 6.6%; intra-assay CV: 4.2%) was measured

by radioimmunoassay as primary outcomes (MilliporeSigma, Darmstadt, Germany). An internal control was utilized for each assay to assess assay performance to calculate the intra-and inter-assay CV. All of the samples for a given participants were run within the same assay (i.e. no individual participant samples were split across assays). Additionally, none of the data fell above or below the lower lipids of quantification or detection.

Statistical Analysis

The SAS version 9.2 statistical package (SAS Institute INC, Cary, NC, USA) was used for all data analysis, and statistical significance was set at $p \leq 0.05$. All results were reported as mean ± SEM unless otherwise noted. A sample size of 72 (18/group) was estimated to detect a difference in LDL cholesterol (Cohen's F of 0.335) based on the results of Prater et al. (16) using G*power 3.19.7 and assuming at least 80% power with an α of 0.05. The decision to use per-protocol analysis was made *a priori*. An analysis of baseline characteristics of participants lost to follow-up and included in the analysis was performed (**Supplementary Table 1**). The normality assumption was tested using the Shapiro-Wilk test and normal probability (Q-Q) plots, and a log transformation was conducted to non-normal data before analysis. All models were adjusted for baseline age (y), sex (male or female), weight (kg), and BMI (kg/m^2). Data was assumed to be missing completely at random. This was validated by conducting a logistic regression analysis to assess whether missingness was associated with group, age, or sex which revealed no significant differences between the missingness indicator and outcomes with missing data. Missing data was handled within our linear mixed model utilizing the restricted maximum likelihood estimation without the need for advanced imputation.

To determine between- and within-group differences from pre to post-intervention for primary outcomes of blood lipids and lipid subfractions repeated measures (RM) linear mixed

models including factors of group and visit were utilized. For primary outcomes for meal response data (NEFA, TG, glucose, insulin) RM linear mixed models including the fixed effect of group and repeated effects of visit and postprandial timepoint were utilized. As secondary analysis, RM linear mixed models for group and visit were used to assess differences between and within groups for hepatic function measures.

For all other outcomes such as compliance, perceived stress, total MET minutes, and self-reported dietary intake RM linear mixed models for group and visit were used to assess differences between and within groups from pre- to post-intervention. An "intervention average" was calculated for total MET minutes (IPAQ from mid-intervention (week 2) and end of intervention (week 4)) and dietary intake (2 weekdays and 1 weekend day food records from mid-intervention) which was compared to baseline averages. If significance was found, a Tukey-Kramer post-hoc test was performed to adjust for multiple comparisons. For all linear mixed models, participants were modeled as a random effect.

Results

Participants

Ninety-five participants were randomly allocated into intervention groups (n=23 LOW; n = 25 MID; n = 24 HIGH; n=23 CON); however, 4 participants were not included in the final analysis (**Figure 1**). Therefore, 91 participants completed the intervention (20 females and 3 males in the LOW group, 20 females and 3 males in the MID group, 20 females and 2 males in the HIGH group, and 21 females and 2 males in the CON group) and were included in this per protocol analysis. Baseline participant demographics are presented in **Table 2.** There were no differences between groups at baseline. For body weight, there was a group x visit interaction (p

= 0.01). Post hoc analyses revealed that this interaction was driven by increases in LOW, MID, and HIGH ($p < 0.01$, $p = 0.01$, $p < 0.001$, respectively) versus no change in CON ($p = 0.17$). For BMI there was a trend for a group x visit interaction ($p = 0.097$). Post hoc analyses showed the trend in the interaction was driven by increases in LOW, MID, and HIGH ($p = 0.03$, $p < 0.01$, $p < 0.001$, respectively) versus CON ($p = 0.20$). There were no other interaction effects in any other characteristics.

On average, participants in the LOW, MID, HIGH, and CON group reported consuming 94 ± 1%, 94 ± 1%, 94 ± 2%, 93 ± 1%, of the provided intervention foods, respectively, and compliance was not different between groups. However, 1 participant from the HIGH group was excluded from final analysis due to poor compliance (defined as <75% study food consumption). Self-reported dietary intake is presented in **Table 3**. There were no differences between groups at baseline for the nutrients analyzed. With respect to total fat intake, there was a group x visit interaction ($p < 0.01$) driven by increases LOW ($p < 0.01$) and HIGH ($p < 0.01$) versus no change in MID ($p < 0.82$) and CON ($p = 0.92$). Further, there was a group x visit interaction ($p < 0.01$) for n-3 fatty acid intake driven by decreases in MID ($p < 0.01$) and HIGH ($p < 0.01$) versus no change in LOW ($p = 0.47$) and CON ($p = 0.99$). Regarding total PUFA as well as n-6 fatty acid intake, there were group x visit interactions ($p < 0.01$ for both), driven by increases in LOW, MID, and HIGH ($p < 0.01$ for all) versus no change in CON ($p = 0.97$ for both). There were no interaction effects for other self-reported dietary intake values.

Fasting blood lipid markers

Fasting biochemical markers are presented in **Figure 2** and **Table 4.** There were no differences at baseline for blood lipids or markers of glycemia between groups.

For TC, there was a group x visit interaction ($p = 0.02$), driven by reductions in the LOW, MID, and HIGH CSO groups (-10.2 mg/dL (estimate of difference of least square means); 95% Confidence Interval (CI): -15.3, -5.1; $p < 0.01$, -12.5 mg/dL; 95% CI: -17.8, -7.2; $p < 0.001$, -13.9 mg/dL; 95% CI: -19.2, -5.5 $p < 0.001$, respectively) versus no change in CON (2.9 mg/dL; 95% CI: -8.1, 2.3; $p = 0.95$). For LDL cholesterol, there was a group x visit interaction ($p = 0.03$) driven by reductions in the MID and HIGH CSO groups (-11.7 mg/dL; 95% CI: -16.6, -6.8; $p <0.001$ and -12.9 mg/dL; 95% CI: -17.7, - 8.0; $p < 0.001$, respectively), and a trend in the LOW group (-6.8 mg/dL; 95% CI: -11.5, -2.2;$p = 0.08$), versus no change in CON (-3.8 mg/dL; 95% CI: -8.6, 0.9; $p = 0.75$). Further, there was an interaction effect for non-HDL cholesterol ($p < 0.01$), driven by reductions in the LOW, MID, and HIGH CSO groups (-10.2 mg/dL; 95% CI: -15.2, -5.2; $p < 0.01$; -15.8 mg/dL; 95% CI: -21.0, -10.6; $p < 0.001$; -16.5 mg/dL; 95% CI: -21.7, -11.3; $p < 0.001$, respectively) versus no change in CON (-4.0 mg/dL; 95% CI: -9.2, 1.0; $p = 0.75$). Similarly, there was a group x visit interaction for TC:HDL ratio ($p < 0.01$), driven by reductions in the MID and HIGH (-0.4; 95% CI: -0.5, - 0.2 and – 0.4; 95% CI: - 0.6, - 0.2, r respectively, $p < 0.001$ for both) and a trend for reductions in LOW (-0.2; 95% CI: -0.4, 0.0; $p = 0.09$) with no change in CON (-0.1; 95% CI: -0.3, 0.0;$p = 0.74$).

For TG, there was a trend for a group x visit interaction ($p = 0.10$); however, post-hoc analyses revealed this interaction to be driven by non-significant decreases in the LOW, MID, HIGH groups, and increases in CON (-7.5 mg/dL; 95% CI: -18.5, 3.5; $p = 0.65$; -6.0 mg/dL; 95% CI: -17.0, 5.0; $p = 0.93$, -12.3 mg/dL; 95% CI: -23.5, -1.0; $p = 0.49$, 7.5 mg/dL, 95% CI: -8.6, 0.9; $p = 0.94$, respectively). There were no other group by visit interactions for any blood lipid variables. there were no interaction effects in fasting HDL cholesterol, NEFAs, LDL small, LDL medium, LDL peak size, LDL-P, HDL large, ApoB, insulin, and glucose.

Postprandial biochemical markers

The meal responses for TGs are presented in **Figures 3 and 4**. Postprandial TGs were suppressed in HIGH (-14.7 mg/dL; 95% CI: -17.2, 0.7; p = 0.02) versus no change in LOW (-7.5 mg/dL; 95% CI: -16.6, 0.5; p = 0.4), MID (-6.0 mg/dL; 95% CI: -15.6, 0.3; p = 0.4), or CON (7.5 mg/dL; 95% CI: -23.3, -6.1; p = 0.4). There were no interaction effects for NEFAs, insulin, and glucose.

Fasting liver function markers

At baseline, albumin was significantly higher in MID ($p < 0.01$) versus LOW and HIGH groups ($p < 0.01$ and $p = 0.04$, respectively). With respect to liver function measures, there was a group x visit interaction ($p = 0.02$) for albumin: globulin ratio, driven by reductions in LOW (-0.2; 95% CI: -0.3, 0.0; $p = 0.02$), versus no change in MID, HIGH, and CON (0.1; 95% CI: 0.0, 0.2 $p = 0.97$; 0.1; 95% CI: -0.1, 0.0; $p = 0.82$; 0.1; 95% CI: - 0.2, 0.0; $p = 0.82$, respectively). Finally, there were no interaction effects in albumin, ALP, GGT, ALT, AST, and total protein.

Discussion

For the first time, in a population at risk for CVD, we have shown that lower doses of CSO (< 30% EEN) resulted in significant improvements in fasting blood lipids. Specifically, we observed decreases in fasting TC, non-HDL cholesterol, and TC:HDL ratio with no worsening of liver function measures in diets enriched with 10%, 20%, and 30% CSO compared to a CON diet. Further, enriching diets with 20% and 30% CSO led to significant reductions in LDL cholesterol, while the 10% CSO dose group displayed only a trend for reductions in LDL cholesterol. Additionally, the 30% CSO dose suppressed postprandial TGs after a HF meal

challenge. Altogether, this provides further evidence of CSO's lipid lowering effects, even at lower doses of consumption.

Cottonseed oil has a unique fatty acid composition because of its high PUFA content, but also high SFA content (27% SFA, 19% MUFA, 54% PUFA; **Supplemental Table 2**). Given its high SFA content, CSO could be regarded as a "less healthy" oil. Yet, CSO is very effective at reducing blood lipid levels (14-16), even when compared to other high n-6 PUFA sources in pre-clinical models (27-32). It was not surprising that we observed improvements in blood lipids in the HIGH group (30% EEN from CSO) based on past literature utilizing this dose to yield similar effects (14, 16). While intake of ~15-20% of energy from canola and corn oil has been identified for optimal changes to blood lipids (33, 34), prior to this study, there had been no dose response clinical trials for CSO. Therefore, this is the first study to demonstrate that doses lower than 30% EEN of CSO improve blood lipids, even when slight, but statistically significant, weight gain occurs. Notably, we did not observe significant changes with respect to lipid subfractions, HDL-cholesterol, or insulin. This is consistent with a previous CSO trial (16). While these subfraction measures are important for getting a complete picture of cardiometabolic health, they are often overlooked in clinical trials where blood lipid changes are being studied. As a result, they are less well understood and the implications for changes in LDL cholesterol, but not changes in subfractions, are unclear. Changes in lipid subfractions can be influenced by genetics and overall diet composition, so that may have influenced the lack of change observed in the present study (35, 36); however, additional research in this area is warranted.

The drawback with utilizing high doses of an intervention oil is that total fat intake typically exceeds the acceptable macronutrient distribution range (AMDR) for total fat as determined by the Dietary Guidelines (current AMDR is 20-35%) (37). Previous studies

demonstrating lipid lowering effects with CSO have corresponded to participants' total fat intake ranging from 40-50% of total energy (14-16). Therefore, the idea behind this dose response study was to determine whether lower doses of CSO would be effective at lowering blood lipids, while hopefully keeping total daily fat intake within the AMDR to encourage healthier dietary patterns. Unfortunately, the former was more successful than the latter. The actual percentage of energy from fat was 45.1%, 44.2%, and 50.6% in the LOW, MID, and HIGH CSO groups, respectively, and 41.5% in the CON. Therefore, even with our LOW dose (and CON) where 10% of energy from fat was provided in the intervention, the fat intake for remaining self-selected meals/snacks was not low enough for participants to fall within the AMDR. Importantly, in our CVD at-risk population, self-reported intake revealed that participants were already following a HF diet before the start of the intervention (LOW: 36.1%; MID: 39.0%; HIGH: 39.3%; CON: 40.0%) which is slightly higher than U.S. averages (~36% of energy) (38). Thus, even with isocaloric integration of the study foods, participants would likely still have been on a HF diet.

This HF diet consumption, particularly in the HIGH group, was a possible cause of the weight/BMI gain that occurred during the intervention in the groups since dietary fat has such a high energy content. Although dietary substitution instructions were provided, and the study foods were well liked and easy for participants to incorporate in their daily lives, complete energy balance was still not achieved. While high doses of CSO provided positive effects with respect to blood lipids, the combined effect of weight gain and a HF diet could have led to the non-significant changes in insulin sensitivity observed in the CSO HIGH group. While the body adapts to HF diets by increasing fat oxidation to match the higher fat intake (39), a positive energy balance can still occur, which can negatively impact insulin sensitivity over time (40). This highlights another potential benefit of utilizing lower doses of CSO for blood lipid

reductions so as not to negatively influence glycemic regulation long-term. Therefore, future studies utilizing lower doses of CSO may need to incorporate dietary counseling centered around total fat reduction in remaining meals to achieve the AMDR. Additionally, emphasizing how to incorporate CSO into one's diet to support cholesterol reduction while maintaining a healthy weight or an overall healthy diet is necessary.

In human studies, it is important to consider not just statistical significance but the clinical relevance of the findings as well. We found that the reductions in TC for the LOW, MID, HIGH CSO groups were 4.9%, 6.3%, and 6.7%, respectively. For LDL cholesterol, the MID and HIGH CSO groups elicited a comparable reduction of 10.4% and 10.8%, respectively, compared to 4.1% reduction in the CON group. These results are clinically meaningful because every 1% of LDL cholesterol reduction equates to a 1.2% to 2.0% reduction of coronary artery disease (CAD) (41, 42), resulting in an estimated ~12.3 to 21% CAD risk reduction with MID and HIGH doses of CSO. Moreover, LDL cholesterol reduction serves as a primary therapeutic target in mitigating and preventing CVD (43-45). Therefore, the magnitude of LDL reduction in our two highest CSO doses was comparable to that of commonly used lipid-lowering pharmacological therapies including bile acid sequestrants, fibrates, and nicotinic acid, without adverse side effects commonly reported while taking these therapies (46).

Although we hypothesized that the LOW, MID, and HIGH CSO groups would elicit improvements in blood lipids in a dose-dependent manner, it is notable that the intake of 20 and 30% EEN from CSO produced a similar magnitude of effect while the LOW dose appeared to have a slightly less magnitude of response. Still, the significant reductions observed within the LOW group for TC, non-HDL cholesterol, and TC-HDL ratio, and a trend for improvements in LDL cholesterol, present a novel finding with such a low intake of CSO. Given that the LOW

group were consuming ≥ 5% EEN from LA (12.0g-16.7g LA/day, dependent on EEN), their reductions align with existing evidence that consuming 5-10% of energy from LA results in improvements in LDL cholesterol and TC (47). We can theorize that a longer intervention period may lead to greater reduction in LDL cholesterol and reveal statistically significant reductions with the LOW dose. This theory is corroborated by the continued reductions observed with a previous CSO intervention from 4 to 8 weeks in a longer-term trial (16). Nevertheless, evaluating doses < 20% in longer-term studies is warranted to understand the relationship of the length of the intervention and magnitude of response versus a control diet.

There are specific components of CSO thought to be responsible for its beneficial effects on lipid metabolism. CSO has the ability to interact with transcription factors through its high PUFA content to regulate lipid and cholesterol metabolism (48). Beyond just the high LA content, though, CSO is unique in that it contains a bioactive lipid, dihydrosterculic acid (DHSA), a known inhibitor of the lipogenic enzyme stearoyl-CoA desaturase-1 (SCD1) (27). Specifically, SCD1 is a lipogenic enzyme that catalyzes the desaturation of SFA to MUFAs, promoting lipid storage rather than free fatty acid oxidation (49). This coupled effect of high PUFA content and DHSA may be necessary to elicit the strong effects for blood lipids observed in previous CSO trials. There are other components of CSO that may contribute to the cholesterol-lowering effect, including tocopherols and beta-sitosterol (50). Nonetheless, it is important to consider that CSO as whole food may be required to elicit the marketed changes in blood lipid metabolism rather than an isolated component.

U.S. adults spend nearly two-thirds of their day in the postprandial state, a period known to exacerbate disease development (51, 52). Our study design allowed us to assess the effects of chronic CSO consumption on postprandial metabolism following a SFA-rich, HF meal typical of

Western dietary patterns. While we did not observe significant suppression of NEFA and glucose with CSO consumption, as reported in a previous 8-week CSO trial following the same meal challenge design (16), we did observe significant suppression of postprandial TGs with our 30% CSO dose group. This aligns with findings from Polley et al. (14) who reported a similar suppression of postprandial TGs with acute CSO consumption. Importantly, the suppression of postprandial TGs in this study may be partially attributed to the non-significant decrease in fasting TGs in the HIGH group. Even so, this finding suggests that CSO may offer protective effects by attenuating postprandial TGs following high SFA meal ingestion, but potentially only at high daily doses of CSO. Further investigation is warranted to understand the relationship between length and dose of CSO consumption and postprandial responses.

