THE IMPACT OF PECAN-ENRICHED DIETS ON CARDIOVASCULAR DISEASE RISK

by

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ABSTRACT

Cardiovascular disease (CVD) is the leading cause of death in America; however, altering the diet to optimize blood lipids, antioxidant status, and body weight may reduce CVD risk. Previous research has shown that daily pecan consumption improves blood lipids and antioxidant status in healthy populations, but its impact in a population at-risk for CVD is unknown. Furthermore, no study has investigated the impact of daily pecan consumption, with and without dietary substitution instructions, on body weight and other health outcomes. The objective was to investigate the impact of pecan-enriched diets on CVD rick factors including blood lipids, angiopoietin-like proteins (ANGPTL), appetite, metabolism, antioxidant status, and body weight. We conducted an 8-week randomized, controlled trial with two pecan groups and a nut-free control group. The ADD group consumed pecans as part of a free-living diet, and the SUB group substituted the pecans for isocaloric foods from their habitual diet. For the first 5 manuscripts (chapters 3-7), we recruited adults at-risk for CVD. For the 6th manuscript (chapter 8), we made the a priori decision to increase sample size and expand the inclusion criteria to obtain adequate power to investigate the impact of pecan-enriched diets on weight outcomes. In manuscript #1 (chapter 3), total cholesterol, low-density lipoprotein cholesterol, triglycerides, and apolipoprotein-B improved in ADD and SUB with no changes in control. In manuscript #2

(chapter 4), postprandial ANGPTL3 improved in ADD and SUB with no changes in control. In manuscript #3 (chapter 5), postprandial CCK, PYY, ghrelin, and subjective markers of appetite improved in ADD but not SUB or control. In manuscript #4 (chapter 6), resting metabolic rate, fasting fat oxidation, and fasting respiratory exchange ratio improved in SUB. In addition, postprandial diet induced thermogenesis increased in ADD with no changes in SUB or control. In manuscript #5 (chapter 7), both pecan-enriched diets improved postprandial lipid peroxidation and total antioxidant capacity with no changes in control. Altogether, the results of the first five manuscripts suggest that daily pecan consumption may reduce risk for developing CVD. Finally, in manuscript #6 (chapter 8), daily pecan consumption did not result in significant weight gain.

INDEX WORDS: TREE NUTS, DIETARY FATTY ACIDS, BLOOD LIPIDS, CHOLESTEROL, TRIGLYCERIDES, ANGPTL, APPETITE, CCK, PYY, GHRELIN, METABOLISM, SUBSTRATE OXIDATION, LIPID PEROXIDATION, TOTAL ANTIOXIDANT CAPACITY, BODY WEIGHT, HIGH-FAT

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DEDICATION

Special thanks to my mom and dad for encouraging me to pursue my passions. Dad, thank you for showing me what it means to be a scientist. Mom, thank you for your endless encouragement and listening ear. Thank you to my husband, Peter, for always making me laugh and reminding me to not take life too seriously. Your support through this process has meant the world to me, and I could not have succeeded without you all.

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CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in America, with 1 in every 4 deaths attributed to this chronic disease [1, 2]. Many forms of CVD are the product of atherosclerosis, an inflammatory lipid storage condition which leads to plaque formation in arterial walls [2]. Obesity-induced inflammation contributes to the generation of CVD by accelerating the atherosclerotic process [3], and the most recent NHANES data from 2017-2018 reported that over forty percent of Americans have obesity [4]. In order to combat CVD, we need to reduce risk factors associated with the disease.

The composition of fatty acids (FA) in the diet has been shown to influence CVD risk [5-7]. Saturated fatty acids (SFA) are associated with negative cardiovascular outcomes, whereas the consumption of unsaturated fatty acids (UFA) may be cardio-protective [5-7]. Additionally, other bioactives in certain foods can lower CVD risk. Dietary antioxidant intake, for example, is inversely associated with many CVD-related risk factors [8]. Tree nuts are rich sources of UFAs and other vitamins, minerals, and phytonutrients [9] and have been shown to improve blood lipid profiles [10-27] and antioxidant status [28-31] and promote weight management [10, 18, 21-25, 27, 32-44]. Although there is substantial health-related research available on tree nut consumption, most studies focus on walnuts [12-19, 34, 36, 38-40, 42, 43, 45-50] and almonds [20-25, 35, 37, 41, 44, 51-53].

A limited number of studies have examined the impact of pecan consumption on chronic disease risk or other markers of health in adults. Two studies of 4- to 8-week interventions

showed that dietary pecan supplementation decreased total cholesterol, low-density lipoprotein cholesterol and fasting triglycerides (TG) in healthy adults (26, 27). Conversely, a more recent study that investigated the impact of a smaller daily dose of pecans (~42.5g/d) in a metabolically at-risk population (overweight with central adiposity) did not show reductions in blood lipids (25). It is unclear if the lack of change in blood lipids was due to the lower dose of pecans or the at-risk population. In addition, another study showed that 4 weeks of a pecan-enriched diet reduced fasting lipid peroxidation with no changes in total antioxidant capacity in healthy individuals [29]. Finally, preliminary evidence suggests that angiopoietin-like-3 (ANGPTL3) may mediate changes in TG following acute pecan consumption [30, 54]. Therefore, more research is needed to investigate the impact of pecan-enriched diets on CVD-risk factors (blood lipids, antioxidant status, and ANGPTL3) in an at-risk population.

As previously mentioned, obesity is also a major risk factor for CVD [55], which highlights the importance of improving weight management. On average, individuals only gain 0.5-1 kg/y, but a small, consistent increase in body weight can lead to obesity over time [56]. Therefore, it is plausible that small adjustments to energy intake or energy expenditure may mitigate the energy imbalances that are fueling annual weight gain, and by extension, obesity. Two promising avenues are to enhance the regulation of appetite and metabolism to decrease energy intake and increase energy expenditure, respectively [57, 58]. Previous research suggests that the long-term intake of UFA may improve appetite and metabolism [59-61]. Similarly, other nutrients such as dietary fiber and protein are known to have stronger effects on satiety [62]. Since pecans are a rich source of UFA and moderate source of fiber and protein, it is plausible that daily pecan intake could suppress appetite or increase energy expenditure, but these outcomes have not been examined previously.

Although some tree nut studies have shown weight maintenance with nut consumption [17, 24, 32, 35, 36, 39, 42, 44], differences in methodological considerations may impact the results. Those different methods include providing no dietary instructions with the nut consumption [22, 25-27, 30, 31, 35, 39], instructions to substitute energy equivalent foods or specific macronutrients in their typical diet [8, 14, 17, 18, 23, 32-34, 36, 37, 44], or the provision of all meals in an outpatient feeding setting [21, 24, 38, 40, 42, 46]. To date, there is limited research comparing the impact of tree nut consumption on body weight outcomes in participants with and without dietary energy substitution instructions. This is an important consideration when establishing public health recommendations concerning nut consumption.

The literature review (Chapter 2) provides an overview of the current body of research surrounding tree nuts and 1) blood lipids/ANGPTLs, 2) body weight, 3) appetite, 4) metabolism, and 5) antioxidant status. Chapters 3 through 7 present data on the impact of pecan-enriched diets, with and without dietary substitution instructions, in adults at-risk for CVD on blood lipids, ANGPTLs, appetite, metabolism, and antioxidant status, respectively. Chapter 8 presents data on the impact of pecan-enriched diets on body weight in adults, with and without risk for chronic disease. We made the a priori decision to increase our sample size and expand our inclusion criteria to obtain adequate power to investigate weight outcomes. All manuscripts (chapters 3 through 8) include two pecan-enriched groups and a nut-free group (control). The ADD group consumed pecans as part of a free-living diet, and the SUB group substituted the pecans for isocaloric foods from the habitual diet. We hypothesized that both the pecan-enriched diets would lead to improvements in blood lipids, appetite, metabolism, antioxidant status, and ANGPTLs from pre- to post-intervention and that this improvement would be greater than the control group. Furthermore, we hypothesized that the ADD group would have an increase in

body weight, and that the change would be greater than that of the control and SUB groups. However, we did hypothesize that the weight gain in ADD would be less than anticipated due to partial energy compensation.