There were some limitations with this study. Our control group received a mixture of oils matching the FA composition of the U.S. diet. These oils were mixed into the same foods that the CSO groups received intentionally to isolate the effect of CSO; however, we did not have a control group that received no food or oils. To keep the groups at 10, 20, and 30% of energy from oils, by design, participants received different amounts of total energy from intervention foods (**Table 1**). The slight, non-significant improvements in some outcome measures in the control group may have prevented the observation of significance in those measures with our LOW group, such as with LDL cholesterol. Also, participants were free-living, making it possible to consume other foods and beverages. Again, this was done by design as our goal was to determine whether incorporation of CSO into one's diet could improve CVD risk measures. Measures of dietary intake, physical activity, stress, and compliance were all self-reported, which may contain some degree of under- or over-reporting, and we did not utilize direct measures or biomarkers to assess compliance such as LA. We did try to control for error in reporting

compliance by counting empty food containers brought back by participants on their weekly visits and comparing that to their compliancy log. While our study was powered to detect changes in LDL cholesterol, we evaluated additional fasting and postprandial markers related to cardiometabolic health to provide a more comprehensive picture of the blood lipid responses to the intervention. However, we made no adjustments for multiple primary and secondary outcome variables; therefore, these results should be interpreted with caution. This study also utilized a per protocol analysis which introduces some degree of bias which should be considered when interpreting these results. While we recruited both males and females, our study had predominantly female participants, so future work should also emphasize male participants. Finally, this design was single blinded, which can introduce bias such as observer or experimenter bias.

In conclusion, we have shown that diets enriched with 10%, 20%, and 30% from CSO reduced TC, non-HDL cholesterol, and TC:HDL ratio versus a control diet in adults at risk for CVD. Diets enriched with 20% and 30% from CSO also had significant reductions in LDL cholesterol while the 10% CSO dose only exhibited a trend in LDL cholesterol improvements. These reductions in blood lipids at lower doses of CSO may enable the adoption of healthier dietary patterns (i.e. lower fat intake) while still reaping the benefits of CSO consumption. Future studies should investigate CSO diet enrichment in different populations and at lower doses, especially the 10% dose, for longer durations. Also, future studies should evaluate other high-PUFA oils compared to CSO to decipher if CSO has superior lipid-lowering effects. Lastly, to provide a more robust understanding of CSO's effects on CVD risk, other studies should asses markers of other chronic disease risk factors.

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Table 3.1. Description of Diet Interventions

Intervention Group	Percentage of energy provided from oil per day	Percentage of energy from provided foods per day	Provided food items that contained oil	Provided food items that do not contain oil
CSO HIGH	30%	47%	Breakfast shake and 1 snack[1,2]	N/A
CSO MID	20%	37%	Breakfast shake[2]	1 snack[1]
CSO LOW	10%	27%	Breakfast shake[2]	1 snack[1]
CONTROL	10%	27%	Breakfast shake[3]	1 snack[1]

Abbreviation: CSO = Cottonseed oil

[1]The snack was either a yogurt or pudding.

[2]Cottonseed oil was utilized in food.

[3]Control oil mixture containing canola, coconut, corn, safflower, and soybean oil was utilized in food.

Table 3.2. Characteristics of adults at-risk of cardiovascular disease at baseline and week 4 in cottonseed oil or control groups[1]

	CSO LOW (n = 23)		CSO MID (n = 23)		CSO HIGH (n = 22)		Control (n = 23)		p values
Characteristic	Baseline	Week 4	Baseline	Week 4	Baseline	Week 4	Baseline	Week 4	Interaction
Female, %	86	—	86	—	91	—	91	—	
Age, y	47 ± 3	—	46 ± 3	—	45 ± 3	—	49 ± 2	—	
"At risk":"High" (n)	7:7	—	9:3	—	12:2	—	10:6	—	
Height, cm	167.0 ± 1.6	167.0 ± 1.7	165.5 ± 2.2	165.3 ± 2.2	165.4 ± 1.4	165.4 ± 1.4	163.8 ± 1.3	163.8 ± 1.3	0.49
Weight, kg	91.5 ± 3.9	92.3 ± 4.0[2]	85.1 ± 3.7	86.0 ± 3.8[2]	90.2 ± 4.3	91.8 ± 4.3[2]	87.8 ± 4.7	88.4 ± 4.7	0.01
BMI, kg/m^2	32.8 ± 1.8	33.0 ± 1.3[2]	31.1 ± 1.3	31.5 ± 1.3[2]	33.0 ± 1.5	33.5 ± 1.5[2]	32.6 ± 1.6	32.9 ± 1.6	0.10
WC, cm	98.2 ± 2.9	98.4 ± 3.1	95.0 ± 2.6	96.5 ± 2.6	99.7 ± 2.8	102.0 ± 3.3	96.8 ± 3.6	95.7 ± 3.7	0.15
HC, cm	117.6 ± 2.1	116.5 ± 2.2	114.1 ± 2.3	114.9 ± 2.4	117.5 ± 3.1	118.8 ± 3.3	116.7 ± 3.4	116.7 ± 3.3	0.21
WHR	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.61
SBP, mmHg	135.4 ± 3.6	132.3 ± 3.2	128.8 ± 4.3	137.9 ± 3.5	133.2 ± 3.2	136.5 ± 3.5	135.2 ± 3.6	132.5 ± 3.5	0.78
DBP, mmHg	84.5 ± 2.9	81.8 ± 2.4	78.7 ± 3.2	78.0 ± 2.9	79.9 ± 1.9	81.0 ± 1.6	82.0 ± 2.6	83.4 ± 2.1	0.31
Body fat, %	40.5 ± 1.6	40.7 ± 1.6	38.5 ± 1.7	38.6 ± 1.8	41.3 ± 2.0	42.0 ± 2.2	40.8 ± 2.0	40.5 ± 2.0	0.36
Total MET[1],min/wk	2007 ± 384	2561 ± 606	2204 ± 463	1918 ± 274	1949 ± 341	2131 ± 401	1494 ± 237	1581 ± 242	0.53
STAI	50.1 ± 2.7	52.3 ± 3.5	53.6 ± 3.1	51.1 ± 2.8	58.8 ± 5.6	59.7 ± 6.9	50.3 ± 3.1	48.0 ± 2.0	0.23
PSS	12 ± 1	11 ± 1	11 ± 1	12 ± 1	12 ± 2	12 ± 2	12 ± 1	10 ±1	0.11

[1]All values are means ± SEMs. Anthropometrics were analyzed with a 2-way (group x visit) linear mixed model. Elevated cholesterol profiles were defined as "At risk" in two or more of the variables (TC: 180-239 mg/dL, LDL cholesterol (110-159 mg/dL), TGs (130-199 mg/dL) or "High" in TC (≥240 mg/dL), LDL cholesterol (≥160 mg/dL) or TGs (between 200 - 350 mg/dL). Week 4 values represents an average of total MET min recorded during the 4-week intervention. BMI, body mass index; CSO, cottonseed oil; DBP, diastolic blood pressure; HC, hip circumference, MET, metabolic equivalent (min/wk); PSS, perceived stress scale; SBP, systolic blood pressure; STAI, state strait anxiety inventory; TFEQ, three factor eating questionnaire; WC, waist circumference; WHR waist-to-hip ratio.

[2] Indicates a significant difference from baseline to week 4 versus control.

Table 3.3 Self-reported nutrient intake at baseline and intervention period in cottonseed oil or control groups.[1]

	CSO LOW (n = 23)		CSO MID (n = 23)		CSO HIGH (n = 22)		Control (n = 23)		p values
Measure	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention	Interaction
Energy, kcal/d	2010 ± 135	2595 ± 142	2369 ± 249	2670 ± 268	2230 ± 223	2782 ± 172	2151 ± 142	2362 ± 184	0.27
Protein, g/d	74.9 ± 5.8	88.1 ± 4.7	86.9 ± 8.5	121. 4 ± 34.7	89.0 ± 10.5	89.0 ± 6.2	76.7 ± 5.2	87.3 ± 5.5	0.47
Carbohydrate, g/d	234.7 ± 18.5	258.4 ± 19.0	249.3 ± 21.8	255.5 ± 28.0	234.7 ± 26.5	236.3 ± 17.3	226.1 ± 19.3	243.5 ± 23.0	0.86
Fiber, g/d	14.7 ± 1.5	14.3 ± 1.4	19.3 ± 2.6	14.5 ± 1.7	16.8 ± 2.0	16.2 ± 2.2	17.8 ± 1.8	13.4 ± 1.7	0.17
Sugar, g/d	94.8 ± 10.3	121.1 ± 8.6	90.0 ± 9.1	125.7 ± 19.7	74.5 ± 9.4	111.3 ± 10.9	89.6 ± 11.0	116.0 ± 11.3	0.90
Fat, g/d	78.0 ± 5.6	124.0 ± 6.5[3]	103.5 ± 15.5	118.7 ± 6.0	93.2 ± 9.5	151.6 ± 10.1[3]	92.9 ± 7.2	105.5 ± 9.0	<0.01
Saturated Fat, g/d	26.0 ± 1.8	38.2 ± 2.2	35.3 ± 4.9	36.4 ± 1.8	30.1 ± 3.6	45.4 ± 1.2	33.2 ± 3.6	38.9 ± 4.5	0.05
Trans fat, g/d	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.8 ± 0.3	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.07
MUFA, g/d	31.8 ± 2.8	34.5 ± 1.8	39.0 ± 5.7	33.4 ± 2.2	38.0 ± 4.1	41.4 ± 3.7	38.3 ± 2.8	41.4 ± 3.4	0.12
PUFA, g/d	19.8 ± 1.6	51.9 ± 3.0[3]	28.7 ± 5.6	48.6 ± 2.5[3]	24.3 ± 2.0	64.7 ± 3.1[3]	21.0 ± 1.8	24.6 ± 2.3	<0.01
n-3 fatty acid, g/d	2.2 ± 0.4	1.4 ± 0.1	2.6 ± 0.5	1.1 ± 0.1[3]	3.0 ± 0.6	1.4 ± 0.2[3]	1.8 ± 0.2	2.1 ± 0.2	<0.01
n-6 fatty acid, g/d	17.7 ± 1.5	50.5 ± 3.0[3]	26.1 ± 5.1	47.5 ± 2.5[3]	21.3 ± 1.8	63.3 ± 3.0[3]	19.1 ± 1.7	22.5 ± 2.1	<0.01
Alcohol, g/d	2.1 ± 1.2	1.8 ± 0.5	4.0 ± 1.4	3.1 ± 2.8	5.0 ± 2.1	5.5 ± 1.8	5.1 ± 1.3	3.6 ± 1.0	0.87

[1]All values are means ± SEMs. Baseline values are an average of 3 food diaries before the intervention. Intervention values are an average of all 6 food diaries kept throughout the intervention. All values were analyzed with a 2-way (group x visit) linear mixed model. CSO, cottonseed oil; d, day; MUFA, monounsaturated fatty acid; n-3, omega 3; n-6 omega 6 PUFA, polyunsaturated fatty acid.

[2]Indicates a trend for a difference from baseline to intervention versus control.

[3]Indiates a significant difference from baseline to intervention versus control.

Table 3.4. Fasting biochemical markers of adults at risk of cardiovascular disease at baseline and week 4 in cottonseed oil or control groups.[1]

	CSO LOW (n = 23)		CSO MID (n = 23)		CSO HIGH (n = 22)		Control (n = 23)		p values
Measure	Baseline	Week 4	Baseline	Week 4	Baseline	Week 4	Baseline	Week 4	Interaction
TC, mg/dL	200.3 ± 9.0	190.3 ± 8.9[2]	193.2 ± 6.8	180.6 ± 6.1[2]	193.9 ± 6.8	180.0 ± 5.9[2]	186.7 ± 7.2	183.7 ± 7.4	0.02
LDL-c, mg/dL	115.5 ± 7.3	109.0 ± 6.4[3]	113.1 ± 5.1	101.3 ± 5.1[2]	114.4 ± 5.5	101.5 ± 5.0[2]	106.9 ± 6.5	103.0 ± 6.6	0.03
HDL-c, mg/dL	59.3 ± 3.3	59.3 ± 3.4	56.3 ± 3.2	59.5 ± 3.5	56.6 ± 3.1	59.2 ± 3.5	55.8 ± 2.2	57.0 ± 2.4	0.20
TG, mg/dL	115.4 ± 11.4	107.4 ± 12.3	108.5 ± 10.5	102.8 ± 10.4	109.5 ± 9.8	97.2 ± 8.0	129.4 ± 14.2	136.9 ± 15.0	0.10
Non-HDL-c, mg/dL	141.0 ± 8.2	131.0 ± 7.6[2]	136.9 ± 6.2	121.1 ± 5.9[2]	137.3 ± 6.3	120.8 ± 5.9[2]	130.8 ± 7.1	126.7 ± 7.0	< 0.01
TC:HDL-c ratio	3.6 ± 0.2	3.3 ± 0.2[2]	3.6 ± 0.2	3.2 ± 0.2[2]	3.6 ± 0.2	3.2 ± 0.2[2]	3.4 ± 0.2	3.3 ± 0.2	< 0.01
LDL-P, nmol/L	1253.9 ± 79.8	1216.8 ± 73.6	1254.2 ± 81.8	1276.5 ± 77.5	1331.4 ± 86.6	1208 ± 77.4	1265.2 ± 80.4	1185.8 ± 63.8	0.42
LDL small, nmol/L	202.0 ± 18.3	212.0 ± 23.8	226.3 ± 26.5	211.7 ± 25.6	235.7 ± 25.3	218.8 ± 22.3	208.2 ± 22.1	192.8 ± 63.8	0.93
LDL medium, nmol/L	259.1 ± 26.3	253.4 ± 22.4	253.1 ± 24.6	238.9 ± 18.9	272.3 ± 19.8	243.4 ± 18.9	268.1 ± 22.6	248.5 ± 16.8	0.63
HDL large, nmol/L	6451 ± 386	6022 ± 370	5855± 414	5851 ± 447	6048 ± 312	5461 ± 219	6011 ± 309	2121 ± 390	0.86
LDL peak size, Å	218.7 ± 1.5	219.3 ± 1.3	218.9 ± 1.4	220.2 ± 1.4	219.1 ± 1.3	219.9 ± 1.3	218.7 ± 1.2	220.4 ± 0.9	0.78
ApoB, mg/dL	92.4 ± 5.2	86.4 ± 5.2	92.2 ± 4.2	83.9 ± 4.0	92.9 ± 4.0	85.9 ± 4.0	88.6 ± 4.8	83.6 ± 4.7	0.68
NEFA, mEq/L	0.49 ± 0.04	0.41 ± 0.03	0.43 ± 0.04	0.44 ± 0.03	0.49 ± 0.05	0.45 ± 0.05	0.51 ± 0.04	0.47 ± 0.04	0.33
Glucose, mg/dL	89.0 ± 2.1	87.4 ± 1.7	87.6 ± 2.2	88.6 ± 2.8	91.2 ± 2.2	92.0 ± 2.5	89.4 ± 2.4	90.6 ± 2.5	0.66
Insulin, μU/L	21.3 ± 3.6	19.5 ± 2.9	17.9 ± 2.3	18.0 ± 2.4	18.5 ± 1.8	20.1 ± 1.9	15.7 ± 1.7	16.0 ± 1.8	0.20
HOMA-IR	3.6 ± 0.3	3.7 ± 0.4	3.3 ± 0.4	3.5 ± 0.5	3.7 ± 0.3	3.9 ± 0.3	3.6 ± 0.5	3.7 ± 0.5	0.75
AST, U/L	20.0 ± 2.3	18.3 ± 1.8	15.9 ± 0.8	16.5 ± 1.0	19.8 ± 2.7	22.2 ± 3.7	17.3 ± 1.4	17.4 ± 1.8	0.24
ALT, U/L	22.3 ± 4.0	20.7 ± 5.0	16.8 ± 1.7	17.9 ± 1.8	24.8 ± 4.8	29.4 ± 5.4	18.4 ± 2.0	18.0 ± 3.0	0.16
ALP, U/L	71.8 ± 3.9	73.6 ± 4.7	65.8 ± 3.3	66.2 ± 3.3	75.6 ± 4.3	74.1 ± 4.2	69.2 ± 9.1	73.7 ± 11.0	0.15
Total protein, g/dL	6.8 ± 0.1	6.8 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	0.95
GGT, U/L	24.8 ± 6.6	28.6 ± 10.2	19.4 ± 2.6	19.7 ± 2.5	19.6 ± 3.5	22.3 ± 3.6	24.1 ± 8.1	19.4 ± 4.3	0.17
Albumin, g/dL	4.4 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.1 ± 0.0	0.13
Albumin:Globulin ratio	1.9 ± 0.1	1.7 ± 0.1[2]	1.7 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	0.04

[1]All values are means ± SEMs. All values were analyzed with a 2-way (group x visit) linear mixed model. No adjustments were made for analyzing multiple primary outcomes. All outcomes were measured in serum, except for NEFA, insulin, and glucose, which were measured in plasma. To convert LDL-c and HDL-c to mmol/L divide by 38.67. To convert TG to mmol/L divide by 88.57. ALP, alkaline phosphatase; ALT, alanine aminotransferase; ApoB, apolipoprotein B; AST, aspartate aminotransferase; CSO, cottonseed oil; GGT, gamma-glutamyl transferase; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-P, LDL particle number; NEFA, nonesterified free fatty acid; TC, total cholesterol; TG, triglyceride; c, cholesterol;

[2]Indicates a significant difference from baseline to week 4 versus control.