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CHAPTER 2

REVIEW OF THE LITERATURE

Cardiovascular Disease and Cholesterol Metabolism

Cardiovascular disease (CVD) is the leading cause of death in America, with 1 in every 4 deaths attributed to this chronic disease [1, 2]. CVD is an umbrella term that refers to many forms of heart disease such as coronary artery disease, myocardial infarctions, ischemic stroke, and heart failure [3]. Many of these forms of CVD are the product of atherosclerosis, an inflammatory lipid storage condition in which diet quality plays an active role in this process [4]. There are a number of modifiable risk factors for CVD including elevated blood pressure, smoking, insulin resistance, and high cholesterol [5].

The consumption of dietary fat influences the total cholesterol content of the blood due to cholesterol's metabolic process [6]. After consumption of dietary fat, a small amount of TG is broken down by lingual lipase in the mouth and gastric lipase in the stomach [7]. The presence of fat in the intestine stimulates the release of CCK from the intestinal cells [8]. Next, CCK prompts the release of bile from the gallbladder to emulsify the hydrophobic fats in the hydrophilic environment and triggers the secretion of pancreatic lipase. The binding of colipase to the fat droplet allows pancreatic lipase to bind and further break down ester bonds of TGs to form FFAs and monoglycerides [9]. The lipid fractions and bile salts complex to form micelles, which passively diffuse into the enterocyte [10].

Once inside the enterocyte, the lipids are re-esterified in the endoplasmic reticulum and packaged with cholesterol and a small amount of protein to form chylomicrons [6]. This

molecule also contains apolipoprotein B-48 (apoB-48), which is only produced by the human intestine [11]. Other apolipoproteins are produced in the liver, thus apoB-48 is a superior marker of CVD risk and nutritional status. The fat-containing portion of the chylomicron is surrounded by phospholipids to allow the molecule to travel in an aqueous environment [6]. The size of the chylomicron corresponds to the amount of TG present within the intestinal enterocyte [12]. During peak TG absorption, the chylomicron is larger in size.

After exocytosis through the basolateral membrane, chylomicrons enter the lymphatic system and travel through the body [13]. During travel, chylomicrons acquire apolipoprotein C (apoC) and apolipoprotein E (apoE) from the HDL [14]. When the chylomicron arrives at a muscle cell or adipocyte, the apoC activates lipoprotein lipase (LPL) which cleaves the TG to deposit FFAs. Muscle cells use the FFAs for energy or fat storage [15], and adipose tissue re-synthesizes the FFAs into a TGs for storage [6]. Notably, LPL is modulated by the angiopoietin-like protein family (ANGPTL), specifically ANGPTL3, ANGPTL8, and ANGPTL4. [16]. In the postprandial state, ANGPTL3 and -8 work synergistically to inhibit lipoprotein lipase (LPL) in oxidative tissues such as the heart and muscles [17]. This action reduces the uptake of TG to increase TG circulation and promote energy storage in the white adipose tissue [18]. Furthermore, ANGPTL4 contributes to the reduction of LPL in adipose tissue, primarily during the fasted state [19]. The process of delivering fatty acids (FA) to body tissues converts the chylomicron into a chylomicron remnant [14]. The low-density lipoprotein (LDL) receptors of the liver have a high affinity for the apoE of the chylomicron remnant, which allows the chylomicron remnant to enter the liver to be converted into bile salts for emulsifying fats, cholesterol esters for storage, or very low density lipoprotein (VLDL) for further circulation.

The endogenous lipoprotein pathway begins with the formation of nascent VLDL from cholesterol, TG, phospholipids and apolipoproteins [20]. Apolipoprotein B-100 (apoB-100) is the predominant apolipoprotein in VLDL [14, 21]. Once in circulation, VLDL becomes mature after acquiring ApoE and apoC from HDL [14]. Once again, apoC activates LPL to allow VLDL to release its TG contents to body tissues, then apoC is returned to HDL. The leftover VLDL remnants, referred to as intermediate density lipoprotein (IDL), contain cholesterol, TG, apoB-100 and apoE [22]. IDL enters either the liver or the adrenal cortex due to the high affinity of these organ's LDL receptors for the apoE of IDL [14]. The expression of these LDL receptors dictate plasma LDL cholesterol concentrations [23]. Once in the liver, cholesterol is used to form bile salts, other lipoproteins, or it is stored [22]. Once apoE is returned to HDL, the molecule becomes LDL which contains predominately cholesterol and apoB-100 with minimal amounts of TGs. As VLDL is metabolized to IDL then LDL, the size of the molecule is decreases and the density of the molecule increases.

The main function of LDL is to deliver cholesterol to peripheral tissues [22]. LDL is available in numerous sizes and densities [24]. Small LDL is more atherogenic than large LDL because small LDL remains in circulation for a longer period of time due to its low affinity for the LDL receptor [22, 25]. Likewise, small LDL enters the arterial wall and can undergo oxidation more easily. Other cholesterol-rich apoB lipoproteins (VLDL, IDL, LDL) also accumulate in the subendothelial space and undergo oxidative reactions [4]. This process causes an inflammatory response which attracts macrophages to devour the oxidized LDL cholesterol, producing foam cells [26]. These foam cells internalize the atherosclerotic lipids until its death by apoptosis, creating the unstable core of the plaque. This process, called atherosclerosis, is associated with increased risk of myocardial infarctions and strokes [27].

Fortunately, the atherosclerotic process is mitigated by HDL and its high content of apoA1 [28]. HDL precursors, devoid of lipid, are secreted by the liver and the intestines. These molecules obtain free cholesterol and phospholipids from peripheral cells through the ATP-binding cassette transporter A1 (ABCA1) [22]. Then the lecithin-cholesterol acyl transferase (LCAT) converts the newly acquired free cholesterol to cholesterol esters. This conversion promotes the incorporation of cholesterol esters into the core of HDL and liberates the HDL surface to scavenge more free cholesterol. This bigger and more spherical HDL is referred to as HDL_3 [28]. This cholesterol scavenging process (reverse cholesterol transport) continues, and the core of the molecule expands further forming HDL_2. Cholesterol-rich HDL_2 then returns the cholesterol to the liver. Increased HDL cholesterol is associated with decreased CVD risk.

Traditionally, LDL cholesterol is used to estimate CVD risk by measuring the cholesterol mass within LDL particles. A LDL cholesterol measurement between 130-159 mg/dL is considered borderline high, and above 160 mg/dL is considered high [29]. However, LDL particle number and LDL size are new measurement techniques that may be a better predictor of CVD risk [30, 31]. An increased number of LDL particles may provide more opportunities for the LDL cholesterol to enter the artery wall and contribute to plaque formation [30]. LDL cholesterol concentrations could be normal but LDL particle number could be elevated, illustrating the importance of the type of LDL measurement. In addition, small and dense LDL increases CVD risk due to its increased potential for oxidation, expanded time spent in plasma, and high permeability [32].

In addition to dietary cholesterol, the body is able to also synthesize cholesterol. Plasma cholesterol levels remain relatively stable through changes in absorption, synthesis, and excretion [33, 34]. Cholesterol synthesis is tightly regulated through negative feedback loops. As

dietary cholesterol increases, endogenous cholesterol synthesis decreases. The synthesis of endogenous cholesterol starts with the conversion of acetyl CoA to mevalonate in the cytosol [35]. 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) is the enzyme that catalyzes this committed step of cholesterol synthesis [36] and is the target of cholesterol lowering drugs such as statins [37]. Then, a three-step process converts mevalonate to isopentyl pyrophosphate, which condenses to the formation of farnesyl pyrophosphate [35]. Two farnesyl pyrophosphates join to form squalene which undergoes several additional reactions before becoming cholesterol. Since endogenous cholesterol synthesis is tightly regulated based on the cellular level of cholesterol [35], individuals should be mindful of exogenous cholesterol intake; however, intake of SFAs is more influential on the development of atherosclerosis [38-40].

Dietary Sources of Fat

Diet is a modifiable lifestyle choice that can affect CVD risk. An important component of the diet that influences CVD risk is the source of FA consumed. Fatty acids are classified as monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) or SFA depending on the number of double bonds in the carbon chain [41]. SFAs (found in milk fat, butter, certain vegetable oils, and animal products) do not contain double bonds [42]. MUFAs contain one double bond and PUFAs contain two or more double bonds [43] and it is the double bonds in the chemical structures that classifies them as USFA. MUFAs are found in high amounts in olive oil, almonds, pecans, cashews, avocados and peanut oil while PUFAs are found primarily in corn, safflower, soybean, flaxseed and cotton seed oils [38].

Two classes of PUFAs are the omega-3 FAs and the omega-6 FAs [43]. The carbon-carbon double bond of omega-3 FAs and omega-6 FAs is positioned 3 and 6 carbons from the methyl end, respectively. Humans cannot produce double bonds before the ninth carbon for the

methyl end, thus omega-3 and omega-6 FAs are essential fatty acids (EFA) that must be consumed in the diet [44]. These essential omega-3 FAs include alpha-linolenic acid (ALA; 18:3n3) and its derivatives, eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3) [45]. ALA can be converted into EPA and DHA, but these FAs still need to be consumed in the diet because this conversion process is not efficient. Likewise, the essential omega-6 FAs includes linoleic acid (18:2n6) and its derivatives, gamma-linolenic acid (GLA; 18:3n6) and arachidonic acid (AA; 20:4n6). Vegetable oils are a predominate source of EFAs, each with a unique combination of FAs. Safflower, corn, sunflower, and soybean oils are rich sources of linoleic acid (omega-6), and flaxseed, canola, and linseed oils and walnuts contain high amounts of ALA (omega-3). Long chain omega-3 FAs such as EPA and DHA are found in fish oils and marine animals.

It is well established that SFAs increase LDL cholesterol concentrations by downregulating LDL receptors [38, 40, 46, 47], upregulating the formation of LDL and decreasing LDL turnover [46], which leads to atherosclerosis [27]. More specifically, many long-chain SFAs block the acyl CoA:cholesterol acyltransferease (ACAT) reaction in the liver, causing incoming dietary cholesterol from the small intestine to be incorporated into the cholesterol regulator pool [48]. Increased levels of cholesterol in the regulatory pool downregulates the LDL receptor activity, resulting in increased plasma LDL cholesterol concentrations. Conversely, some long-chain unsaturated fatty acids (UFA) shift cholesterol away from the regulatory pool to improve LDL receptor function [49]. This shift results in the incorporation of cholesterol into cholesteryl esters, which are packaged into VLDL. Therefore, diets rich in SFAs are more atherogenic than diets rich in USFAs.

The 2020-2025 Dietary Guidelines for Americans recommend limiting SFA intake to less than 10% of total calories and incorporating nutrient-dense foods such as fruits, vegetables, whole grains, seafood, legumes, nuts, seeds, low-fat dairy, and lean meats [50]. There is strong evidence to support the substitution of SFAs with PUFAs [40, 51]. For example, a highly-controlled, eight-year intervention which replaced SFAs with PUFAs resulted in a significant 34% reduction of myocardial infarctions, sudden death, and ischemic stroke [38]. In addition, the experimental diet reduced serum cholesterol by thirteen percent. Furthermore, two meta-analyses that included nineteen studies concluded that substituting PUFA-rich vegetable oils for SFAs lowered cardiovascular risk or events by approximately twenty-eight percent [39, 40]. Finally, the World Health Organization (WHO) conducted a regression analysis that indicated for every 1% of energy from SFA replaced isocalorically with PUFA, LDL cholesterol decreased by 2.1 mg/dL [52].

Conversely, the evidence for substituting SFAs with MUFAs is not as strong [39, 53]. Schwingshackl & Hoffman [53] conducted a meta-analysis on the impact of replacing SFAs with MUFAs, and they found that the limited reported evidence on this substitution was contradictory and unclear. The results of this analysis indicated that MUFA-rich diets may improve blood pressure and glycemic control in the long-term and TG and HDL cholesterol in the short-term. In addition, Vasilopoulou et al. [54] reported that consuming modified dairy products in which there was a partial substitution of SFA for MUFA prevented the rise in fasting LDL cholesterol that was observed in the group that consumed conventional dairy products. Finally, the WHO reported that for every 1% of energy from SFA replaced isocalorically with MUFA, LDL cholesterol decreased by 1.6 mg/dL [52]. Overall, more long-term trials that investigate that

impact of MUFA-rich diets need to be conducted, but there is preliminary evidence that suggests these diets are beneficial for reducing CVD risk.

Tree Nuts and Blood Lipids

Tree nuts are substantial sources of vegetable protein, MUFAs, PUFAs, dietary fiber, vitamins E and K, and other minerals and phytonutrients [55]. Pecans and almonds are a moderate source of protein and a substantial source of UFAs, specifically MUFA. In comparison, walnuts provide more PUFA than MUFAs. Macadamia nuts and Brazil nuts contain the highest content of SFAs. Epidemiological research associates frequent nut consumption with reduced risk of CVD, type 2 diabetes mellitus, and all-cause mortality [56]. In addition, Kelly and Sabaté [57] reviewed four major epidemiological studies and concluded that the consuming nuts more than four times a week leads to an average risk reduction of 37% for coronary heart disease compared to those that rarely consume nuts.

These positive findings are also observed with randomized, controlled trials on nut consumption. Del Gobbo et al. [58] conducted a meta-analysis on the effect of a variety of tree nuts on blood lipids in adults without prior CVD. Sixty one trials, ranging from three to twenty-six weeks, were included in the analysis. The results indicated that nut intake significantly decreases TC, LDL, apoB, and TG. Stronger impacts on blood lipids were observed when the dose of nuts was at least 60 g/d, which is equivalent to approximately 2 ounces. There was also a more prevalent impact on apoB in populations with type 2 diabetes compared to healthy individuals. The results from this study, however, should be interpreted with caution. Tree nuts do not have the same nutrient composition, thus they likely do not individually have the same influence on blood lipids. This meta-analysis included eight varieties of nuts, and the representation of each nut was disproportional [58]. For instance, there were 21 studies on

walnuts and only 2 studies on pecans. In addition, this analysis was conducted in healthy populations, so the results cannot be extrapolated to individuals with hypercholesterolemia at baseline.

Liu et al. [59] addressed one of these gaps in the literature by conducting a meta-analysis that assessed the comparative effects of walnuts, pistachios, hazelnuts, cashews, and almonds on blood lipid profiles. This analysis of 34 studies indicated that pistachio-enriched diets were more effective in lowering TG, LDL cholesterol, and TC compared to the other tree nuts. Walnut-enriched diets were ranked as second best for reducing TG and TC, while almond-enriched diets were ranked as second best for reducing LDL cholesterol. In addition, Altamimi et al. [60] addressed the other gap in the literature by conducted a systematic review of the impact of tree nut consumption on blood lipid profiles in patients with hypercholesterolemia. The authors concluded that tree nut consumption is favorable for blood lipid reduction in this at-risk population.

Walnuts are one of the most frequently researched nuts with respect to cardiovascular health. This is likely due to the relatively high ALA content of walnuts compared to other tree nuts. Guasch-Ferré et al. [61] recently published an updated systematic review and meta-analysis examining the effect of walnut consumption on blood lipid profiles. The analysis included 26 studies in which 15-108 g/d of walnuts were consumed for 4 weeks to 1 year as 5-24% of total energy. Participants in the studies had normal cholesterol concentrations, hypercholesterolemia, type 2 diabetes, overweight, obesity, or metabolic syndrome. The walnut-containing diets significantly reduced TC by 7.0 mg/dL, LDL cholesterol by 5.5 mg/dL, TG by 4.7 mg/dL, and apolipoprotein B by 3.7 mg/dL more than the control group. These findings support the results from the aforementioned meta-analysis of general tree nut consumption.

Almonds have also been shown to have protective effects against CVD. Asbaghi et al. [62] recently conducted a meta-analysis evaluating the impact of almond intake on blood lipids. The final analysis of 36 effect sizes from 27 studies reported that almond intake significantly reduced TG, TC, and LDL cholesterol (but not HDL cholesterol) more than the control group. The included studies incorporated 10-168 g/d of almonds individuals with overweight, obesity, hypercholesterolemia, coronary artery disease, prediabetes, type 2 diabetes, or no chronic conditions. The findings of this meta-analysis are in accordance with previous analyses conducted in almonds and general tree nuts.