[3]Indicates a trend for a significant difference from baseline to week 4 versus control.

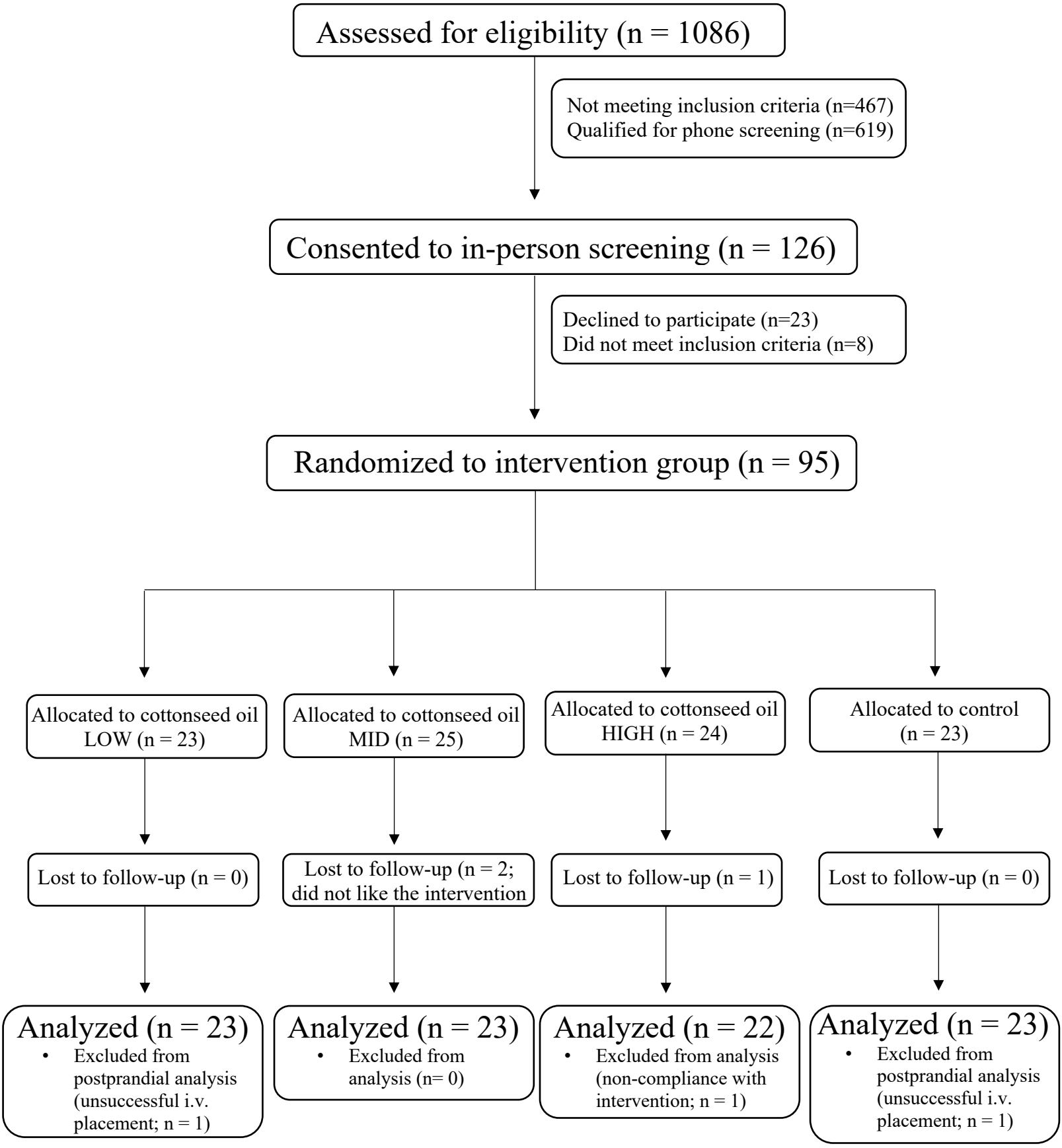

Figure 3.1. CONSORT flow diagram selection of participants.

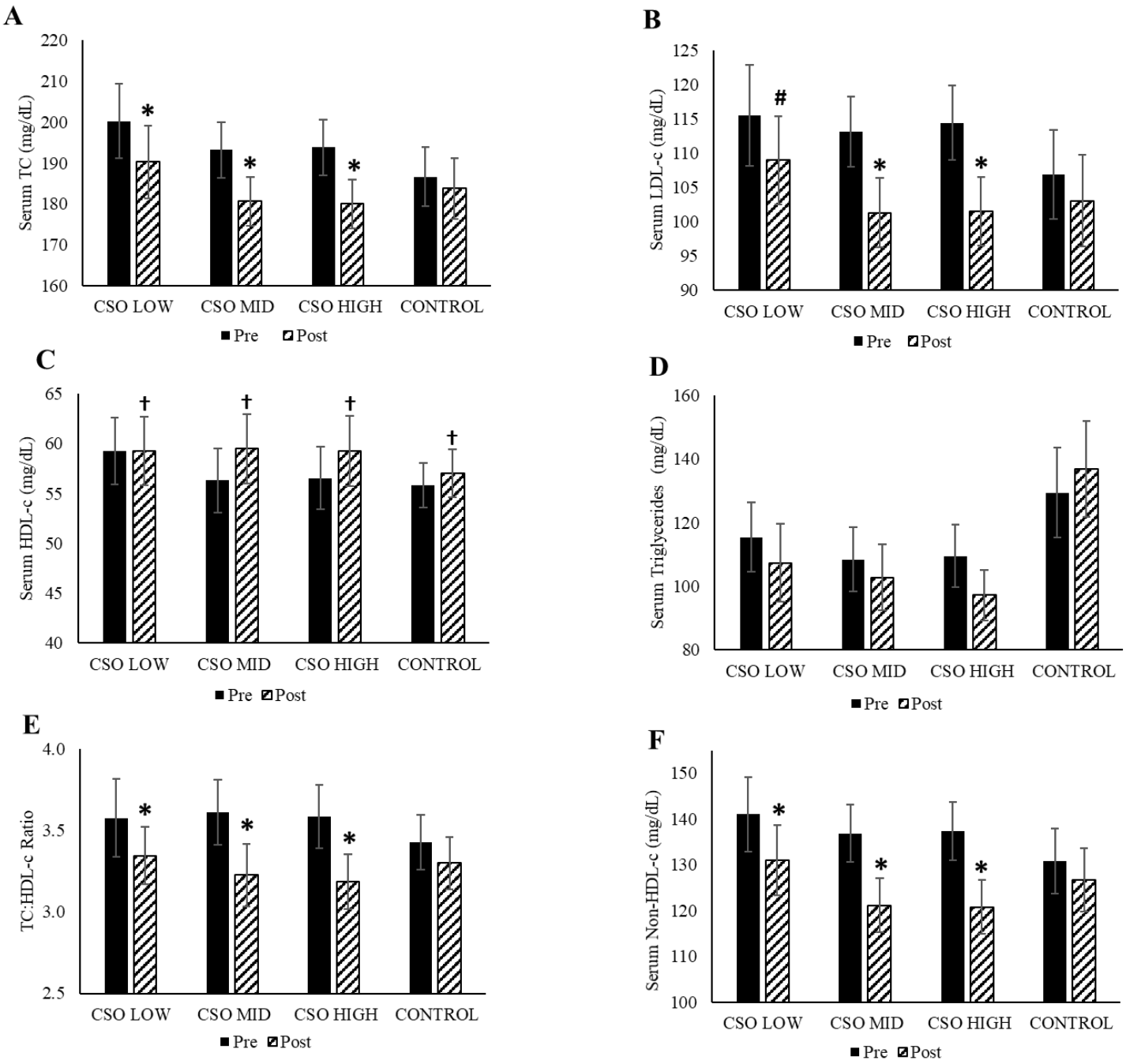
A
Serum TC (mg/dL)
220
210
200
190
180
170
160
*
*
*
CSO LOW
CSO MID
CSO HIGH
CONTROL
Pre
Post
B
Serum LDL-c (mg/dL)
125
120
115
110
105
100
95
90
#
*
*
CSO LOW
CSO MID
CSO HIGH
CONTROL
Pre
Post
C
Serum HDL-c (mg/dL)
65
60
55
50
45
40
†
†
†
†
CSO LOW
CSO MID
CSO HIGH
CONTROL
Pre
Post
D
Serum Triglycerides (mg/dL)
160
140
120
100
80
CSO LOW
CSO MID
CSO HIGH
CONTROL
Pre
Post
E
TC:HDL-c Ratio
4.0
3.5
3.0
2.5
*
*
*
CSO LOW
CSO MID
CSO HIGH
CONTROL
Pre
Post
F
Serum Non-HDL-c (mg/dL)
150
140
130
120
110
100
*
*
*
CSO LOW
CSO MID
CSO HIGH
CONTROL
Pre
Post

Figure 3.2. Fasting serum levels of (A) total cholesterol (TC), (B) low-density lipoprotein cholesterol (LDL-c), (C) high density lipoprotein cholesterol (HDL-c), (D) serum triglycerides (E) TC:HDL-c ratio, and (F) non-HDL-c from pre- to post-diet intervention in adults at risk of cardiovascular disease (n=23 LOW; n = 23 MID; n = 22 HIGH; n=23 CON). * indicates a difference vs. control ($p \leq 0.05$); # indicates a trend for differences vs. control ($p = 0.06$ to 0.10). † Significant visit effect for higher HDL-c at post vs. pre-intervention, regardless of group. All data are presented as mean ± SEM. CSO, cottonseed oil; LOW, received 10% energy needs from cottonseed oil; MID, received 20% energy needs from cottonseed oil; HIGH, received 30% energy needs from cottonseed oil; CON, received 10% energy needs from a mixture of oils representative of the fatty acid composition of the U.S. diet.

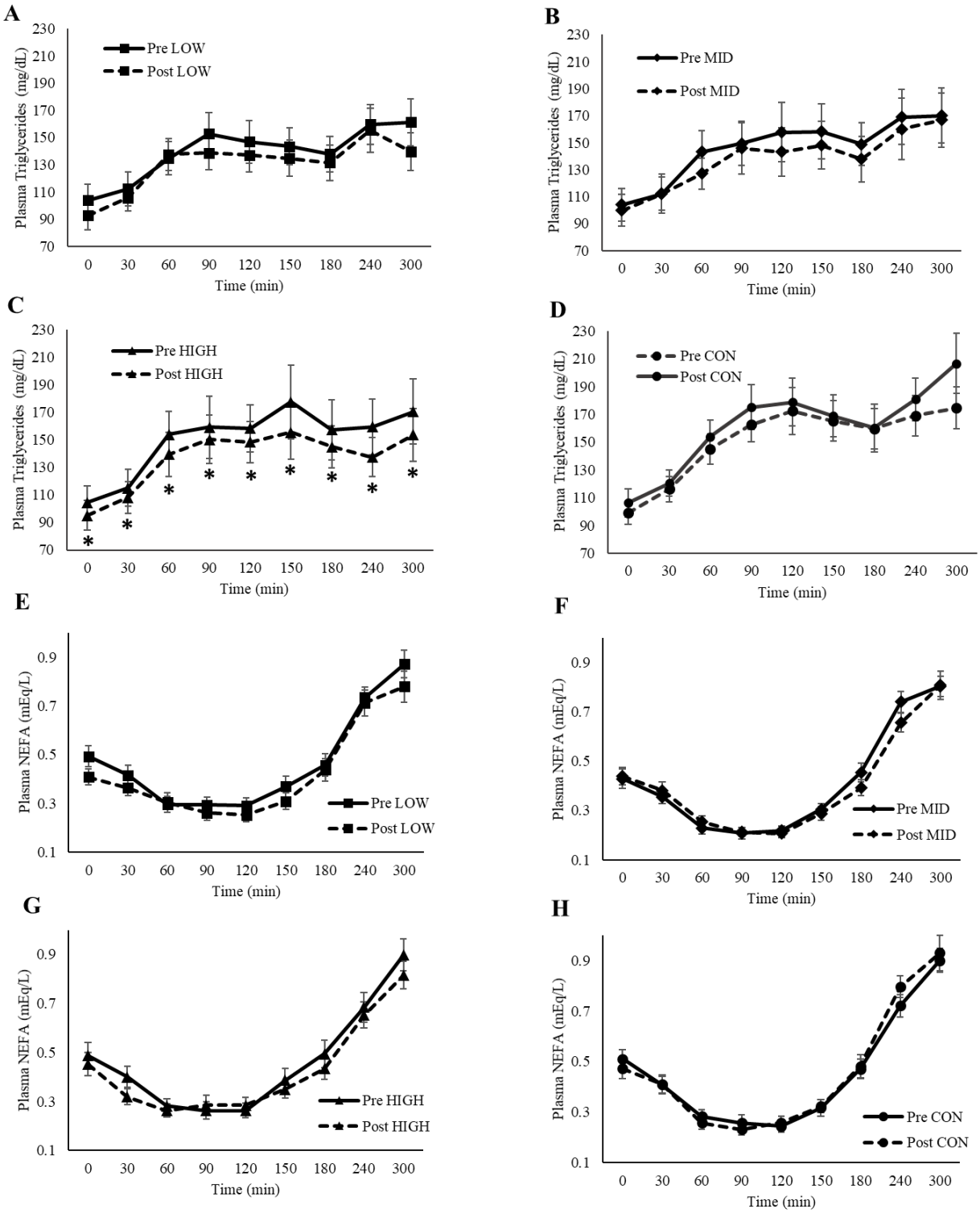
A
Plasma Triglycerides (mg/dL)
Pre LOW
Post LOW
Time (min)
B
Plasma Triglycerides (mg/dL)
Pre MID
Post MID
Time (min)
C
Plasma Triglycerides (mg/dL)
Pre HIGH
Post HIGH
Time (min)
D
Plasma Triglycerides (mg/dL)
Pre CON
Post CON
Time (min)
E
Plasma NEFA (mEq/L)
Pre LOW
Post LOW
Time (min)
F
Plasma NEFA (mEq/L)
Pre MID
Post MID
Time (min)
G
Plasma NEFA (mEq/L)
Pre HIGH
Post HIGH
Time (min)
H
Plasma NEFA (mEq/L)
Pre CON
Post CON
Time (min)

Figure 3.3. Time course for plasma triglycerides (A-D) and plasma unesterified fatty acids (NEFAs) (D-F) for each group at pre- and post-intervention in adults at risk of cardiovascular disease (n=23 LOW; n = 23 MID; n = 22 HIGH; n=23 CON). Participants consumed a high-saturated fat meal after time 0. *Indicates a significant difference between pre and post-intervention meal responses vs. control ($p \leq 0.05$). All data are presented as mean ± SEM. CSO, cottonseed oil; LOW, consumed 10% energy needs from cottonseed oil; MID, received 20% energy needs from cottonseed oil; HIGH, received 30% energy needs from cottonseed oil; CON, received 10% energy needs from a mixture of oils representative of the fatty acid composition of the U.S. diet.