There is less literature available on the impact of pistachios on blood lipid profiles. Hadi et al. [63] recently conducted a meta-analysis on 12 studies and reported that pistachio consumption resulted in greater reductions in TC, LDL cholesterol, and TG levels (but not HDL cholesterol). Previous research indicates that the fats in pistachios are resistant to absorption, yet the fiber is readily absorbed [64]. Thus, the overall limited fat absorption may lead to favorable changes in TC, LDL and HDL. Furthermore, pistachios are 2-3 times more abundant in phytosterols than walnuts and almonds, and phytosterols effectively improve blood lipid profiles by preventing the absorption of cholesterol in the gastrointestinal system [65]. Overall, daily consumption of pistachios likely improves blood lipid profiles due to the nut's unique dietary composition.

Only three human feeding trials have been conducted using pecans. The first recruited normal weight adults with optimal blood lipid profiles [66]. The participants consumed 68 g/d of pecans or a habitual diet without nuts for eight weeks. LDL cholesterol was significantly lower than baseline in the pecan intervention group at weeks four and eight. Notably, LDL cholesterol was 10% lower than baseline at week four and only 6% lower than baseline at week eight. In

addition, TC and HDL cholesterol were significantly lower at week eight in the pecan group compared to the control group. The significant difference in TC between the two groups was in part due to an increase in TC in the control group.

The second study on pecans was a highly controlled feeding study of healthy adults with normal blood lipid profiles [67]. Participants first underwent a two-week lead-in diet comprised of 34% fat with 15% of fat from SFA. Notably, this lead-in diet provided substantially more than the 10% of energy from SFA that is recommended by the 2020-2025 Dietary Guidelines for Americans [50]. Next, participants were provided with all meals from the university kitchen for four weeks [67]. They received the Step-I control diet for four weeks then an isocaloric pecan-enriched Step-I diet for another four weeks or vice versa. Both of the diets had favorable effects on participants' blood lipid profile, but the pecan-enriched Step-I diet reduced TC, LDL and TGs significantly more than the Step-I control diet. Serum apoB and lipoprotein(a) significantly decreased, and apoA1 significantly increased, when subjects consumed the pecan-enriched diet compared to the control diet.

Finally, the third study on pecans was also a 4 week controlled feeding trial in adults that were metabolically at-risk [68]. In this crossover trial, participants were provided with all meals during a two-week run-in period and two four-week feeding periods. The control diet was designed to reflect the average American diet (low in fruits, vegetables, fiber, and n-3 fatty acids). The pecan diet isocalorically substituted pecans for 15% of the total energy (42.5 g/2000 kcal) provided in the control diet. There was a trend for a greater reduction in TC and LDL cholesterol in the pecan vs control diet, but these outcomes did not reach statistical significance. Contrary to the study by Rajaram at al. [67], there was no change in TG. It is unclear if the lack of change in blood lipids in the study by Mckay et al. [68] was due to the lower dose of pecans or

the at-risk population. The data for pecans and blood lipid reduction is promising, but more research is needed to clarify this gap in the literature.

The mechanism by which nut consumption improves TC and LDL cholesterol is not fully understood [69]. Nuts are rich sources of MUFAs and PUFAs and minimal contributors of SFAs. Previous research has shown that diets rich in UFAs produce favorable effects on LDL cholesterol by upregulating LDL receptor function (described in more detail above) [39, 40, 49, 70]. Furthermore, tree nuts contain dietary fiber, which is associated with decreased CVD risk due to the reduction of LDL cholesterol [71, 72]. The lipid-lowering effect of fiber is not completely understood [73]. Some researchers propose that soluble dietary fiber lowers cholesterol by preventing the reabsorption of the cholesterol-containing bile acids [74, 75]. Another proposed mechanism is that soluble dietary fiber reduces the glycemic index of foods, resulting in less insulin stimulation of hepatic cholesterol synthesis [76]. Finally, the third possible mechanism is that dietary fiber alters the gut microbiota to produce cardioprotective short-chain fatty acids [77].

Regarding the mechanism for TG reduction with tree nuts, it is possible that the ANGPTL family of proteins are involved. As previously mentioned, ANGPTL3 and -8 work synergistically in the postprandial state to inhibit lipoprotein lipase (LPL) in oxidative tissues such as the heart and muscles [17], resulting in increased circulating TG [18]. Our lab previously showed that acute meals and diets rich in UFAs (including pecans and walnuts) suppress fasting and postprandial ANGPTL3 and TG [78-82]. Previous research demonstrated that oxidative stress modulates the liver X receptor (LXR) [83], which regulates the transcription of ANGPTL3 in the liver [84]. Furthermore, there is evidence that polyphenolic compounds decrease the expression of LXR and hepatic ANGPTL3 mRNA and protein while upregulating the expression

of adipose tissue LPL mRNA to reduce TG circulation [85-87]. Based on this evidence, we theorize that oxidative stress modulates LXR to increase the expression of ANGPTL3 mRNA, and that phenolic compounds inhibit this pathway by decreasing oxidative stress. There is also evidence that UFAs, especially PUFAs, also downregulate LXR [88, 89]. Altogether, it is possible that the antioxidants and UFAs in tree nuts work alone or synergistically to downregulate ANGPTL3 expression via LXR.

Obesity and Weight Management

Obesity is an important contributor to CVD risk, in part because the inflammation affiliated with obesity may expedite the atherosclerotic process [4]. The most recent NHANES data from 2017-2018 reported that almost forty percent of Americans have obesity, which is a 30% increase from the 1999-2000 data [90]. Obesity significantly reduces one's quality of life due to its association with depression, shame, limited mobility, sexual dysfunction and diminished work achievement [91]. Likewise, obesity is not only associated with CVD but other medical conditions such as type 2 diabetes mellitus, hypertension, certain types of cancer, sleep apnea, and nonalcoholic fatty liver disease.

The complications and co-morbidities associated with obesity highlight the need for effective treatments. National and international guidelines for obesity treatment recommend dietary manipulation to induce energy deficits, exercise and behavior change [92]. However, even successful weight loss interventions often lead to weight regain during the weight maintenance period [93]. Thus, individuals often seek pharmacologic therapies to assist with weight loss when previous efforts are unsuccessful. These therapies are indicated when energy restriction, exercise and behavior modification do not achieve weight loss goals among individuals with a body mass index (BMI) of 30 kg/m^2 or a BMI of 27 kg/m^2 and at least one

obesity-related comorbidity [92]. One must weigh the risk versus the benefit when considering the commencement of an obesity drug. There is limited literature that evaluates the cost-effectiveness of long-term obesity pharmacotherapy use, and the drugs may have a large out-of-pocket expense [94]. In addition, some drugs are associated with potent side effects such as oily stools, decreased absorption of fat soluble vitamins, cramps, insomnia, anxiety, nausea or constipation, to name a few [92, 95, 96]. Finally, bariatric surgery is indicated for individuals with a BMI greater or equal to 40 kg/m^2 [97]. This extreme method is associated with long-term risk of bowel obstruction, dumping syndrome, vitamin deficiencies, hypoglycemia, ulcer and vomiting [98]. Lifestyle modifications are still recommended post-operative to prevent the regain of weight and complications.

Since weight loss through energy deficits and exercise is not effectively sustained, and pharmacologic or surgical treatments aggravate quality of life, obesity prevention through weight maintenance may be the best approach to managing the obesity epidemic. On average, individuals only gain 0.5-1 kg/y, but a consistent increase in body weight (BW) can lead to obesity overtime [99]. Therefore, small dietary changes or small changes in EE may prevent the onset of obesity in the long run. The incorporation of nutrient-dense foods may limit overall energy consumed preventing gradual weight gain. Preventing obesity improves comprehensive quality of life and prevents further medical complications like CVD.

Tree Nuts and Weight Maintenance

Tree nuts are rich in protein, fiber and energy, which may prevent further food intake by inducing satiety [100]. Interestingly, not all the energy in some nuts is fully metabolized and absorbed [64, 101, 102]. The metabolizable energy (ME) of almonds [101], walnuts [102], cashews [103], and pistachios [64] is 32%, 21%, 16%, and 5% less than predicted by the Atwater

factors, respectively. Research on the ME of pecans has not yet been determined. An analysis of the NHANES 2005-2010 data compared the impact of frequent tree nut consumption (≥ ¼ ounce/d) to non-tree nut consumers (< ¼ ounce/d) [104]. The data revealed that tree nut consumption was associated with lower BMI, waist circumference (WC), systolic blood pressure, and higher HDL compared to the non-tree nut consumption. However, this is observational data, so cause and effect cannot be determined.

Numerous randomized controlled trials (RCT) also report that regular nut consumption, even in large quantities, does not cause weight gain [66, 105-122]. However, these studies manipulate the diet or provide instructions on how to incorporate nuts into one's diet to varying degrees, and it is unclear if the type of diet instructions provided to subjects impacts weight and body composition outcomes. Those different methods include providing no dietary instructions with the nut consumption [66, 105, 107, 109, 117, 120, 121, 123], instructions to substitute energy equivalent foods or specific macronutrients in their typical diet for the nuts provided [110-116, 124-127], or the provision of all meals in an outpatient feeding setting designed to keep participants in energy balance [67, 128-132].

To our knowledge, only two studies on chronic consumption of tree nuts have compared weight outcomes in participants who received diet instructions versus those who did not receive diet instructions. In the first research study, participants did not receive diet instructions and their background diet was not controlled for the first 8-week phase of the study [133]. In the next 3-week phase, subjects incorporating peanuts into a prescribed background diet without diet instructions. Finally, in the last 8-week phase, subjects were instructed to substitute the peanuts with 50% of fat intake in the prescribed background diet. Subjects significantly increased weight

during the first and second phases, but not during the last phase when subjects were instructed to substitute the peanuts.

In the second study by Njike et al., [134] there were 2 groups with and without dietary advice to control energy intake. Within each group, subjects were further randomized to receive a supplement of 56 g/d of walnuts or no walnuts for six months. The group that consumed walnuts and did not receive dietary advice to adjust energy intake resulted in a significant increase in percent body fat and visceral fat in comparison to baseline. The group that consumed walnuts and received dietary advice resulted in a significantly improved waist circumference. The findings of these two studies suggest that weight outcomes are substantially influenced by the diet instructions that are provided.

To further address this gap in the literature, we conducted a systematic review and meta-analysis of clinical trials with parallel or crossover designs to examine the impact of no dietary substitution instructions or some type of dietary substitution instructions on BW and body composition during interventions >3 weeks in adults [135]. We hypothesized that studies without substitution instructions would result in a significant increase in BW, BMI, waist circumference (WC), and total body fat percentage (BF%) while studies with energy or fat substitution instructions would not result in changes in these aforementioned outcome variables. Our meta-analysis included 55 studies involving almonds, cashews, hazelnuts, macadamia nut, mixed nut, peanut, pecan, pistachio, walnut, and a nut-based snack bar interventions. In studies where participants received substitution instructions, those instructions included: (1) substituting energy (kilocalories) from habitual diet or prescribed background diet [113, 136-144], substituting fat energy [114, 145-148], substituting starchy foods [149, 150] or meat sources [151], substituting a combination of foods or macronutrients [152, 153], substituting specific food items

recommended in a background diet [106, 154], or substituting specific foods based on individualized advice or exchange lists [155, 156].

In studies without dietary substitution instructions, there was no change in any outcome [135]. In studies with dietary substitution instructions, there were no changes in BW, BMI, or WC. However, there was a significant effect of the intervention on BF% when substitution instructions were incorporated. There were only 5 effects included in this analysis of BF% and the significant effect was lost when we conducted a sensitivity analysis that removed all studies in which the control group also received a dietary intervention. Contrary to our hypothesis, the results of the meta-analysis suggest that nut consumption, regardless of the presence or absence of dietary substitution instructions, does not lead to weight gain. Since the conclusions from our meta-analysis and the clinical trials that directly compared the impact of diet instructions on weight outcomes [133, 134] conflict, further research is needed in this area.

Energy Balance

Obesity results from chronic imbalance of energy intake (EI) and EE [157]. Small deviations in intake or expenditure overtime can have a momentous impact on weight change. A number of studies have examined the impact of dietary FA composition, as well as tree nuts specifically, on energy balance. These studies primarily examine one side of the equation; either EI (via appetite measures) or EE (via metabolism measures). The next two sections explore the literature on both sides of that equation.

Appetite Regulation

The hormonal regulation of food intake is one mechanism that affects weight regulation. Hunger and satiety hormones act through both neural and peripheral pathways to influence food intake [158]. When gut peptides bind to the vagal afferent neuron, electrical signals are

transported to the nucleus tractus solitaries (NTS or brainstem), which results in neurotransmitter release to the arcuate nucleus (ARC) in the hypothalamus [159]. In addition to neuronal signals, the ARC also receives circulatory signals, which reflect current energy status [158, 160]. The ARC has circulatory signal access due to its close proximity to the third ventricle, which maintains a less restricted blood-brain barrier. There are two neuronal populations within the ARC that have opposing effects on appetite regulation [161]. The pro-opiomelonocortin (POMC) and cocoaine and amphetamine-regulated transcript (CART) neurons suppress appetite. POMC neurons release α-melanocyte stimulating hormone (α-MSH) which then binds to melanocortin-4 receptors (MC4Rs) on second order neurons in the paraventricular nucleus (PVN) to decrease appetite [161-163]. MC4R deficiency is associated with increased hunger and obesity in mice [164, 165] and humans [166, 167]. CART is co-localized with POMC to increase satiety, yet its distinct mechanism is not well understood [168]. In humans, mutations in the CART gene is associated with obesity, decreased metabolic rate, and greater prevalence of type II diabetes mellitus [169].

Conversely, the neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons of the ARC stimulate appetite through various mechanisms. First, the NPY/AgRP neurons releases gamma-amino butyric acid (GABA) to inhibit the anorexigenic action of α-MSH [163, 170], but this action is downregulated when leptin or serotonin bind to the orexigenic neurons [163, 171, 172]. Next, the binding of ghrelin onto AgRP neuron receptors results in hyperpolarization and reduced anorexigenic activity of POMC neurons [172, 173]. In addition, the binding of NPY to Y1 receptors in the hypothalamus and AgRP to MC4R both promote feeding [161, 174]. In more recent studies, activation of AgRP neurons through photostimulation or drugs in mice induced intense feeding [175, 176]. Thus, NPY/AgRP neurons have a potent role in stimulating feeding.

As previously mentioned, circulating hormones act on the NPY/AgRP and POMC/CART neurons to dictate energy intake [177]. Peptide YY, CCK, glucagon-like-peptide-1 (GLP-1) and ghrelin respond acutely in the fed and fasted states while leptin and insulin act as both acute and chronic regulators of appetite homeostasis [178-181]. PYY is released from L cells in the ileum and colon in response to meals [182]. High-fat meals elicit a greater PYY response [183]. PYY_{1-36} and PYY_{3-36} are the circulating forms of PYY, but PYY_{3-36} is the active form which binds to Y_2 receptors to inhibit the orexigenic NPY neurons and trigger the anorexigenic POMC neurons [184, 185]. Studies in both rats and humans indicate that obesity is associated with low levels of PYY, a mechanism that potentiates excess adiposity [186]. CCK, in addition to enhancing satiety, contributes to digestion by stimulating pancreatic releases, gallbladder contractions, and intestinal motility and inhibiting gastric emptying [187]. It is released from endocrine I cells in the duodenum and jejunum following a fat- or protein-rich meal. Similarly, GLP-1 is secreted from the L-cells in the distal ileum and colon [187] and functions to delay gastric emptying, enhance the postprandial secretion of insulin, and inhibit glucagon secretion [188]. CCK and GLP-1 enhances satiety by acting on the ARC neurons, possibly by inhibiting the actions of ghrelin on the POMC neurons [189-191]. In a study in rats, intravenous infusion of GLP-1 reduced meal size, independent of an intact vagus nerve [192]. This supports the role of GLP-1 acting as a satiety signal in the periphery.

Insulin and leptin have similar appetite-suppressing pathways, and they respond through both acute and chronic mechanisms [193]. In the acute setting, insulin is secreted by the pancreatic islet beta cells in the presence of glucose in the gastrointestinal tract [194], and leptin is released predominately from the adipocytes, but also from the stomach, liver, and fetal tissues [195-197]. In regards to long-term appetite regulation, circulating concentrations of both insulin

and leptin are positively associated with the degree of adiposity of an individual [198-203]. Binding of insulin to its receptor in the central nervous system (CNS) initiates autophosphorylation of the receptor which activates the phosphatidylinositol 3 kinase (PI3 kinase) and mitogen activated protein kinase (MAPK) cascades [204]. The binding of leptin to the ObRb receptor results in the activation of the JAK/STAT pathway [205] and slight activation of the PI3 kinase cascade [206]. Both pathways of insulin and leptin result in the phosphorylation of the transcription factor FOXO, which transcribes POMC neurons and inhibits NPY/AgRP neurons to produce appetite suppression [205, 207, 208].