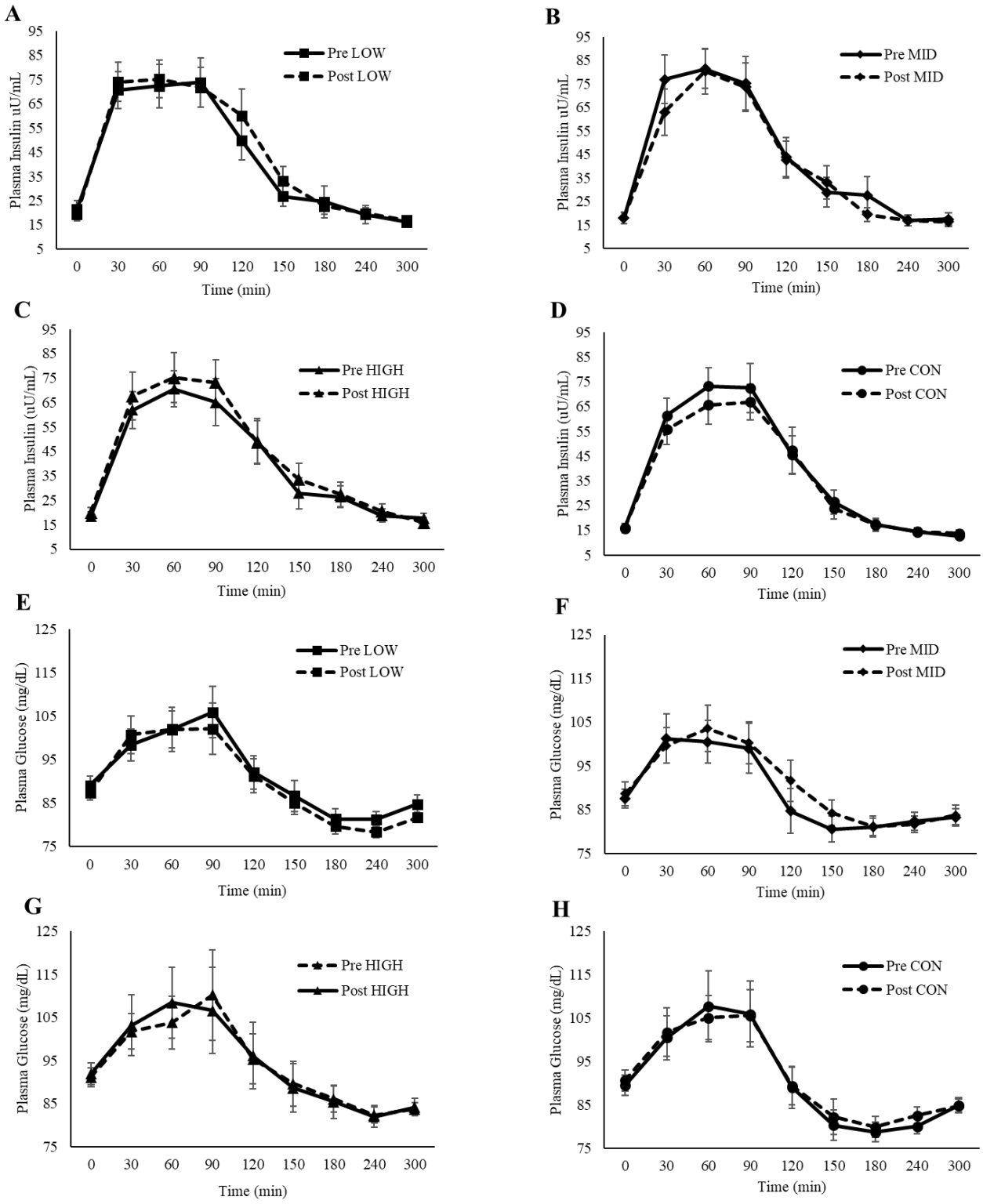
A
Plasma Insulin uU/mL
Pre LOW
Post LOW
Time (min)
B
Plasma Insulin uU/mL
Pre MID
Post MID
Time (min)
C
Plasma Insulin (uU/mL)
Pre HIGH
Post HIGH
Time (min)
D
Plasma Insulin (uU/mL)
Pre CON
Post CON
Time (min)
E
Plasma Glucose (mg/dL)
Pre LOW
Post LOW
Time (min)
F
Plasma Glucose (mg/dL)
Pre MID
Post MID
Time (min)
G
Plasma Glucose (mg/dL)
Pre HIGH
Post HIGH
Time (min)
H
Plasma Glucose (mg/dL)
Pre CON
Post CON
Time (min)

Figure 3.4. Time course for plasma insulin (A-D) and plasma glucose (D-F) for each group at pre- and post-intervention in adults at risk of cardiovascular disease (n=23 LOW; n = 23 MID; n = 22 HIGH; n=23 CON). Participants consumed a high-saturated fat meal after time 0. All data are presented as mean ± SEM. CSO, cottonseed oil; LOW, received 10% energy needs from cottonseed oil; MID, received 20% energy needs from cottonseed oil; HIGH, received 30% energy needs from cottonseed oil; CON, received 10% energy needs from a mixture of oils representative of the fatty acid composition of the U.S. diet.

CHAPTER 4

APPETITE RESPONSES TO DIFFERENT DOSES OF COTTONSEED OIL IN ADULTS AT RISK OF CARDIOVASCULAR DISEASE

[1] Hawkins, C. D., Prater M.C., Ward C. A., Paton C. M., Cooper J. A. Submitted to *Physiology and Behvavior,* 5/3/2025.

Abstract

High doses of cottonseed oil (CSO) have improved markers of appetite. The objective of this study was to examine dose response effects of CSO vs. a control (CON) diet on appetite responses in adults at risk for cardiovascular disease (CVD). This 28-day parallel outpatient feeding intervention randomized participants into a CSO dose group: LOW (10% estimated energy needs (EEN) CSO; n=23), MID (20% EEN CSO; n=23), or HIGH (30% EEN CSO; n=22) or CON (10% EEN from oil mixture; n=23). At pre- and post-intervention, participants consumed a high fat meal challenge with 5 hr postprandial blood draws to evaluate cholecystokinin (CCK), peptide YY (PYY), and ghrelin. Participants completed VAS questionnaires for subjective appetite at the same timepoints and hourly the remainder of the day with a food record. From pre- to post-intervention, postprandial ghrelin and PYY were suppressed in LOW and HIGH ($p < 0.01$ for all) vs. MID and CON ($p > 0.05$ for all). Subjective hunger was suppressed in LOW, MID, and HIGH ($p < 0.01$ for all) vs. CON ($p=0.93$). Fullness in all CSO groups increased ($p < 0.01$ for all) vs. a reduction in CON ($p=0.04$). Desire to eat was suppressed in LOW, MID, and CON ($p < 0.01$ for all) vs. HIGH ($p=0.95$), and overall subjective appetite decreased in LOW and MID ($p < 0.01$ for both) vs. HIGH and CON ($p > 0.05$ for both). Altogether, enriching diets with 10%, 20%, and 30% CSO improved markers of subjective appetite in adults at-risk for CVD.

1. Introduction

Obesity prevalence continues to rise with rates nearly doubling since 1990 (World Health Organization, 2024). Obesity is a risk factor for a number of chronic diseases (Diseases, 2023); thus, enhancing weight management interventions remains a key component in the prevention of chronic disease. One accepted hypothesis for effective weight management suggests improving appetite regulation (J. C. Halford & J. A. Harrold, 2012). Appetite is the physiological system that influences energy intake (EI) (Gibbons et al., 2019), consequentially, influencing energy balance. There are multiple gastrointestinal hormones known to influence appetite. Ghrelin is the "hunger hormone" and is responsible for promoting hunger and feeding behavior in low energy states through stimulation of orexigenic neurons (Howick et al., 2017). Conversely, following nutrient consumption, cholecystokinin (CCK) and peptide YY (PYY) promote an anorexigenic response and suppress orexigenic neurons in the proximal and distal gut which increase satiety to influence meal cessation (Cooper, 2014; J. C. G. Halford & J. A. Harrold, 2012; Lau & Herzog, 2014; Nakamura & Nakamura, 2018).

High-fat (HF) diets have been observed to be obesogenic due to their energy density, which contributes to EI and a positive energy balance (Prater et al., 2023; Romieu et al., 2017; Stevenson et al., 2015). This has been disputed within the literature, however, because of the observance of foods such as nuts and oils eliciting no weight gain when added to the diet (Maki et al., 2015; Romieu et al., 2017). Therefore, the fatty acid (FA) composition of the meal may impact appetite regulation by modifying the response of hunger and satiety hormones. Acute meal challenges have evaluated subjective (assessed through visual analog scale (VAS) questionnaires), physiologic, and applied appetite (estimated EI) (Kaviani & Cooper, 2017); some found no differences based on FA composition (Alfenas & Mattes, 2003; Casas-Agustench

et al., 2009; Flint et al., 2003; Graybeal et al., 2021; MacIntosh et al., 2003; Stevenson et al., 2015; Strik et al., 2010), while others report differences (Kozimor et al., 2013; Lawton et al., 2000; Maljaars et al., 2009; Valente et al., 2018). Previously, our lab has shown that a single meal rich in polyunsaturated fat (PUFA) induces a heightened PYY response compared to a monounsaturated fat (MUFA) rich meal (Kozimor et al., 2013; Stevenson et al., 2015). Additionally, we have shown that a 7-day, PUFA-rich diet increased fasting and postprandial PYY and decreased fasting ghrelin (Stevenson et al., 2017). Despite the limited evidence, there is trend for beneficial appetite responses from high PUFA meals, but further research is indicated to make well-supported conclusions.

Few longer-term dietary interventions have examined a HF, PUFA-rich diet on appetite outcomes, and even fewer have utilized a whole foods approach. Cottonseed oil (CSO), an oil rich in PUFAs, has displayed beneficial outcomes with respect to lipid metabolism and mitigating chronic disease risk (Davis et al., 2012; Paton et al., 2017; Polley et al., 2018; Prater et al., 2022). Despite its prominence within our food system (Mundi, 2023), CSO has only been evaluated for its effects on appetite in two dietary interventions. Notably, these dietary interventions resulted in consumption of HF diets (≥ 35% estimated energy needs (EEN)). Specifically, one placed healthy males on a 5-day HF diet (50% EEN) enriched with CSO (44% EEN) and showed improvements in physiologic and subjective measures of appetite (Polley et al., 2019). Additionally, an 8-week partial outpatient feeding trial providing a HF diet (49% EEN) that included a high dose of CSO (30% EEN) elicited greater appetite suppression than a MUFA-rich diet. The purpose of this study was to determine the effects of different doses of CSO vs. a control (CON) diet over a 4-week period on fasting and postprandial appetite measures in adults at risk of cardiovascular disease (CVD). We evaluated self-reported EI,

subjective ratings of appetite, and hunger and satiety hormones. We hypothesized that, in a dose-dependent manner, the CSO-enriched diets would result in improved fasting and postprandial appetite regulation, and that all groups would be different than CON.

2. Methods

2.1 Study Design

This was a single-blind, parallel randomized control trial (clinicaltrials.gov: NCT05686954). There were four groups in which participants were randomized to: the LOW group received 10%, the MID group received 20%, and the HIGH group received 30% of their EEN from CSO. The fourth group was a CON group that received 10% of their EEN from an oil mixture designed to match the FA profile of the typical American diet (USDA, 2021). This 28-day protocol included six visits: one screening visit (v0), a pre-intervention visit (v1), short weekly visits throughout the intervention (ends of weeks 1,2, and 3; v2-4), and a post-intervention visit (end of week 4; v5). This study was approved by the University of Georgia Institutional Review Board, and written informed consent was obtained from all participants prior to any study procedures taking place.

2.2 Participants

Ninety-one (n=23 LOW; n = 23 MID; n = 22 HIGH; n=23 CON) sedentary adults between the ages of 25 and 75y at increased risk of CVD were included in the per-protocol analysis (**Figure 1)**. Increased risk for CVD was defined by either elevated cholesterol profiles or overweight/obesity. Elevated cholesterol profiles were defined as "At risk" in two or more of the variables (total cholesterol (TC): 180-239 mg/dL, low density lipoprotein cholesterol (LDL-c) (110-159 mg/dL), triglycerides (TGs) (130-199 mg/dL) or “High” in TC (≥240 mg/dL), LDL-

c (≥160 mg/dL) or TGs (between 200 - 350 mg/dL). Overweight/obesity was defined by body mass index (BMI ≥ 25 kg/m^2).

Individuals with TC >290 mg/dL or LDL > 190 mg/dL plus a family history of myocardial infarction (MI) before 50y in a 2nd-degree relative, or below age 60 in a 1st-degree relative were not eligible to participate in this study to rule out participants with probable familial hypercholesterolemia (Lozano P, 2016). Additionally, other exclusion criteria included women on hormone replacement therapy for < 2y, women who are pregnant or nursing, individuals who regularly exercised > 3h/wk, weight instability (gain or loss >5% of body weight in the past 3mo), plans to begin a weight loss/exercise regimen during the trial, history of medical or surgical events that could affect digestion or swallowing, gastrointestinal surgeries, any chronic diseases (including moderate to severe asthma, chronic lung disease, and kidney disease), metabolic diseases, atherosclerosis, previous MI or stroke, and cancer. Also, those taking fish oil supplements, excessive alcohol use (> 3 drinks/d for men; > 2 drinks/d for women), tobacco or nicotine use, or underweight BMI (<18.5 kg/m^2) were excluded. Fasting blood glucose levels > 126 mg/dL or blood pressure > 180/120 mmHg were also excluded. Individuals were excluded if taking medications influencing digestion and absorption, metabolism, lipid-lowering medications, medications for diabetes, steroid/hormone therapies, or current antibiotic cycles. Individuals on a medically prescribed or special diet or had food allergies (specific to the foods in the study) also were excluded.

2.3 Protocol

2.3.1 Screening Visit (v0)

Participants completed an 8-12h overnight fast and 24h abstaining from alcohol and exercise prior to arriving to the Human Nutrition Lab (HNL). Height, weight, and blood pressure were recorded, and BMI inclusion criteria was confirmed. A fasting blood sample was obtained to evaluate blood lipids and glucose. An Alcohol Use Disorders Identification Test (AUDIT) was given to participants to determine alcohol consumption habits (Bohn et al., 1995). Next, indirect calorimetry measurement (TrueOne 2400, Parvo Medics, Sandy, UT) was used, following standard conditions, to determine resting metabolic rate (RMR; kilocalories/day) (Cooper et al., 2009). Participants' RMR were multiplied by an activity factor of 1.55 to determine total daily EEN (Weir, 1949). This calculation was used for the test meal at pre- and post-intervention visits, and for the 28-day diet intervention. Using a random number generator, qualifying participants were then randomized into one of the four groups.

2.3.2 Pre-Intervention Visit (v1)

The night before the pre-intervention visit, all participants consumed a standardized dinner and snack for dinner (~35% of total energy from fat, 15% protein, and 50% carbohydrate). Participants arrived at the HNL in the morning fasted (8-12h fast) and abstaining from alcohol or exercise (24h). Upon arrival, participants turned in a 3-day food record (2 weekdays, 1 weekend day) (Gersovitz et al., 1978). Next, height, weight, waist and hip circumference, blood pressure, and body composition were measured. Body composition was measured using a Bod Pod (Cosmed USA, Inc.). After anthropometric measurements, participants completed a 100 mm VAS questionnaire assessing feelings of hunger, fullness, prospective consumption, and desire to eat to evaluate subjective appetite (Kral et al., 2004). Then, an intravenous (IV) catheter was placed and a fasting blood sample was collected. Throughout the protocol, the line was kept patent with a saline solution.

After fasting measures were taken, participants were instructed to consume a high-fat meal rich in saturated fatty acids (SFA) within a 10-minute period. The liquid meal contained a milk-chocolate, ready-to-drink shake (Ensure, Abbott Laboratories, Inc.), unsalted butter, red palm oil, coconut oil, soy lecithin granules, and powdered chocolate drink mix. As described by Prater et al. (Prater et al., 2023), the meal provided 35% of the participant's total EEN with 5%, 25%, and 70% of energy from protein, carbohydrate, and fat, respectively. The FA composition of the meal was 47%, 16%, and 7% of energy provided from SFA, MUFA, and PUFA, respectively. A high SFA meal was chosen to represent an "unhealthy" (high SFA) meal, which is commonly noticed in weekend eating habits (An, 2016). Therefore, this allows us to evaluate appetite responses to changes in fat composition from the habitual diet. The container used to hold the liquid meal was rinsed with four ounces of water to ensure the participant ingested the entire meal. A validated sensory questionnaire, using a modified 9-point hedonic scale with '1' indicating 'dislike extremely' and '9' indicating 'like extremely,' was given to assess perception of the high SFA meal (Peryam, 1957). Following the SFA-rich meal challenge blood draws occurred, and VAS questionnaires were administered, intermittently for 5h (timepoints 30, 60, 90, 120, 150, 180, 240, and 300 min) postprandially. The VAS measures taken at these time points were defined as "in lab" (LAB) measures. Four ounces of water was provided to participants hourly. The IV was removed after 5h, and participants were instructed to consume a self-selected lunch within 1h of leaving the lab and a self-selected diner 4h later. Additionally, participants were given VAS questionnaires and ask to complete once per hour for the next 7h (starting at 360 min post SFA test meal). VAS measures taken at these timepoints were defined as at-home" (HOME) measures. Participants were instructed to record all food and drink for the rest of the day on a food record. Food records were analyzed with The Food Processor SQL

software (version 11.11.32; Salem, OR, USA); the analysis included the SFA test meal and self-reported intake for the remainder of the day. This combined assessment made up total EI data. Lastly, participants completed the following questionnaires: the fat preference questionnaire, perceived stress scale (PSS) (Cohen et al., 1983), the State Trait Anxiety Inventory (STAI) (Gaudry et al., 1975), the Three Factor Eating Questionnaire (TFEQ) (Shearin et al., 1994), and the international physical activity questionnaire (IPAQ) (Lee et al., 2011).

2.3.3 28-day Dietary Intervention

The day following v1, participants began the 28- diet intervention. At v1, participants were sent home with a one-week supply of daily shakes and snacks that corresponded to their assigned diet group. The shakes and snacks were all were provided in multiple flavors to enhance compliance. Specifically, the shake flavors were either vanilla or chocolate, the yogurt flavors were vanilla, blueberry, and strawberry, and the pudding flavors were vanilla, chocolate, tapioca, and lemon. Participants were instructed to consume all provided foods, while treating the shake as a meal replacement and the snack as a replacement for an isocaloric item in their diet. Otherwise, they were asked to maintain their normal dietary habits for their remaining meals each day. Participants in the MID, LOW, and CON group received all their oil in the form of a breakfast shake with an additional snack item that did not contain oil. The HIGH group received oil through both their breakfast shake and the daily snack **(Table 1).** Participants were instructed to prepare this breakfast shake by mixing the proportioned shake mix with a milk of choice and assigned intervention oil. The ingredients for the breakfast shakes and snacks were identical between groups with the only differences being the amount of the assigned oil incorporated into the foods.

Throughout the intervention, participants were asked to complete daily Compliance Checklists to record study foods consumed and sensory evaluation questionnaires the first time they ate a new flavor of any of the provided foods (shakes and snacks). Participants that consumed less than 75% of provided foods were deemed non-compliant and removed from the final data analysis. Additionally, participants completed 3-day food records (two weekdays and one weekend day) at the mid- and end-point of the intervention (weeks 2 and 4).

2.3.4 Visits 2, 3, and 4 (v2-4)

During the 28-day intervention participants returned to the HNL each week for short study visits (v2-v4). Participants consumed their breakfast shake in the lab, collected their foods for the next 7 days and turned in study documents. Those documents included (1) compliance checklist, (2) saved food containers, (3) 3-day food records (v3 only), and (4) the sensory evaluation logs (as completed). At v3 participants completed an IPAQ questionnaire, and at v4 participants were additionally provided the lead-in meal to be consumed the night before v5.