Ghrelin, the only known orexigenic gut hormone, is predominately secreted by the X/A-like endocrine cells of the stomach [187]. Concentrations of ghrelin in circulation increase during fasting and decline after a meal [178]. Ghrelin binds to the growth hormone secretagogue receptor which is present in the hypothalamus and the brain stem [209]. Ghrelin's mechanism is not well understood, yet it is strongly suggested that ghrelin plays an important role in stimulating the NPY/AgRP neurons and inhibiting the POMC neurons of the ARC to promote food intake [210]. Recent research in rodent models has elucidated the complexities of the role of ghrelin in feeding. Ghrelin also signals to the ventral hippocampus neurons, an area of the brain that is known for memory [211]. The downstream effects of this signaling lead to the release of orexin from neurons in the lateral hypothalamus (LHA) to induce appetite.

The regulation of these aforementioned hunger and satiety hormones is essential for maintaining energy balance. However, there is some evidence that alterations in these metabolites may circumvent weight loss and weight maintenance efforts. Sumithran et al. [212] enrolled obese individuals in a very-low-energy diet for 10 weeks and measured circulating levels of appetite-regulating hormones and subjective appetite. As expected, weight loss

contributed to significant decreases in hormones that suppress hunger (leptin, PYY, CCK insulin) and increased ghrelin. In addition, subjects experienced increases in subjective appetite. One year after the weight loss intervention concluded, the significant differences in hunger-regulating hormones and subjective appetite persisted, highlighting the importance of obesity prevention. Likewise, the positive association of adiposity and leptin levels may be a mechanism to prevent obesity [198, 202, 203]; however, obesity is also associated with reduced sensitivity to satiety signals from leptin [213, 214]. This phenomenon, known as leptin resistance, illustrates the complexities of the role of hunger and satiety hormones in weight management [213].

There are fundamental methods for assessing appetite that encompass physiologic, subjective, or applied measurements. The first is to measure aforementioned gastrointestinal hormones that are thought to regulate physiological appetite. The second method involves the assessment of subjective ratings of appetite, usually through visual analog scale (VAS) questionnaires [215]. The final method is designed to be an applied measure where total energy intake (EI) and/or macronutrient intake is determined either through an ad libitum buffet meal or food records [215-217].

There is evidence that diets high in MUFAs and PUFAs may improve subjective and physiological appetite [218]. Acute consumption or infusion of meals rich in MUFA or PUFA resulted in improved GLP-1, gastric inhibitory peptide (GIP), ghrelin, PYY, and/or subjective ratings of hunger and fullness when compared to a meal rich in SFA [219-223]. However, results are conflicting when the impact of PUFA- and MUFA-rich meals are compared. Stevenson et al. [221] and Polley et al. [224] reported improved PYY, ghrelin, and/or CCK following a PUFA- vs MUFA-rich meal. However, Sun et al. [225] reported improved ghrelin and GIP following a MUFA- vs PUFA-rich meal.

Regarding chronic diets, only a limited number of studies investigated the impact of various dietary FA on markers of appetite. Three studies involving 3d to 2 week interventions reported that the FA composition of the diet did not impact subjective appetite, physiological appetite and/or energy intake at a buffet meal [226-228]. However, a more recent study from our lab demonstrated that subjective hunger was lower following a 5d, PUFA-rich diet [224]. Altogether, it appears that meals rich in UFAs may improve markers of appetite, but more research is needed that compares MUFA vs PUFA, especially for longer durations.

Tree Nuts and Appetite Regulation

Positive changes in subjective or physiological appetite could explain how tree nuts promote weight stability, despite their caloric density. However, the evidence to support this mechanism is limited. Acute meal challenges containing pistachios, black walnuts, English walnuts, almonds, and pecans increased GLP-1 and/or suppressed subjective appetite more than the control [81, 229-232]. For longer-term studies incorporating tree nuts, results are mixed. We showed that a 7d highly-controlled PUFA-rich diet containing walnuts decreased fasting ghrelin and increased fasting and postprandial PYY, but there were no changes in VAS scores [233]. Furthermore, two 4-week studies of daily pistachio or almond intake without additional diet instructions reported conflicting findings for subjective appetite [109, 234]. Preliminary research suggests that tree nuts improve subjective and physiological markers of appetite, especially during an acute meal, but more research on longer-term nut intake is needed.

Energy Metabolism

The other side of the energy balance equation relates to EE. Energy expenditure is a function of total daily energy expenditure (TDEE), which is comprised of RMR, diet-induced thermogenesis (DIT), and activity EE [193]. RMR is the energy required to sustain body

functions at complete rest, and it is affected by fat-free mass, sex, age and familial characteristics. RMR comprises 60-75% of TDEE [235]. DIT is the increase in EE above RMR for the metabolism of food consumed [236]. It is the smallest component of EE which comprises 10% of TDEE [235]. Activity EE, the most modifiable component of TDEE, is the energy utilized for all physical activities [236]. Activity EE encompasses 15-30% of TDEE [235]. Slight decreases in any aspect of TDEE may result in obesity overtime.

RMR is not easily predicted based solely on changes in body mass. Multiple studies have shown that energy restriction and weight loss result in disproportionate declines in RMR beyond the expected changes from a decrease in body mass [237-239]. In fact, these metabolic adaptations to weight loss persist over time. Fothergrill et al. [239] measured the changes in RMR and body weight of participants on "The Biggest Loser" weight loss competition television show. They evaluated the participants at baseline, the end of the 30-week competition, and six years after the competition. Although 70% of the initial weight loss was regained after six years, RMR decreased an additional 221 kilocalories/d when measured six years after the competition. The overall decrease in RMR from baseline was 704 kilocalories/d. Therefore, accurately measuring metabolism allows individuals to manipulate their EI and EE more appropriately, and it is a reliable method of evaluating the effects of an intervention.

In addition to optimizing EE, substrate utilization and macronutrient balance also influences weight regulation [240]. Macronutrient balance is achieved when there is a balance between the rate at which individual macronutrients are consumed and subsequent oxidation. There is 100 times greater TG stores than glycogen stores in the body [241]. Carbohydrate balance is tightly regulated to maintain appropriate blood glucose levels [242]. Therefore, regulatory mechanisms adjust the rate of glucose oxidation based on glycogen stores. Likewise,

protein balance is tightly regulated to maintain nitrogen balance [241]. Therefore, the regulation of fat oxidation is the lowest metabolic priority, so daily intake of fat does not substantially affect the body's lipid stores [243]. The amount of fat oxidized is governed by the difference between energy needs and the supply of energy from carbohydrate and protein. Therefore, after introducing a high-fat (HF) diet, there is a delay in the shift towards increased fat oxidation, which results in positive fat balance and weight gain.

Unsaturated Fats and Metabolism

Since weight maintenance is achieved through energy balance and macronutrient balance [240], it is essential to promote the efficient transition between macronutrient fuel sources or metabolic adaptation [244]. Schrauwen et al. [245] demonstrated that it takes lean individuals seven days to obtain fat balance after switching from a low-fat diet to a HF diet. Many studies have demonstrated that low levels of fat oxidation lead to weight gain [246]. For example, a 10-year longitudinal study of non-obese Caucasian males indicated that a RER of 0.85 or greater was a significant predictor of weight gain [247]. Since this period of positive fat balance is a contributing factor to obesity, researchers have investigated how to mitigate the delay in adaptation. Increasing exercise is the most efficient method of improving adaptation to a HF diet. [240].

In addition to increasing exercise, more recent research suggests that altering the FA content of meals may also reduce the delay in adaptation. The results are conflicting in studies that use indirect calorimetry to measure changes in RER or DIT following an acute HF meals of varying FA composition [240]. Piers et al. [248] and Soares et al. [249] found that individuals who consumed a MUFA-rich meal demonstrated higher fat oxidation versus individuals who consumed a SFA-rich meal. Also, DIT significantly increased after the MUFA-rich meal, but it

was sometimes dependent on waist circumference [248]. Likewise, Polley et al. [250] reported lower RER and greater DIT following a MUFA-rich meal compared to a PUFA-rich meal. Conversely, Flint et al. [251] and Paton et al. [252] found no differences in postprandial EE between meals rich in PUFA, MUFA and trans FA or SFA, respectively. Thus, it remains unclear how macronutrient utilization or EE, DIT, and substrate utilization changes following an acute HF meal.

Investigating the long-term impact of HF diets with varying FA composition on metabolism is beneficial because it more accurately illustrates these diets' impact on body weight and blood lipids. Polley et al. [250] and Stevenson et al. [253] reported that there was a greater improvement in fat oxidation following a 5-7d PUFA-rich diet versus a MUFA-rich diet or control diet. Furthermore, two longer studies of 3-4 weeks reported that MUFA-rich diets increase EE compared to a SFA-rich diet [254, 255]. However, Lovejoy et al. [256] compared diets containing 9% of energy from MUFA, SFA, and trans FA, and the trans FA meal induced greater postprandial fat oxidation compared to MUFA or SFA with no difference between MUFA and SFA. There was also no difference in RMR or DIT between the three groups. The unexpected findings from this study may be explained by the design of the HF diets. Each diet only contained 30% of energy from fat with 9% of energy from MUFA or SFA. Contrastingly, the two studies conducted by Piers et al. [254] and Kien et al. [255] incorporated diets with greater than 40% of energy from fat.

The literature on the metabolic impact of long-term HF diets containing PUFA versus SFAs is also inconsistent. Jones and Scholler [257] reported significantly higher RMR and postprandial fat oxidation after consuming a high PUFA:SFA (PS) ratio diet for 7d compared to consuming a low PS diet. There was no difference in DIT between the two groups. In another

study with a high PS or low PS diet for 14d, there were no differences in fasting fat oxidation among the obese or lean participants regardless of the type of diet consumed [258]. However, the lean individuals who consumed either PS diet had greater postprandial fat oxidation compared to the obese individuals who consumed the low PS diet. Thus, adiposity may be an important factor in the rate of fat oxidation following HF diets. Lichtenbelt et al. [259] is the only study that demonstrated that a high PS ratio diet for 14d increased DIT. Due to limited literature available, more research is needed to fully understand if high PS ratio diets reduce the delay in adaptation to HF diets and how adiposity influences this mechanism.

In summary, although conflicting results exist, many acute meal challenges and long-term studies support the notion that UFAs induce greater fat oxidation and DIT when compared to SFAs. There is currently no consensus of whether MUFAs or PUFAs are more beneficial to maintaining energy balance [240]. Tree nuts are rich sources of UFAs [55], which may explain why they are associated with weight maintenance despite their high energy density. There are limited studies available that measure fat oxidation and DIT after consuming an acute meal or diet rich in tree nuts.

Tree Nuts and Metabolism

Since tree nuts are rich sources of unsaturated fats, nuts may also produce positive metabolic effects observed. There are three studies that measure changes in metabolism following an acute meal containing walnuts. Tapsell et al. [260] demonstrated that meals containing walnuts induce greater fat oxidation compared to meals containing olive oil, but there were no difference in DIT. In contrast, Casas-Agustench et al. [261] observed significantly higher DIT and no changes in fat oxidation following acute meals containing MUFAs (olive oil) or PUFAs (walnuts) compared to SFAs (dairy). Finally, Brennan et al. [262] found no difference

in REE following short-term consumption of meals containing walnuts or placebo. The results of these three studies indicate that an acute meal including walnuts may increase fat oxidation or DIT, but the literature is limited

For almonds, there are two longer-term diet intervention studies that measured changes in metabolism. Hollis and Mattes [128] and Fraser [107] observed no differences in RMR or DIT following an almond enriched diet for 10 weeks to 6 months compared to the control. Based on these two studies, it appears that supplementation of habitual diets with almonds does not impact RMR or DIT. Conversely, peanuts may increase RMR and fasting fat oxidation, especially in overweight populations. Alper et al. [133] and Coelho et al. [263] observed a 5-11% increase in RMR following 8-19 weeks of peanut or peanut oil consumption. Notably, the improvement in RMR in the study by Coelho et al. occurred in the subjects with higher BMIs but not normal BMIs [263]. Similarly, Claesson et al. [264] reported a 5% increase in RMR and no change in BW following 2 weeks of over-feeding with peanuts. Finally, Alves et al. reported an increase in fasting fat oxidation in men with overweight or obesity following the consumption of conventional and high-oleic peanuts for 4 weeks [265].

In summary, there is limited but growing research on the impact of nut consumption on metabolic markers such as RMR and DIT. To date, there are no long-term intervention studies in walnuts, but results of acute studies reveal that walnuts may increase postprandial fat oxidation and DIT. Studies involving almonds are limited, but there is currently no evidence that long-term almond supplementation impacts RMR or DIT. Finally, the four studies incorporating peanuts or peanut oil suggest that its acute intake may increase RMR, especially in overweight individuals, and chronic intake may increase fasting fat oxidation. Importantly, to our knowledge, there are no studies that measure changes in metabolism after the consumption of pecans. More research

on tree nuts and metabolism is need to provide conclusive evidence, along with initial research in pecans.

Antioxidants

Free radicals are unstable molecules with an unpaired electron that are naturally formed through endogenous (metabolism, mitochondria, peroxisomes) and exogenous sources (pollution, alcohol, heavy metals) [266]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are two major sources of free radicals in the body [266]. ROS/RNS are beneficial at low levels for proper immune function and intracellular signaling [267]; however, oxidative stress occurs when free radical development supersedes antioxidant protection [268]. This occurrence damages proteins, lipids, deoxyribonucleic acid (DNA) [269], and it is associated with cancer, CVD, neurological disease, and other inflammatory diseases [270].

Antioxidants are defined as substances that eradicate ROS by preventing radical formation or blocking chain reactions (free radical propagation) of oxidation [271]. Antioxidants are either produced within the body (endogenous) or ingested through foods or supplements (exogenous) [270]. Endogenous sources are divided into enzymatic antioxidants and non-enzymatic antioxidants. Superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase are examples of enzymatic antioxidants that are actively engaged in the neutralization of ROS and RNS. Endogenous sources of non-enzymatic antioxidants are produced through metabolic processes in the body and include glutathione, coenzyme Q10, melatonin, uric acid, and transferrin. Finally, exogenous antioxidants (vitamin E, vitamin C, carotenoids, flavonoids, omega-3 FAs, omega-6 FAs) must be provided through the diet.

A single antioxidant may act differently depending on the reaction system, or it may operate by more than one mechanism in one system [272]. For example, in neurons, the ROS

generated from the mitochondria triggers glutamate to initiate oxidative stress and apoptosis [273]. Flavonoids undergo combat the oxidative stress by modifying glutathione metabolism, suppressing ROS activity, and inhibiting cell death [274]. These three complex mechanisms illustrate why one antioxidant assay cannot measure all radicals and antioxidants in one system. Thus, when choosing an antioxidant assay method, one must consider the radical source, distinctive system characteristics, and the antioxidant mechanism to appropriately measure antioxidant capacity [272].

There are two mechanisms by which antioxidants mitigate the effects of ROS [272]. The classic mechanism is the Hydrogen Atom Transfer (HAT) in which the antioxidant donates a hydrogen atom to the ROS. Bond dissociation enthalpy (BDE) of the antioxidant is measured to quantify the strength of the bond of the hydrogen-donating group. The lower the BDE, the more efficiently an antioxidant will deactivate a ROS. This mechanism of antioxidant activity is best measured by Oxygen Radical Absorbance Capacity (ORAC) assays and the other mechanism, Single Electron Transfer (SET) method, is best measured by Ferric Reducing Ability of Plasma (FRAP) assays [275]. In the SET mechanism, the antioxidant itself becomes a free radical after providing an electron to the ROS. The ionization potential (IP) is the energy required to abstract an electron from a neutral atom. The IP of the antioxidant is the best measure of an antioxidant's capacity when undergoing the SET mechanism. Finally, the TBARS assay is another common method used to measure the decline of lipid peroxidation in an antioxidant environment [276]. Since oxidative stress contributes to the aging process and the onset of chronic disease such as CVD and cancer, TAC and lipid peroxidation assays are important tools for quantifying protective effects of specific foods. The assay mechanisms are described below in the "Measurement" section.