2.3.5 Post-Intervention Visit (v5)

Participants returned to the HNL for their post-intervention visit after completing their 28-day intervention. Participants turned in their compliance checklist and saved food containers. Otherwise, all procedures from the pre-intervention visit were repeated at the post-intervention visit.

2.4 Sample Analysis

All blood samples were drawn into K2 EDTA-coated vacutainers (Becton, Dickinson, and Company) containing4-benzenesulfonyl fluoride hydrochloride (AEBSF) and Dipeptidyl

Peptidase IV (DPP IV), immediately placed on ice, then centrifuged for 15min at 4°C. The plasma was aliquoted and stored at -80°C until further analysis. Active ghrelin and total PYY were analyzed utilizing radioimmunoassay (Millipore, Billerica, MA, USA). Total CCK was measured using an extraction free enzyme immunoassay (Phoenix Pharmaceuticals, Burlingame, CA, USA). Every participant's total amount of samples was analyzed within the same assay.

2.5 Statistical Analysis

The SAS version 9.2 statistical package (SAS Institute INC, Cary, NC, USA) was used for all data analysis, and statistical significance was set at $p \leq 0.05$. All results were reported as mean ± SEM unless otherwise noted. A sample size of 86 (~21/group) was estimated to detect a difference in postprandial ghrelin (Cohen's F of 0.306) based on the results from a previous CSO intervention by Prater et al. (Prater et al., 2023) using G*power 3.19.7 and assuming at least 80% power with an α of 0.05. The decision to use per-protocol analysis was made *a priori*. Repeated measures (RM) linear mixed models including factors of group and visit were used to determine between- and within group differences from pre- to post-intervention for fasting hormone and VAS data, EI on test days, anthropometric data, compliance, and sensory evaluation data of SFA test meals. An intervention average was calculated for dietary intake (2 week days and 1 weekend day food records from mid- and end-points of the intervention) which was compared to baseline averages. Linear mixed models for group were used to determine differences in sensory perception of study foods between groups. For all postprandial hormone and VAS data, RM linear mixed models for group, visit, and timepoint was utilized to assess between- and within-group differences. Change from baseline responses were calculated by subtracting fasting values from each postprandial timepoint. For VAS data, the time course data was analyzed separately in subgroups including: all time points (0-720min), LAB (0-300min), and HOME (360-720 min)

using similar linear mixed models. If significance was found, a Tukey-Kramer post-hoc test was performed. For all linear mixed models, participants were modeled as a random effect.

3. Results

3.1 Participants

Ninety-five participants were assigned to an intervention group (n=23 LOW; n = 25 MID; n = 24 HIGH; n=23 CON), but 4 participants were not included in the final analysis (**Figure 1**). Thus, 91 individuals (20 women and 3 men in the LOW group, 20 women and 3 men in the MID group, 20 women and 2 men in the HIGH group, and 20 women and 2 men in the CON group) finished the intervention and were included in this per protocol analysis. Participant characteristics are presented in **Table 2**. At baseline, there were no significant differences between groups. There was an increase in body weight ($p = 0.01$) driven by increases in LOW, MID, and HIGH ($p < 0.01$, $p = 0.01$, $p < 0.001$, respectively) from pre- to post-intervention with no significant increase in CON ($p = 0.17$). Further, there was a trend for a group x visit interaction ($p = 0.097$) in BMI driven by increases in LOW, MID, and HIGH ($p = 0.03$, $p < 0.01$, $p < 0.001$, respectively) from pre- to post-intervention vs. CON ($p = 0.20$). No other participant characteristics changed throughout the intervention. Compliance in the LOW, MID, HIGH, and CON group averaged 94 ± 1%, 94 ± 1%, 94 ± 2%, 93 ± 1%, respectively, and compliance was not different between groups. However, based on poor compliance (defined as <75% consumption of study food), 1 participant was excluded from final analysis.

3.2 Physiologic responses

Fasting plasma CCK, PYY and ghrelin levels are presented in **Table 3**. At fasting, there was a group effect in PYY ($p < 0.01$) driven by a difference in LOW vs. MID ($p = 0.04$) and

CON ($p = 0.02$). regardless of study visit. For CCK, there was a visit effect ($p < 0.01$) for increases from pre- to post-intervention, regardless of study group. There were no changes in ghrelin at fasting. The time course for the meal response for CCK, PYY, and ghrelin are displayed in **Figure 2**. For ghrelin, there was a significant group by visit interaction ($p < 0.01$) driven by greater postprandial suppression in LOW ($p < 0.01$) and HIGH ($p < 0.01$) vs. no change in MID ($p = 0.99$) and CON ($p = 0.27$). However, for PYY there was a significant group by visit interaction ($p < 0.01$) for postprandial suppression ($p < 0.01$) in LOW ($p < 0.01$) and HIGH ($p < 0.01$) vs. no change in MID ($p = 0.94$) and CON ($p = 0.07$). There were no differences between groups across the intervention in CCK meal responses.

3.3 Subjective ratings of appetite

3.3.1 Fasting subjective appetite

Fasting subjective appetite ratings are shown in **Table 3**. At fasting, there were significant visit effects for increases in hunger, appetite score, and prospective consumption from pre- to post-intervention ($p \leq 0.05$), regardless of group. Also, there was a group by visit interaction in fullness ($p = 0.026$); however, post-hoc analyses showed non-significant differences between groups.

3.3.2 All-day subjective appetite

Subjective ratings of appetite across the whole day (0-720 min) are displayed in **Figures 3-5**. The LAB and HOME measures mimicked all-day measures except in desire to eat; for specific analyses of all measures of subjective appetite for LAB and HOME refer to supplementary materials. For all-day measures of appetite score, there was an interaction effect ($p < 0.01$), driven by reductions in LOW ($p < 0.01$) and MID ($p < 0.01$) vs. no change in HIGH

(p = 0.98) and CON (p = 0.93) (**Figure 3**). For hunger, there was a group by visit interaction driven by suppression in LOW (p < 0.01), MID (p < 0.01), and HIGH (p = 0.03) vs. no change in CON (p = 0.95). There was an all-day group by visit interaction in fullness (p < 0.01); post-hoc analyses showed this was driven by increases in LOW (p < 0.01), MID (p < 0.01), and HIGH (p < 0.01) vs. suppression in CON (p = 0.04) (**Figure 4**). For prospective consumption ("How much do you think you can eat right now?") there was a visit effect (p < 0.01) for suppression from pre- to post-intervention, regardless of group. The only differences between the three measurements of LAB, HOME, and all-day subjective appetite was in desire to eat. For all-day measures of desire to eat, the interaction effect (p < 0.01) observed was driven by suppression in LOW (p < 0.01), MID (p < 0.01), and CON (p < 0.01) vs. no change in HIGH (p = 0.95). For LAB, there was an interaction (p < 0.01) driven by reduction in MID (p < 0.01) and CON (p < 0.01) vs. no change in LOW (0.64) or HIGH (p = 1.0). While the HOME measures showed a group by visit interaction (p < 0.01) driven by suppression in LOW (p = 0.02) and MID (p < 0.01) vs. no change in HIGH (p = 0.89) or CON (p = 0.15) (**Figure 5**).

3.5 Sensory evaluation

Sensory evaluations of the SFA meal and study foods are presented in **Table 4**. There were no differences between groups for any characteristics of the CSO- or control oil- enriched study foods. Regarding the high SFA meal challenge at the pre- and post-intervention study visits, there was visit effect (p < 0.01) for taste driven by decreases from pre- to post-intervention, regardless of group. Additionally, there was a trend for a group effect (p = 0.06) for taste; post-hoc analysis revealed this effect to be driven by a trend for differenced in MID vs. CON and LOW (p = 0.08 and 0.09, respectively). Further, there was a group x visit interaction (p

= 0.03) for overall acceptability of the high SFA meal driven by a trend for reduction in HIGH (p= 0.09).

3.6 Applied responses

Self-reported total energy and macronutrient intake from pre- and post-intervention visit days are presented in supplementary materials (**Figure S1**). Based on the analysis of food diaries and provided SFA meal at pre- and post-intervention visit, there were no significant differences between groups for total energy intake or carbohydrate, fat, or protein and macronutrient intake.

4. Discussion

Diets enriched with all three doses of CSO resulted in improvements in subjective, but not physiologic, postprandial appetite markers compared to a control diet in adults at risk for CVD. Specifically, consuming 10% and 20% of EN from CSO resulted in postprandial suppression of overall subjective appetite, while all three doses of CSO improved postprandial hunger and fullness ratings. Moreover, consuming 10% and 30% energy from CSO suppressed the hunger hormone ghrelin; however, those doses also suppressed the satiety hormone PYY, postprandially. Therefore, none of the CSO doses has a clear impact or improvement on physiologic measures of appetite regulation. Additionally, we did not observe any dose response patterns for measures of subjective or physiologic appetite, so it appears that even low doses of CSO can have beneficial effects on subjective appetite suppression.

The changes we observed for VAS hunger, fullness, and overall appetite score represent a novel finding with the LOW and MID doses since previous trials on CSO and appetite have utilized a high dose (≥ 30% EEN) (Polley et al., 2019; Prater et al., 2023). Specifically, acute CSO consumption has previously shown suppression of hunger and increased fullness (Polley et

al., 2019) when compared to a meal rich in olive oil. Another study utilizing an 8-week CSO intervention, and a similar HF meal challenge design to the present study, also found an increase in postprandial fullness following chronic CSO consumption (Prater et al., 2023). Therefore, the suppression in hunger, and increases in fullness, in the current study aligns with previous high-dose research.

Interestingly, changes in overall appetite scores were not in a dose-dependent manner and were only observed in the LOW and MID groups. This is likely due to the decrease in "desire to eat" within the control group and lack of change in that measure within the HIGH group. We do not have a clear scientific explanation for this null response; however, this "desire to eat" question has not yielded any changes with previous CSO interventions either (Polley et al., 2019; Prater et al., 2023). It is speculated that assessing desire to eat or "wanting" may be influenced by more environmental and psychological factors when participants are interpreting this measure compared to others (Blundell et al., 2010; Rakha et al., 2022). For example, desire to eat may be influenced by environmental triggers like time, situation, and appropriateness of consumption (Blundell et al., 2010) and psychological factors such as sensory inputs, mood, and food cravings (Rakha et al., 2022). Nevertheless, the suppressive effects in our LOW and MID groups may have some clinical relevance.

The improvement in overall subjective appetite scores in the LOW and MID CSO groups is meaningful. It is generally accepted that an 8-10% change in subjective VAS can indicate a clinically significant change regarding energy balance (Flint et al., 2000). In this study, the LOW and MID group's change in overall appetite score for the full day was 26% and 27% different than control, respectively, indicating a meaningful reduction in appetite. Changes in subjective appetite have been shown to influence EI. However, in this present trial, we saw no significant

differences in EI between the CSO groups and control group. These results should be interpreted with some degree of caution though as EI was self-reported and may be susceptible to some degree of over- or under-reporting (Wehling & Lusher, 2019). Additionally, the EI data was only gathered for one day on the pre- and post-intervention study visit day, so the high SFA meal challenge was not a self-selected amount. Still, an 8-week trial evaluating CSO consumption (30% EEN) with a similar study design saw a reduction in overall EI. This suggests that a longer intervention may be necessary for changes in EI to occur with CSO consumption.

It was not surprising that we observed a suppression in ghrelin with CSO consumption since this has been observed previously (Polley et al., 2019; Prater et al., 2023). However, it was unexpected that suppression occurred only in the HIGH and LOW, but not the MID, dose. Accordingly, we can assume that the changes in ghrelin were not a dose-dependent effect of CSO, and it is unclear why we did not observe suppression in our MID group. The same pattern of suppression of the LOW and HIGH groups was also unexpectedly observed for the satiety hormone, PYY. Previously, CSO consumption has not elicited a significant change in PYY; however, acute consumption of PUFAs (rich in CSO) has heightened PYY responses (Kozimor et al., 2013; Stevenson et al., 2015; Stevenson et al., 2017). Additionally, suppression of PYY did not align with the increased fullness we observed following also doses of CSO consumption. It is possible that measuring total, rather than active, PYY contributed to these unexpected results. This is also not the first study to show contradictory results for subjective vs. physiologic data (Doucet et al., 2008; Naharudin et al., 2020; Shabat-Simon et al., 2018). Despite the discrepancies in physiologic vs. subjective appetite measures, subjective appetite is recognized as a reliable predictor of EI (Gibbons et al., 2019).

Chronic HF diet consumption above the AMDR for fat (20-35% of EN (USDA, 2020)) is associated with positive energy balance and weight gain (Prater et al., 2023; Romieu et al., 2017; Stevenson et al., 2015). Previously, improved appetite regulation has been observed with high doses of CSO (≥ 30% EEN), resulting in total fat intake above the AMDR (~50% EEN) (Polley et al., 2019; Prater et al., 2023). Therefore, we aimed to assess whether lower doses could elicit appetite suppression and encourage healthier dietary patterns to support weight management. The appetite suppressive effects observed with the 10% and 20% doses of CSO in this trial is promising for the future integration of CSO into a diet without placing individuals above the AMDR; Unfortunately, in the present study, participants failed to integrate the study foods into their diet and stay within the AMDR. The LOW, MID, and HIGH CSO groups consumed 45.1%, 44.2% and 50.6% energy from fat, respectively, and 41.5% in CON. Importantly though, self-reported intake demonstrated that participants were already following a HF diet at the start of the intervention (LOW: 36.1%; MID: 39.0%; HIGH: 39.3%; CON: 40.0%). Nevertheless, the improved appetite regulation we observed with lower doses of CSO vs. a control diet may be beneficial for overall health. Further research is warranted to assess the effects of lower doses of CSO consumption as part of a diet that falls within the AMDR on appetite regulation.

This study is not without limitations. As mentioned previously, we used self-reported measures of dietary intake, which may comprise some degree of under- or over-reporting. We also did not have a control group that received no food or oils. Our control group received a mixture of oils that mimicked the composition of the U.S. diet (USDA, 2021), which was done intentionally to ensure that our control group consumed the same study foods as the CSO groups to isolate the impact of CSO on appetite responses. Next, we did not measure active PYY, so it is possible that the unexpected postprandial responses for that hormone may not indicate the same

response as the active 3-36 hormone. Finally, we did not take direct measures or biomarkers to ensure compliance; however, we did assess compliance via empty food container returns and compliance logs.

In conclusion, we observed improvements in overall subjective appetite with diets enriched with 10% and 20% energy needs from CSO as well as hunger suppression and increased fullness in all CSO doses. Intake of 10% and 30% suppressed postprandial ghrelin; however, this result was contraindicated by suppression of PYY in those same groups. While we did not find dose-dependent responses in physiologic measures of appetite, all doses of CSO showed improvements in subjective appetite measures. Future research may need to include additional physiologic measures of appetite and more data on subsequent EI to better determine the overall effects of multiple doses of CSO on appetite.

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Table 4.1. Description of Diet Interventions

Intervention Group	Percentage of energy provided from oil per day	Percentage of energy from provided foods per day	Provided food items that contained oil	Provided food items that do not contain oil
CSO HIGH	30%	47%	Breakfast shake and 1 snack[1,2]	N/A
CSO MID	20%	37%	Breakfast shake[2]	1 snack[1]
CSO LOW	10%	27%	Breakfast shake[2]	1 snack[1]
CONTROL	10%	27%	Breakfast shake[3]	1 snack[1]

Abbreviation: CSO = Cottonseed oil

[1]The snack was either a yogurt or pudding.

[2]Cottonseed oil was utilized in food.

[3]Control oil mixture containing canola, coconut, corn, safflower, and soybean oil was utilized in food.

Table 4.2. Characteristics at baseline and week 4 in cottonseed oil or control groups.

	CSO LOW		CSO MID		CSO HIGH		Control		p values		
Characteristic	Baseline	Week 4	Baseline	Week 4	Baseline	Week 4	Baseline	Week 4	Group	Visit	Interaction
Female, %	86	—	86	—	91	—	91	—			
Age, y	47 ± 3	—	46 ± 3	—	45 ± 3	—	49 ± 2	—			
Height, cm	167.0 ± 1.6	167.0 ± 1.7	165.5 ± 2.2	165.3 ± 2.2	165.4 ± 1.4	165.4 ± 1.4	163.8 ± 1.3	163.8 ± 1.3	.65	.47	.49
Weight, kg	91.5 ± 3.9	92.3 ± 4.0*	85.1 ± 3.7	86.0 ± 3.8*	90.2 ± 4.3	91.8 ± 4.3*	87.8 ± 4.7	88.4 ± 4.7	.52	.001	.02
BMI, kg/m^2	32.8 ± 1.8	33.0 ± 1.3*	31.1 ± 1.3	31.5 ± 1.3*	33.0 ± 1.5	33.5 ± 1.5*	32.6 ± 1.6	32.9 ± 1.6	.54	.001	.10
WC, cm	98.2 ± 2.9	98.4 ± 3.1	95.0 ± 2.6	96.5 ± 2.6	99.7 ± 2.8	102.0 ± 3.3	96.8 ± 3.6	95.7 ± 3.7	.54	.16	.15
HC, cm	117.6 ± 2.1	116.5 ± 2.2	114.1 ± 2.3	114.9 ± 2.4	117.5 ± 3.1	118.8 ± 3.3	116.7 ± 3.4	116.7 ± 3.3	.69	.51	.21
WHR	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	.72	.41	.61
SBP, mmHg	135.4 ± 3.6	132.3 ± 3.2	128.8 ± 4.3	137.9 ± 3.5	133.2 ± 3.2	136.5 ± 3.5	135.2 ± 3.6	132.5 ± 3.5	.30	.15	.78
DBP, mmHg	84.5 ± 2.9	81.8 ± 2.4	78.7 ± 3.2	78.0 ± 2.9	79.9 ± 1.9	81.0 ± 1.6	82.0 ± 2.6	83.4 ± 2.1	.43	.73	.31
Body fat, %	40.5 ± 1.6	40.7 ± 1.6	38.5 ± 1.7	38.6 ± 1.8	41.3 ± 2.0	42.0 ± 2.2	40.8 ± 2.0	40.5 ± 2.0	.55	.37	.36
Total MET, min/wk	2007 ± 384	2561 ± 606	2204 ± 463	1918 ± 274	1949 ± 341	2131 ± 401	1494 ± 237	1581 ± 242	.40	.37	.53
STAI	50.1 ± 2.7	52.3 ± 3.5	53.6 ± 3.1	51.1 ± 2.8	58.8 ± 5.6	59.7 ± 6.9	50.3 ± 3.1	48.0 ± 2.0	0.07	0.73	0.23
PSS	12 ± 1	11 ± 1	11 ± 1	12 ± 1	12 ± 2	12 ± 2	12 ± 1	10 ±1	0.92	0.11	0.11
Fat Preference											
Taste Score, %	74 ± 3	74 ± 4	71 ± 3	73 ± 3	72 ± 3	74 ± 3	67 ± 4	71 ± 4	0.79	0.02	0.70
Frequency Score, %	56 ± 4	59 ± 5	58 ± 4	57 ± 5	50 ± 4	52 ± 4	55 ± 3	59 ± 3	0.64	0.04	0.39
Difference Score, %	18 ± 3	16 ± 3	13 ± 2	16 ± 3	22 ± 4	22 ± 4	13 ± 3	13 ± 3	0.11	0.76	0.48
TFEQ											
Cognitive Restraint	8.5 ± 0.7	8.9 ± 0.7	9.1 ± 0.6	10.2 ± 1.2	9.2 ± 0.9	9.7 ± 0.7	9.1 ± 0.7	8.8 ± 0.8	0.48	0.14	0.48
Disinhibition	5.1 ± 0.6	4.9 ± 0.4	4.8 ± 0.5	5.6 ± 0.5	4.8 ± 0.5	4.9 ± 0.6	4.1 ± 0.5	4.7 ± 0.62	0.73	0.07	0.75
Hunger	32.2 ± 1.4	29.7 ± 1.3	30.5 ± 1.2	30.7 ± 1.2	29.7 ± 1.3	30.0 ± 1.4	29.5 ± 1.3	28.8 ± 1.3	0.89	0.60	0.26

All values are means ± SEMs. Characteristics were analyzed with a 2-way (group x visit) linear mixed model. Week 4 values represents an average of total MET min recorded during the 4-week intervention. BMI, body mass index; CSO, cottonseed oil; DBP, diastolic blood pressure; HC, hip circumference, MET, metabolic equivalent (min/wk); PSS, perceived stress scale; SBP, systolic blood pressure; STAI, state strait anxiety inventory; TFEQ, three factor eating questionnaire; WC, waist circumference; WHR waist-to-hip ratio.

* Indicates a significant difference from baseline to week 4 vs. control.

Table 4.3. Fasting physiological and subjective markers of appetite.

	CSO LOW		CSO MID		CSO HIGH		Control		p values		
	Baseline	Week 4	Baseline	Week 4	Baseline	Week 4	Baseline	Week 4	Group	Visit	Interaction
CCK (ng/ml)	0.51 ± 0.04	0.60 ± 0.06	0.51 ± 0.06	0.55 ± 0.07	0.48 ± 0.05	0.62 ± 0.08	0.52 ± 0.05	0.52 ± 0.03	0.88	<0.01	0.19
PYY (pg/ml)	120.0 ± 8.4	122.8 ± 8.4	97.0 ± 5.7	99.9 ± 6.0	107.2 ± 6.2	113.5 ± 8.4	98.0 ± 6.7	93.1 ± 4.4	0.01	0.52	0.55
Ghrelin (pg/ml)	103.9 ± 8.9	107.9 ± 12.0	92.9 ± 9.2	101.8 ± 11.1	113.5 ± 12.8	114.7 ± 14.7	117.3 ± 10.2	120.9 ± 11.1	0.48	0.10	0.78
Hunger (mm)	36.0 ± 6.2	51.2 ± 6.7	32.2 ± 4.3	49.9 ± 6.5	35.5 ± 5.5	44.5 ± 5.8	33.8 ± 6.0	38.3 ± 6.1	0.47	<0.01	0.70
Fullness (mm)	28.8 ± 4.4	19.7 ± 4.6	37.2 ± 5.3	27.4 ± 6.8	19.3 ± 4.3	25.9 ± 5.3	28.0 ± 5.5	29.6 ± 5.6	0.34	0.38	0.03*
Prospective Consumption (mm)	43.4 ± 4.1	46.5 ± 4.6	48.6 ± 4.2	53.6 ± 6.0	42.6 ± 3.8	47.5 ± 4.3	37.5 ± 2.7	43.9 ± 5.2	0.19	0.05	0.98
Desire to Eat (mm)	38.2 ± 6.4	42.0 ± 7.2	39.5 ± 5.2	51.4 ± 6.3	43.0 ± 6.3	39.8 ± 4.4	33.9 ± 4.1	44.4 ± 6.1	0.81	0.07	0.30
Appetite Score (mm)	47.2 ± 4.4	55.0 ± 5.1	45.8 ± 3.6	56.9 ± 5.7	50.4 ± 3.7	51.5 ± 3.8	44.3 ± 3.8	49.3 ± 4.9	0.60	0.01	0.51

All values are means ± SEMs. All fasting data was analyzed with a 2-way (group x visit) linear mixed model. CCK, Cholecystokinin; CSO, cottonseed oil; mm, millimeter; PYY, Peptide YY.
*Indicates an interaction driven by non-significant differences between groups.

Table 4.4. Sensory evaluation of cottonseed oil or control groups.

	CSO LOW		CSO MID		CSO HIGH		Control		p values		
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Group	Visit	Interaction
Sensory evaluation of the high SFA meal											
Appearance	6.4 ± 0.3	6.5 ± 0.4	5.9 ± 0.3	5.9 ± 0.4	6.4 ± 0.3	6.0 ± 0.5	6.1 ± 0.4	5.9 ± 0.4	0.45	0.69	0.73
Taste/Flavor	7.0 ± 0.3	6.9 ± 0.4	6.2 ± 0.5	6.0 ± 0.5	7.3 ± 0.2	6.3 ± 0.4	7.3 ± 0.2	6.8 ± 0.3	0.06	<0.01	0.32
Texture/Consistency	5.7 ± 0.5	6.3 ± 0.4	5.9 ± 0.4	5.4 ± 0.5	5.6 ± 0.5	5.7 ± 0.5	6.3 ± 0.4	5.9 ± 0.4	0.41	0.76	0.13
Aroma/Smell	6.3 ± 0.4	5.8 ± 0.4	5.9 ± 0.4	6.0 ±0.5	6.1 ± 0.4	5.9 ± 0.4	6.3 ± 0.3	6.4 ± 0.4	0.72	0.41	0.76
Overall Acceptability	6.5 ± 0.5	6.9 ± 0.3	6.1 ± 0.4	6.0 ± 0.5	6.8 ± 0.3	6.0 ± 0.4#	7.0 ± 0.2	6.9 ± 0.3	0.06	0.10	0.03
Sensory evaluation of intervention food											
Appearance	7.2 ± 0.2	—	7.2 ± 0.2	—	6.6 ± 0.3	—	7.1 ± 0.2	—	0.18	—	—
Taste/Flavor	7.1 ± 0.2	—	7.2 ± 0.2	—	7.0 ± 0.2	—	7.3 ± 0.2	—	0.67	—	—
Texture/Consistency	7.1 ± 0.2	—	7.2 ± 0.2	—	6.7 ± 0.3	—	7.2 ± 0.2	—	0.27	—	—
Aroma/Smell	7.2 ± 0.2	—	7.3 ± 0.2	—	6.8 ± 0.3	—	7.2 ± 0.2	—	0.32	—	—
Overall Acceptability	7.2 ± 0.2	—	7.3 ± 0.3	—	7.0 ± 0.3	—	7.4 ± 0.1	—	0.46	—	—

All values are means ± SEMs. Sensory evaluation of SFA meal was analyzed with a 2-way (group x visit) linear mixed model while a 1-way (group) linear mixed model was used to analyze sensory evaluation of the intervention food. Pre, Pre-Intervention; Post, Post-intervention; SFA, saturated fatty acid. Evaluations of intervention foods was done the first day of the intervention only.
#Indicates a trend for a difference from pre-intervention to post-intervention vs. control.

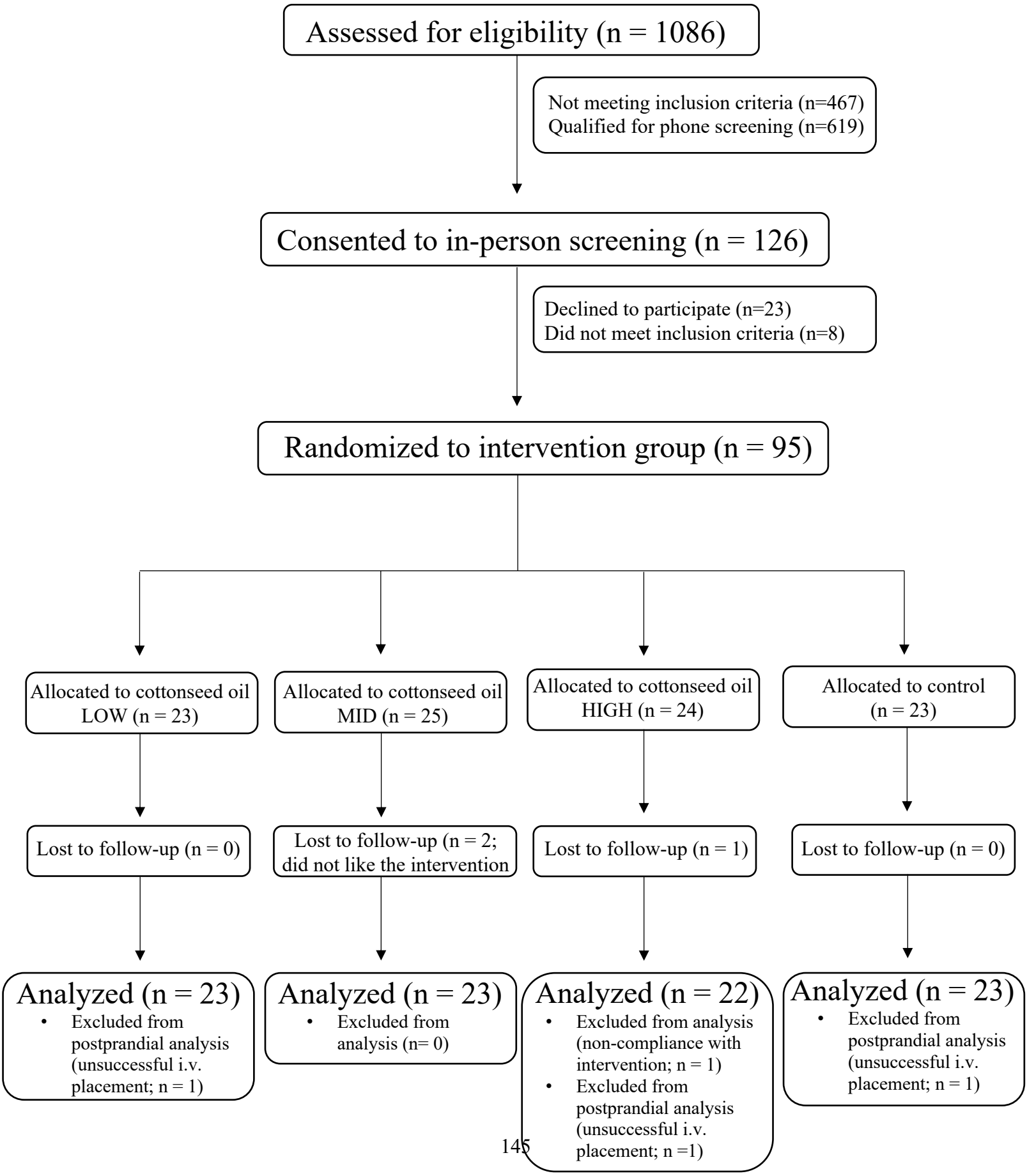
Assessed for eligibility (n = 1086)
Not meeting inclusion criteria (n=467)
Qualified for phone screening (n=619)
Consented to in-person screening (n = 126)
Declined to participate (n=23)
Did not meet inclusion criteria (n=8)
Randomized to intervention group (n = 95)
Allocated to cottonseed oil LOW (n = 23)
Allocated to cottonseed oil MID (n = 25)
Allocated to cottonseed oil HIGH (n = 24)
Allocated to control (n = 23)
Lost to follow-up (n = 0)
Lost to follow-up (n = 2; did not like the intervention
Lost to follow-up (n = 1)
Lost to follow-up (n = 0)
Analyzed (n = 23)
• Excluded from postprandial analysis (unsuccessful i.v. placement; n = 1)
Analyzed (n = 23)
• Excluded from analysis (n= 0)
Analyzed (n = 22)
• Excluded from analysis (non-compliance with intervention; n = 1)
• Excluded from postprandial analysis (unsuccessful i.v. placement; n =1)
Analyzed (n = 23)
• Excluded from postprandial analysis (unsuccessful i.v. placement; n = 1)

Figure 4.1. CONSORT flow diagram selection of participants.

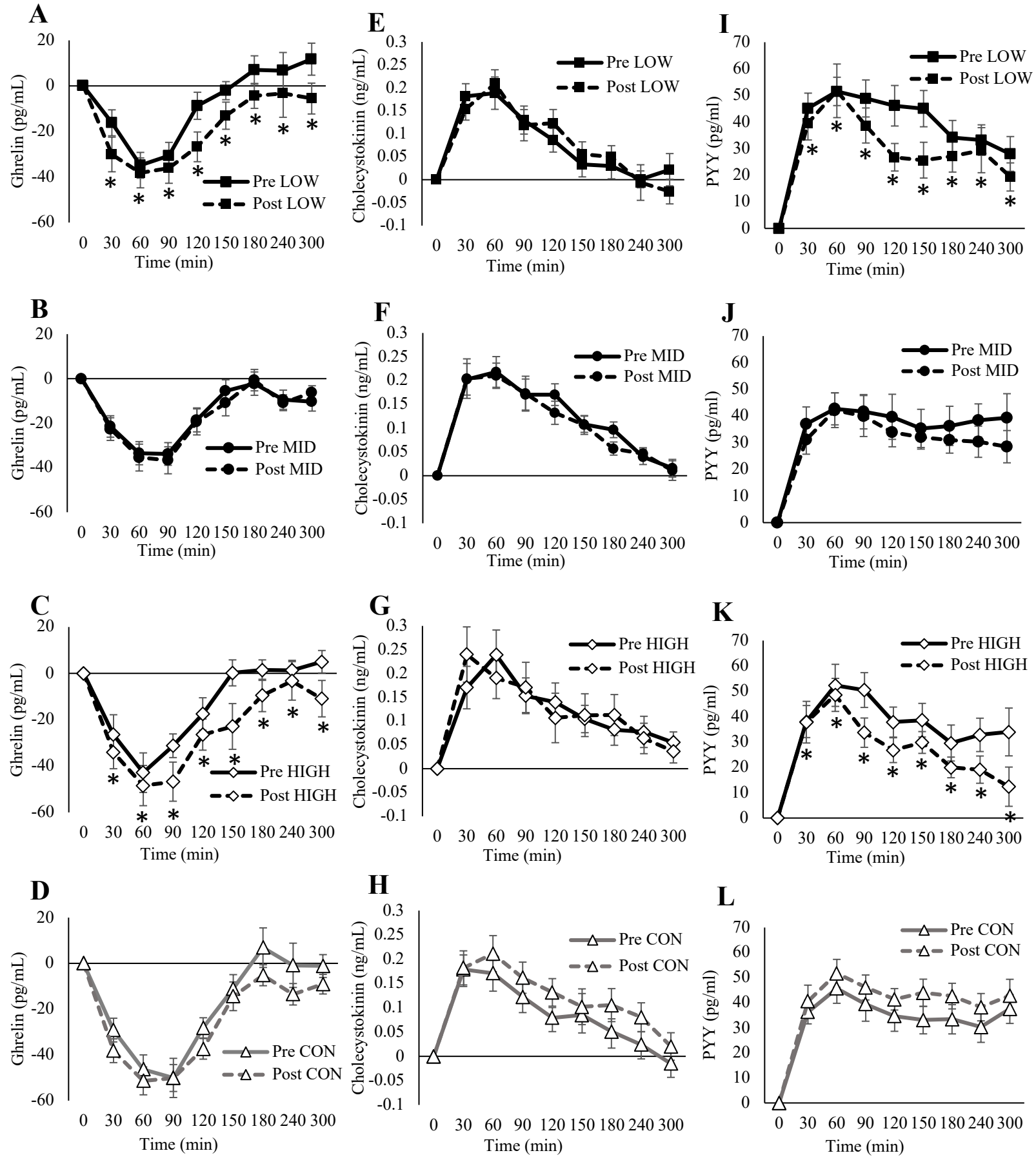
A
Ghrelin (pg/mL)
20
0
-20
-40
-60
Pre LOW
Post LOW
0 30 60 90 120 150 180 240 300
Time (min)
E
Cholecystokinin (ng/mL)
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
Pre LOW
Post LOW
0 30 60 90 120 150 180 240 300
Time (min)
I
PYY (pg/ml)
70
60
50
40
30
20
10
0
Pre LOW
Post LOW
0 30 60 90 120 150 180 240 300
Time (min)
B
Ghrelin (pg/mL)
Pre MID
Post MID
0 30 60 90 120 150 180 240 300
Time (min)
F
Cholecystokinin (ng/mL)
Pre MID
Post MID
0 30 60 90 120 150 180 240 300
Time (min)
J
PYY (pg/ml)
Pre MID
Post MID
0 30 60 90 120 150 180 240 300
Time (min)
C
Ghrelin (pg/mL)
Pre HIGH
Post HIGH
0 30 60 90 120 150 180 240 300
Time (min)
G
Cholecystokinin (ng/mL)
Pre HIGH
Post HIGH
0 30 60 90 120 150 180 240 300
Time (min)
K
PYY (pg/ml)
Pre HIGH
Post HIGH
0 30 60 90 120 150 180 240 300
Time (min)
D
Ghrelin (pg/mL)
Pre CON
Post CON
0 30 60 90 120 150 180 240 300
Time (min)
H
Cholecystokinin (ng/mL)
Pre CON
Post CON
0 30 60 90 120 150 180 240 300
Time (min)
L
PYY (pg/ml)
Pre CON
Post CON
0 30 60 90 120 150 180 240 300
Time (min)

Figure 4.2. Postprandial change from baseline for plasma ghrelin (A-D), peptide YY (E-H), and cholecystokinin (I-J) at pre- and post-intervention visits. Participants consumed a high-saturated fat meal immediately after time 0. Data were analyzed using a linear mixed model for group, visit, and timepoint. * Denotes a significant difference between pre- and post-invention vs. control. ($p \leq 0.05$). All data are presented as mean ± SEM. CSO, cottonseed oil; peptide YY (PYY); LOW, consumed 10% energy needs from cottonseed oil; MID, received 20% energy needs from cottonseed oil; HIGH, received 30% energy needs from cottonseed oil; CON, received 10% energy needs from a mixture of oils representative of the fatty acid composition of the U.S. diet.

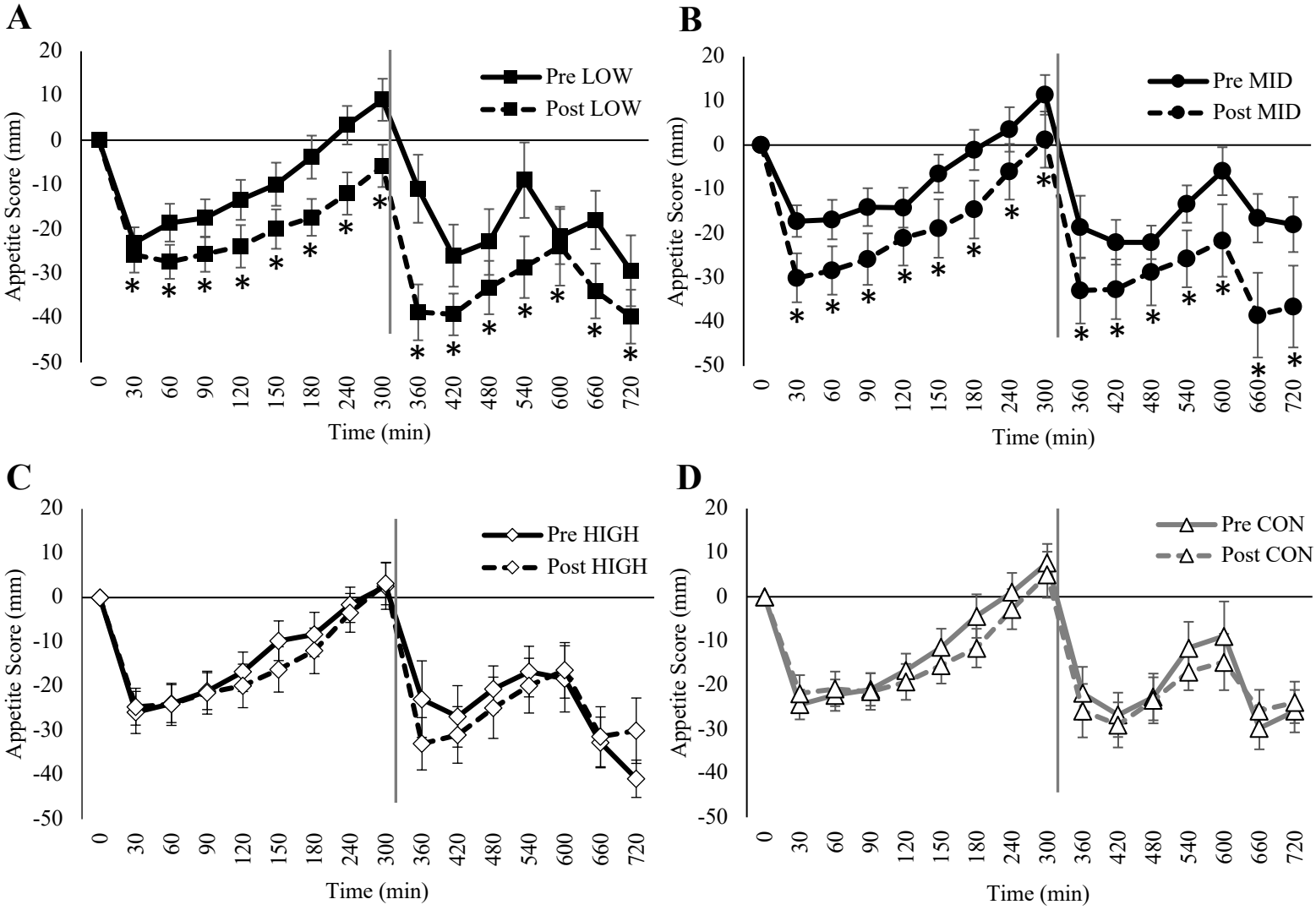
A
Pre LOW
Post LOW
Appetite Score (mm)
Time (min)
B
Pre MID
Post MID
Appetite Score (mm)
Time (min)
C
Pre HIGH
Post HIGH
Appetite Score (mm)
Time (min)
D
Pre CON
Post CON
Appetite Score (mm)
Time (min)

Figure 4.3. Postprandial change from baseline subjective VAS (visual analog scale) calculated appetite score (A-D) at pre- and post- intervention visits. Participants consumed a high-saturated fat meal immediately after time 0. The vertical line represents the division of "in lab"(LAB) measures (time 0-300 minutes) and "at-home" (HOME) measures (360-720 minutes) which were combined to make up all-day measures. Data were analyzed using a linear mixed model for group, visit, and timepoint. * Denotes a significant difference between pre- and post-intervention vs. control for all-day ($p \leq 0.05$). All data are presented as mean ± SEM. CSO, cottonseed oil; LOW, consumed 10% energy needs from cottonseed oil; MID, received 20% energy needs from cottonseed oil; HIGH, received 30% energy needs from cottonseed oil; CON, received 10% energy needs from a mixture of oils representative of the fatty acid composition of the U.S. diet.

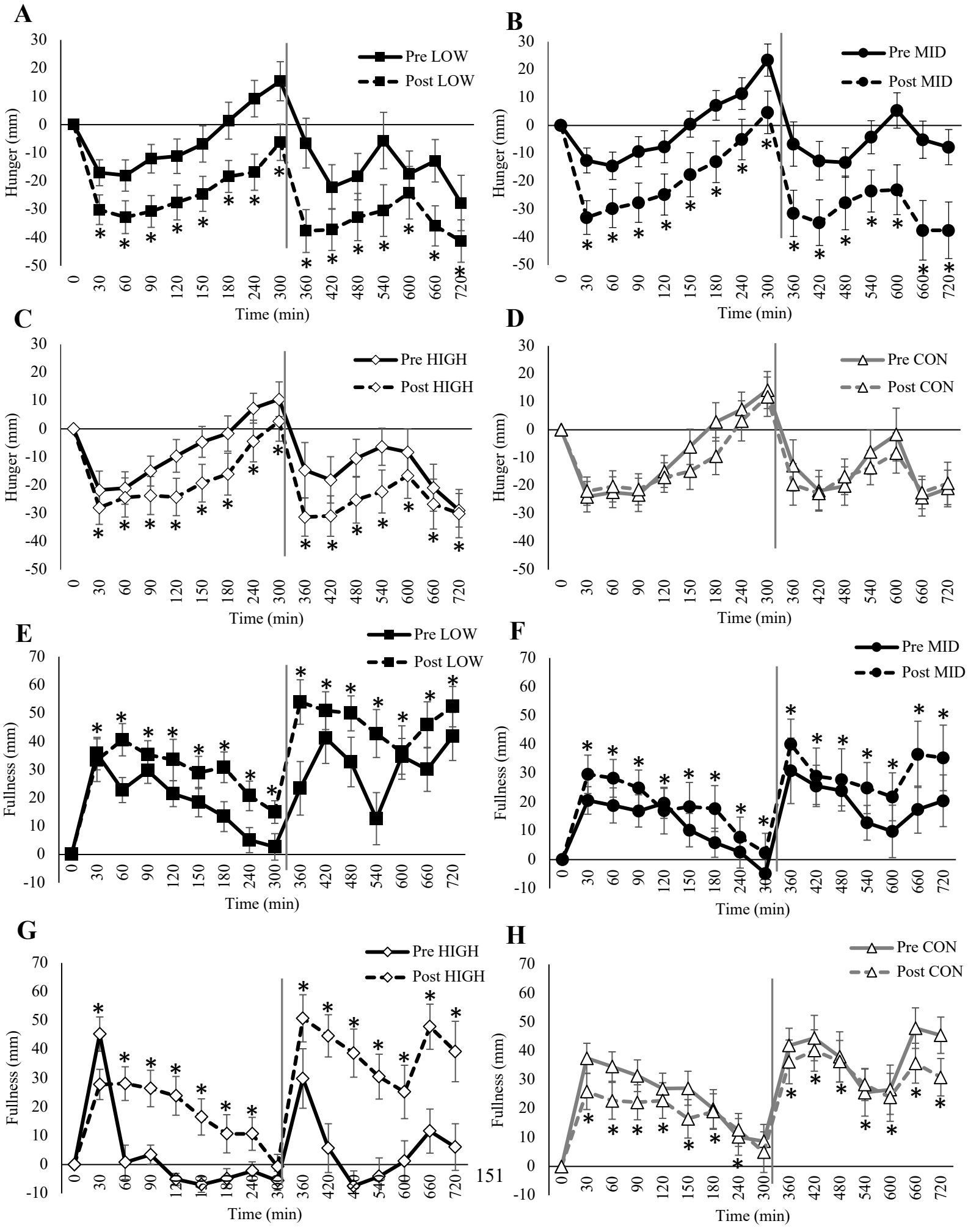
A
Pre LOW
Post LOW
Hunger (mm)
Time (min)
B
Pre MID
Post MID
Hunger (mm)
Time (min)
C
Pre HIGH
Post HIGH
Hunger (mm)
Time (min)
D
Pre CON
Post CON
Hunger (mm)
Time (min)
E
Pre LOW
Post LOW
Fullness (mm)
Time (min)
F
Pre MID
Post MID
Fullness (mm)
Time (min)
G
Pre HIGH
Post HIGH
Fullness (mm)
Time (min)
H
Pre CON
Post CON
Fullness (mm)
Time (min)

Figure 4.4. Postprandial change from baseline subjective VAS (visual analog scale) hunger (A-D) and fullness (E-H) at pre- and post- intervention visits. Participants consumed a high-saturated fat meal immediately after time 0. The gray line represents the division of "in lab" (LAB) measures (time 0-300 minutes) and "at-home" (HOME) measures (360-720 minutes) which were combined to make up all-day measures. Data were analyzed using a linear mixed model for group, visit, and timepoint. * Denotes a significant group by visit interaction ($p \leq 0.05$). For hunger, there was significant suppression from pre- and post-invention in all CSO groups vs. control across the whole day ($p \leq 0.05$). For fullness, there was a significant increase from pre- and post-invention in all CSO groups vs. a suppression in control across the whole day. All data are presented as mean ± SEM. CSO, cottonseed oil; LOW, consumed 10% energy needs from cottonseed oil; MID, received 20% energy needs from cottonseed oil; HIGH, received 30% energy needs from cottonseed oil; CON, received 10% energy needs from a mixture of oils representative of the fatty acid composition of the U.S. diet.

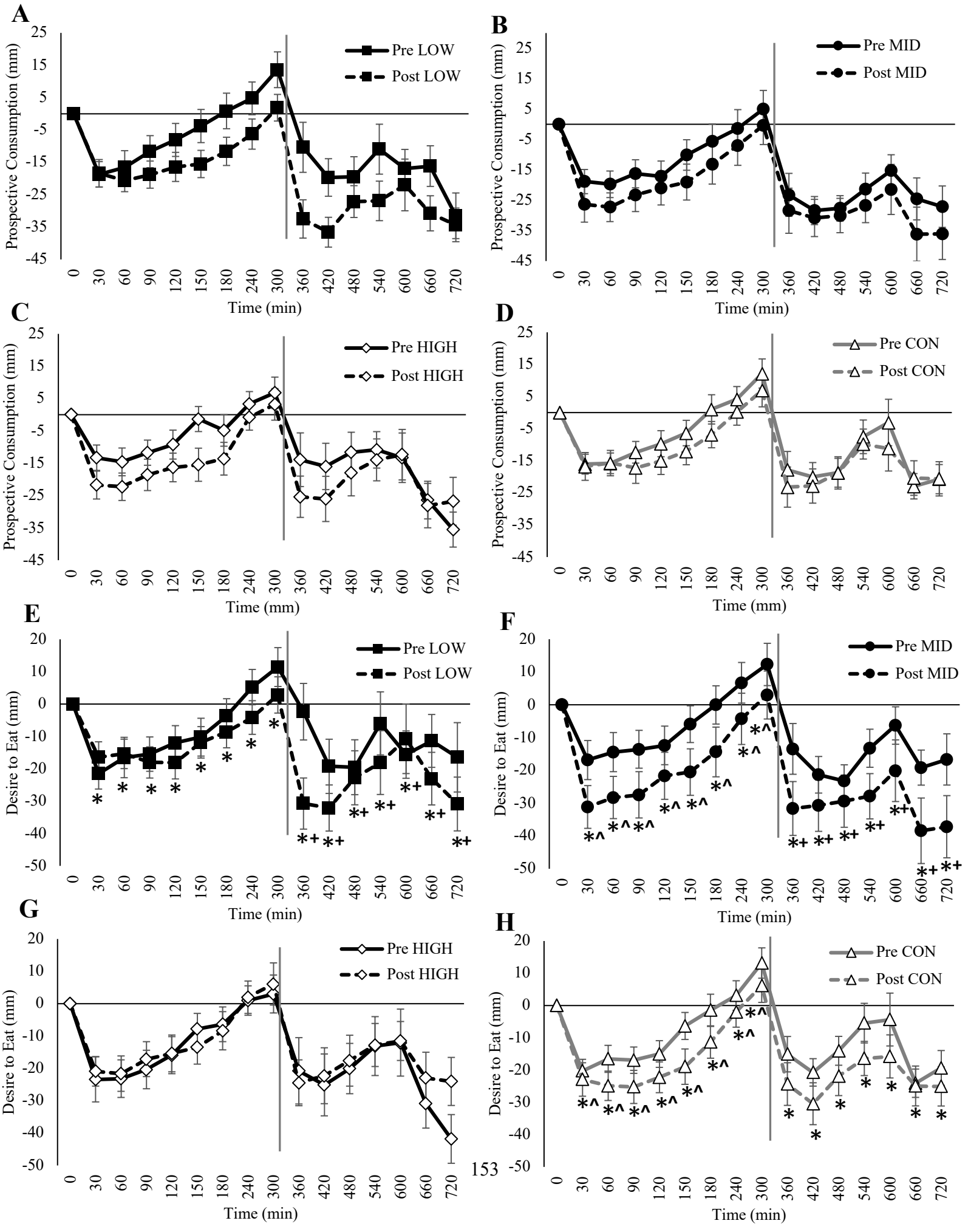
A
Pre LOW
Post LOW
Prospective Consumption (mm)
Time (min)
B
Pre MID
Post MID
Prospective Consumption (mm)
Time (min)
C
Pre HIGH
Post HIGH
Prospective Consumption (mm)
Time (mm)
D
Pre CON
Post CON
Prospective Consumption (mm)
Time (mm)
E
Pre LOW
Post LOW
Desire to Eat (mm)
Time (min)
F
Pre MID
Post MID
Desire to Eat (mm)
Time (min)
G
Pre HIGH
Post HIGH
Desire to Eat (mm)
Time (min)
H
Pre CON
Post CON
Desire to Eat (mm)
Time (min)

Figure 4.5. Postprandial change from baseline subjective VAS (visual analog scale) prospective consumption (A-D) and desire to eat (E-H) at pre- and post- intervention visits. Participants consumed a high-saturated fat meal immediately after time 0. The gray line represents the division of "in lab" (LAB) measures (time 0-300 minutes) and "at-home" (HOME) measures (360-720 minutes) which were combined to make up all-day measures. Data were analyzed using a linear mixed model for group, visit, and timepoint. *Denotes a significant suppression from pre- and post-invention for LOW, MID, and CON vs. HIGH across the whole day ($p \leq 0.05$). ^ Denotes suppression from pre-to post-intervention in MID vs. LOW and HIGH for LAB measures ($p \leq 0.05$). + denotes suppression from pre- to post-intervention in LOW and MID vs. HIGH and control for HOME measures ($p \leq 0.05$). All data are presented as mean ± SEM. CSO, cottonseed oil; LOW, consumed 10% energy needs from cottonseed oil; MID, received 20% energy needs from cottonseed oil; HIGH, received 30% energy needs from cottonseed oil; CON, received 10% energy needs from a mixture of oils representative of the fatty acid composition of the U.S. diet.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The main purpose of this thesis project was to evaluate the effects of different doses of CSO on cardiometabolic responses (blood lipids and appetite markers) in adults at-risk for CVD. To assess our research question, we completed a 28-day parallel, randomized control trial in which we allocated participants into one of three CSO dose groups: LOW (10% EEN from CSO; n=23), MID (20% EEN from CSO; n=23), or HIGH (30% EEN from CSO; n=22), or CON in which participants received 10% EEN from a mixture of oils (n=23).

The results displayed in Chapter 3 demonstrate that diets enriched with 10%, 20% or 30% CSO improved TC, non-HDL-c, and TC:HDL ratio vs. a control diet. Additionally, diets enriched with 20% and 30% from CSO had significant reductions in LDL-c, while the 10% CSO dose only displayed a trend in LDL-c improvements. In addition to these fasting outcomes, the 30% dose suppressed postprandial TGs after HF meal consumption. The LDL-c reduction in the MID and HIGH group corresponded to an estimated 12.3-21% coronary artery disease risk reduction (1, 2). Moreover, the MID and HIGH doses elicited a similar magnitude of reduction for LDL-c while the LOW group had a slightly less magnitude of response.

There were some additional analyses of interest that did not fit within the manuscripts of this study. Therefore, they will be evaluated in this chapter. To further explore the relationship between length of intervention and blood lipid reduction, we performed an exploratory slope analysis for LDL-c (**Figure 5.1**). Upon analysis, we observed a significant reduction in slope

from week 3 to week 4 within the LOW group ($p = 0.05$). This finding supports our hypothesis that with a longer intervention, the LOW dose may exhibit the same magnitude of response as the MID and HIGH group. For instance, the slope of LDL-c reduction in the HIGH group was most pronounced for week 1 (-9.5mg/dL), followed by relatively stable values through the subsequent weeks (1.3, 0.9, and -4.6mg/dL for weeks 2-4, respectively). In contrast, the slope of the LOW remained relatively unchanged over weeks 1-3 (-0.8, 2.9, 2.0mg/dL, respectively), with a marked decline in week 4 (-9.9mg/dL). These findings suggest that extending the intervention period may be necessary to achieve a clinically meaningful reduction in LDL-c with the LOW CSO dose. A similar analysis was then completed for TC. The reductions in TC for the LOW, MID, and HIGH CSO groups were 4.9%, 6.3%, and 6.7%, respectively. Slope analysis revealed no significant differences between weekly slopes within the LOW or HIGH groups (**Figure 5.1**). However, there was trend for reduction ($p= 0.07$) for the slope from week 3 to 4 in the CSO MID group (-11.5mg/dL) following relatively stable values from weeks 1-3 (-2.4, -2.2, 3.9mg/dL, respectively). A similar pattern was observed in the LOW group from weeks 1-4 (-5.4, 4.2, 0.9, -10.2mg/dL, respectively). However, the more pronounced decline from week 3-4 in the MID group may explain the why this group reached a similar magnitude of reduction for TC as the HIGH group.

In Chapter 4, the results presented show the improvements of subjective markers of appetite with all three CSO dose groups vs. a control diet. Specifically, we reported an improvement in overall subjective appetite with diets enriched with 10% and 20% EEN from CSO, and hunger suppression and increased fullness in all CSO doses. Moreover, the change in overall subjective appetite in the LOW and MID group was 26% and 27% different than control, respectively, which indicates a meaningful reduction in appetite (3). Additionally, intake of 10%

and 30% CSO suppressed postprandial ghrelin; however, PYY was also suppressed in the same groups. Therefore, none of the groups had a clear impact or improvement on hormonal regulation of appetite. Nonetheless, none of the observed changes of subjective or physiologic appetites corresponded to any dose-response pattern. This suggests that low doses of CSO can have beneficial effects on subjective appetite suppression.

The rationale behind this dose-response study was to evaluate whether lower doses of CSO would be effective in improving cardiometabolic outcomes in hopes to keep dietary fat intake within the AMDR (20-35% EEN; (4)) to encourage healthier dietary patterns. However, regardless of intervention group, our participants were consuming a HF diet throughout the intervention. The LOW, MID, and HIGH CSO groups consumed 45.1%, 44.2% and 50.6% energy from fat, respectively, and 41.5% in CON. Notably, our participants were also following a HF diet prior to the start of the intervention (LOW: 36.1%; MID: 39.0%; HIGH: 39.3%; CON: 40.0%). Therefore, further research is warranted to assess whether the observed cardiometabolic effects can be achieved with lower doses of CSO in a diet that aligns with the AMDR of fat intake. To obtain this result in this population, more intensive dietary counseling around total fat reduction may be needed. Another consideration for future studies may be to screen participant's dietary intake prior to the participant beginning the intervention to gather a more robust understanding of their dietary habits and macronutrient distribution ranges of their current diet.

To further explore the timing of weight gain across the intervention of this trial, a slope analysis was conducted (**Figure 5.1**). There were no significant within group differences in weekly slopes for weight change for the MID, HIGH, and CON group. However, there was a significant increase in slope in week 1 in the LOW group (1.0kg; $p = 0.04$), followed by weight fluctuations from weeks 2-4 (-0.1, 0.2, -0.4kg, respectively). In contrast, the HIGH group

exhibited steady weight gain over weeks 1-3 (0.8, 0.5, 0.3kg, respectively), and the slope tapered off from week 3-4 (0.0kg). Additionally, to examine the relationship between substitution of the study foods and weight gain in our participants, we performed a theoretical weight gain analysis utilizing the NIH body weight planner (https://www.niddk.nih.gov/bwp) (**Figure 5.2**). This analysis served to evaluate the differences between projected weight gain if no energy substitution of the study foods occurred compared to the actual weight gain observed within each group. There was a significant difference between theoretical vs. actual weight gain ($p < 0.01$) driven by greater predicted weight change in all CSO dose groups and CON ($p < 0.01$ for all). These findings suggest that participants partially incorporated these foods into their diet, but complete isocaloric substitution may not be feasible for this type of outpatient feeding intervention.

The data presented in this body of work provide evidence that lower doses of CSO (<30% EEN) provide cardioprotective effects such as blood lipid reduction and improved appetite regulation. Therefore, these results indicate the potential for the adoption of healthier dietary patterns (lower dietary fat intake), although fat intakes within the AMDR were not achieved in this study. Additionally, future studies should evaluate other high-PUFA oils compared to CSO to decipher if CSO has superior cardiometabolic protective effects. Finally, to obtain a more robust understanding of CSO's effects on CVD risk, other studies should assess markers of other chronic disease risk factors, such as inflammation.

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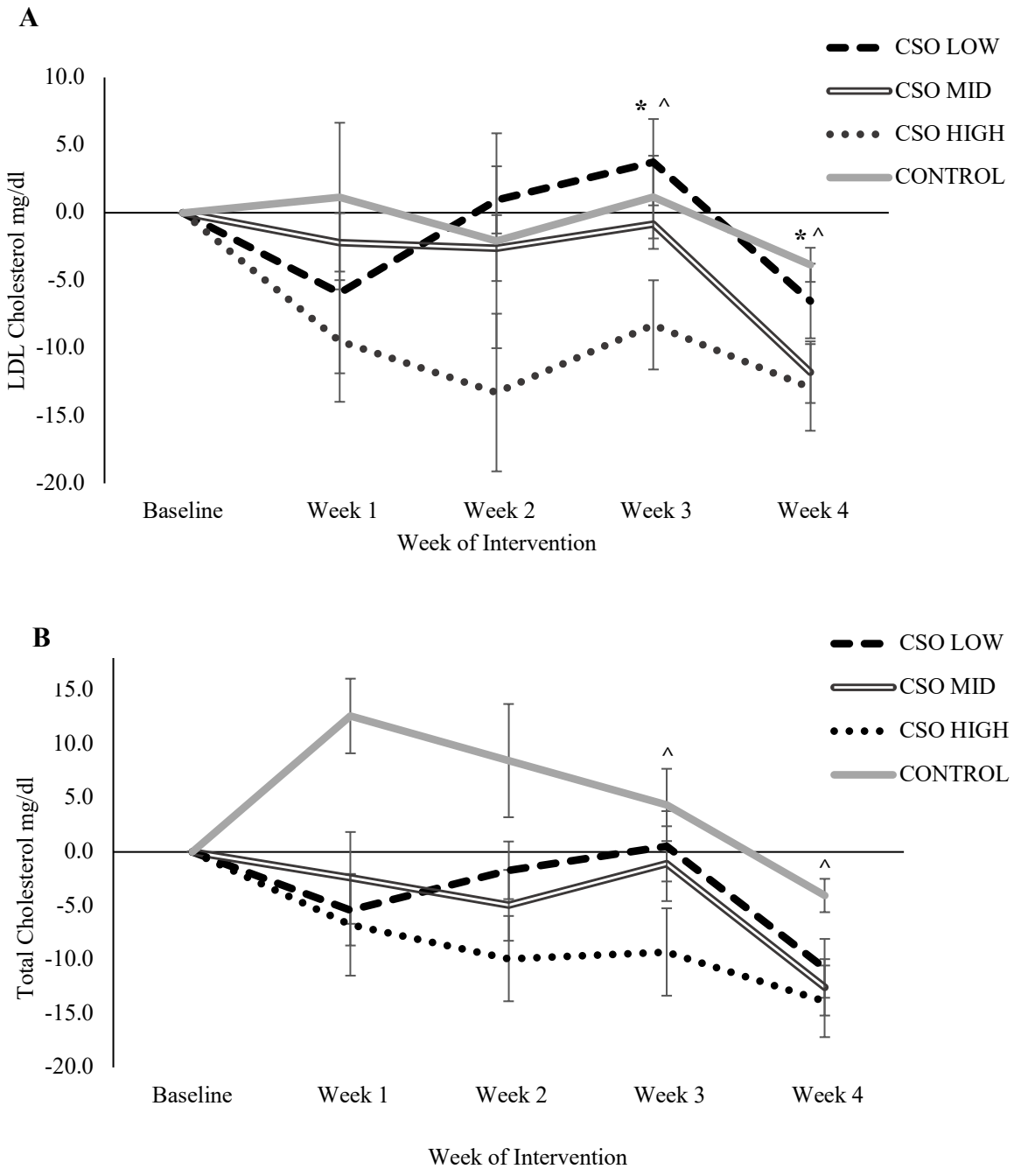
Figure 5.1


A
CSO LOW
CSO MID
CSO HIGH
CONTROL
10.0
5.0
0.0
-5.0
-10.0
-15.0
-20.0
LDL Cholesterol mg/dl
* ^
* ^
Baseline
Week 1
Week 2
Week 3
Week 4
Week of Intervention
B
CSO LOW
CSO MID
CSO HIGH
CONTROL
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
-20.0
Total Cholesterol mg/dl
^
^
Baseline
Week 1
Week 2
Week 3
Week 4
Week of Intervention

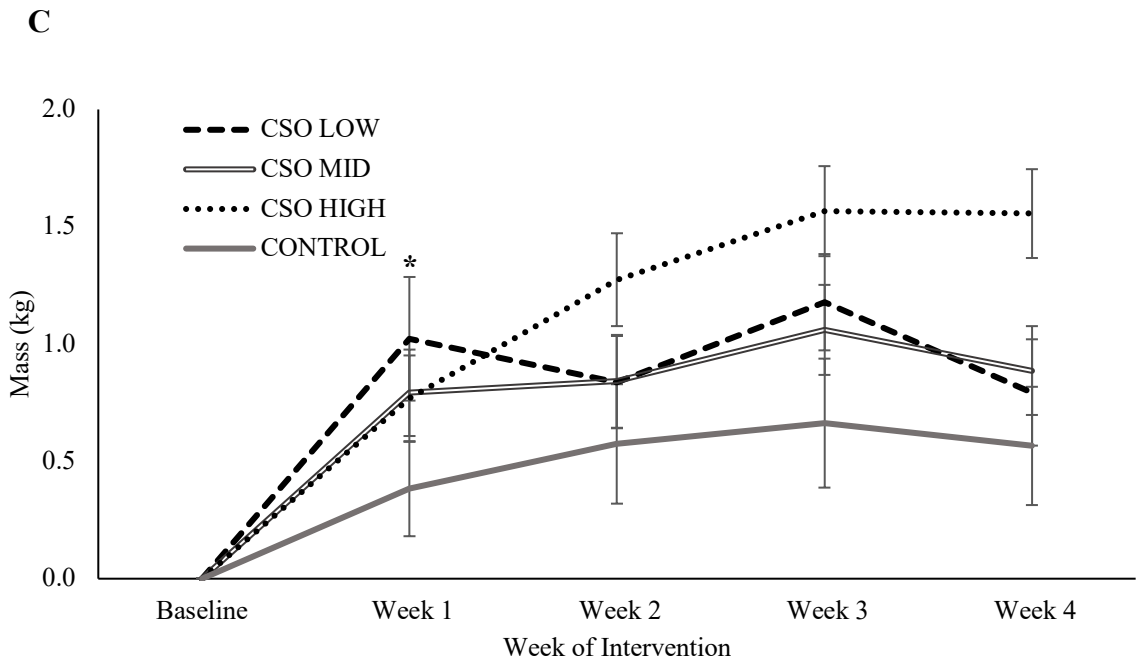


Figure 5.1 Weekly change in LDL-c (**A**), Total Cholesterol (**B**), and Weight (**C**) for each group throughout the 4-week intervention. * indicates a significant within group difference for weekly slope in LOW. ^ indicates a trend for a within group difference for weekly slope in MID. CSO, cottonseed oil; LOW, received 10% energy needs from cottonseed oil; MID, received 20% energy needs from cottonseed oil; HIGH, received 30% energy needs from cottonseed oil; CON, received 10% energy needs from a mixture of oils representative of the fatty acid composition of the U.S. diet.

Figure 5.2

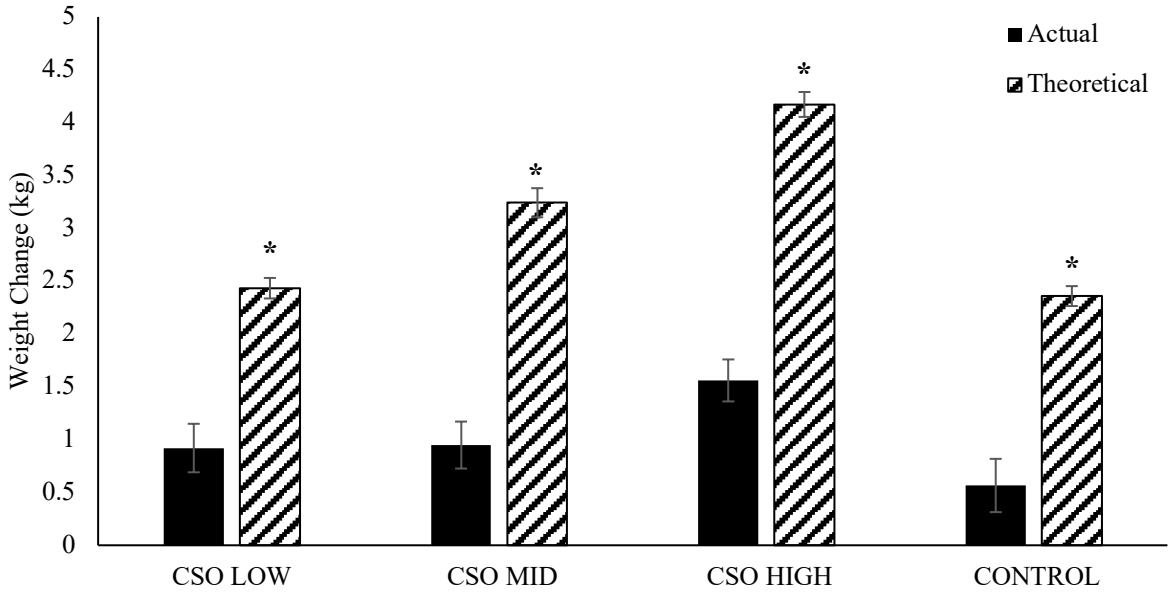


Figure 5.2. Changes in actual and theoretical body weight. *indicates a significant difference between actual and theoretical weight ($p \leq 0.05$). CSO, cottonseed oil; LOW, received 10% energy needs from cottonseed oil; MID, received 20% energy needs from cottonseed oil; HIGH, received 30% energy needs from cottonseed oil; CON, received 10% energy needs from a mixture of oils representative of the fatty acid composition of the U.S. diet.