Pecans and Antioxidant Activity

Tree nuts contain phytochemicals which are non-essential nutrients found in plants that promote health [277]. Phytochemicals are broken down into six subclasses: alkaloids, organosulfurs, phenolics, carbohydrates, proteins, and lipids. Phenolics contains other subgroups such as tannins, phenolic acids, and flavanoids. Polyphenol refers to any molecule with one or more phenolic groups. Some phytochemicals contain antioxidant properties, which prevent cellular damage to cells by quenching free radicals [278].

One of the most well established health benefits of pecans is related to its high antioxidant content, even compared to other tree nuts [279]. According to the US Department of Agriculture ORAC database, pecans have the greatest concentration of phenols among all tree nuts [280]. More specifically, pecans are rich in flavonoids in the form of flavon-3-ols and anthocyanins [281]. However, there are often inconsistencies in reported phenolic content of nuts due to varying methods of phenolic extraction, nut processing, and different nut cultivars [277].

There are extraneous factors that can impact the concentration of phytochemicals within tree nuts such as gene expression, growing cycles and post-harvest food processing [277]. Vilarreal-Lozoya et al. [282] found differences in phenolic content, condensed tannin content and TAC among six different pecan cultivars. In addition, the FA profile and tocopherol content also varied among the pecan cultivars. Pecan trees bear fruit in 2y cycles, in which there is a large crop followed by little or no crop [283]. The unique growing pattern, along with differences in gene expression, may explain the variability of FA profiles, tocopherol content and phenolic compounds in pecans [277]. The literature that investigates the impact of post-harvest food processing and storage methods on nutrient and polyphenol content in pecans in inconsistent, indicating a need for further evaluation.

Once a tree nut is harvested, processed and shipped to the store, there are remaining factors that may impact the bioavailability of nutrients and antioxidants during human consumption. Yang et al. [284] demonstrated that stir-frying pecans for a short time initially increased the nuts' antioxidant activity, but further roasting at higher temperatures resulted in decreased values. Likewise, variations in the length of chewing may impact the nut's lipid bioavailability and the satiety response [285]. Increased chewing of almonds led to significant improvements in subjective appetite, decreases in fecal fat excretion, and increases in GLP-1, with no impact on ghrelin or PYY. Reduced fecal fat excretion suggests increased bioavailability of almonds after more chewing. Greater fecal fat loss, independent of chewing duration, has also been documented after consumption of pecans [286].

Only 3 studies have investigated the impact of acute or chronic pecan consumption on antioxidant status. Hudthagosol et al. [287] evaluated antioxidant measures following three test meals: whole pecans, blended pecans, or a control meal matched for energy and macronutrients. The results of the study indicated that antioxidant capacity, measured by hydrophilic- and lipophilic-ORAC, increased 12% and 10% from baseline after the pecan test meals, respectively, but there was no change when measured by FRAP. In addition, there was a significant decrease in LDL oxidation and an increase in epigallocatechin-3-gallate concentrations. More recently, we showed that partially substituting pecans for butter in a breakfast muffin resulted in increased total antioxidant capacity (TAC) and lower lipid peroxidation compared to the traditional control muffin [81]. In this study, TAC and lipid peroxidation were measured by the ORAC and TBARS assays, respectively. Finally, Haddad et al. [288] conducted a crossover, controlled-feeding trial in which 24 participants consumed a heart-healthy control diet and the same diet enriched with pecans for 4 weeks each. The pecan-enriched diet resulted in increased serum gamma-tocopherol

and decreased alpha-tocopherol. Lipid peroxidation, measured by TBARS assay, significantly decreased, and there was no change in antioxidant capacity measured by FRAP and Trolox Equivalent Antioxidant Capacity (TEAC) assays.

These three studies suggest that acute and chronic consumption of pecans improves TAC, LDL oxidation and/or lipid peroxidation [81, 287, 288]. It appears that the ORAC assay is a better measure TAC than the FRAP assay [287, 288]. The lack of response to the FRAP assay in two of the studies is a reflection of differences in antioxidant mechanisms [287]. Metabolism of foods containing fructose increase plasma uric acid, a potent antioxidant, which stimulates a postprandial increase in FRAP, but there is limited fructose in pecans. Furthermore, the lipid-lowering effect of nuts is often attributed to their high unsaturated FA:saturated FA ratio; however, their strong antioxidant properties likely contribute to the observed lipid benefits.

Summary

In conclusion, pecans are a rich source of UFA and antioxidants, which may promote health. In past studies, pecan consumption has been linked to reductions in lipid peroxidation [288], increases in TAC acutely [287], and lowering of cholesterol in healthy adults [66, 67]. Importantly, it is unknown if the same effects would be found in populations that are at risk for CVD. Furthermore, it is unknown if the type of diet instruction that is provided during longer-term pecan consumption impact weight outcomes. Finally, the mechanism by which pecans promote weight maintenance is unknown. No studies have examined the impact of a long-term pecan-enriched diet on subjective and physiological measures of appetite or metabolism to examine overall energy balance. Therefore, more research is needed on how long-term pecan-enriched diets impact weight, metabolism, appetite, and cholesterol in adults at risk for CVD.

Methodology Used in Study Design

Calorimetry

Direct calorimetry and indirect calorimetry are two methods used to measure EE and determine substrate oxidation [193]. Direct calorimetry assumes that all energy utilized during physiological processes is eventually released as heat. This technique measures all heat transfers via a whole-room chamber system. The design of direct calorimetry is often impractical and difficult to monitor, so indirect calorimetry is a more reliable technique. Indirect calorimetry assesses EE by measuring oxygen consumption and carbon dioxide expiration. The Weir equation calculates total heat output (kilocalories) as: 3.9 x oxygen used (L) + 1.11 x carbon dioxide produced (L) [289]. The RER is the ratio of oxygen consumption to carbon dioxide production and is an indicator of the source of substrate for oxidation [241]. Macronutrient utilization is calculated by the following equation [290]: fat (g/min) = (1.67*VO_2 (L/min))-(1.67*VCO_2 (L/min)) and carbohydrate (g/min) 0 = (4.56*VCO_2 (L/min))-(3.21*VO_2 (L/min)). After an overnight fast, RER values of about 0.70 indicating fat is the most substantial energy substrate, and values close to 1.00 indicate carbohydrate is the predominate source of fuel.

Radioimmunoassays

In an RIA, a labeled tracer antigen (such as I-125) competes with an unlabeled antigen for binding sites on the antibody [291]. As the concentration of unlabeled antigen increases, the amount of labeled antigen that is bound to the antibody will decrease. Finally, a second antibody is added into solution which binds to the antibody/antigen complex. This complex precipitates out of the solution, allowing for the separation of the bound and unbound antigens.

Enzyme-Linked Immunosorbent Assays

The ELISA follows the same principles as the RIA, but does not you radioactive tracer antigens [292]. The 96 well plate is coated with an immobilized antibody, which binds with the target protein within the unknown sample. Following washing, another antigen with a marker is added and it binds to the target protein. The color changes with respect to the amount of labeled antibody that is bound to the target protein. The intensity of the color produced is measured spectrophotometrically.

Enzyme-Based Colorimetric Assays

In an enzyme-based colorimetric assay, a course of enzymatic reactions is followed, then changes in the intensity of light is measured spectrophotometrically [293].

Oxygen Radical Antioxidant Capacity Assay

The ORAC assay is a widely accepted test for measuring HAT-based antioxidant mechanisms [275]. The ROS in the system causes the fluorescent probe to become non-fluorescent [294]. Thus, the assay measures the ability of the antioxidant in the system to prevent damage to the fluorescent probe. The inhibition of free radical activity quantifies the antioxidant capacity of the sample. ORAC is the only assay that takes both inhibition time and inhibition amount into consideration.

Ferric Reducing Ability of Plasma Assay

The FRAP assay measures SET-based antioxidant mechanisms [275]. Ferric iron (Fe^{3+}) is reduced by the antioxidant from the sample to form ferrous iron (Fe^{2+}) which produces a dark blue color [295]. The antioxidant activity is proportional to the total Fe^{2+} in the system. It is measured by a spectrophotometric microplate reader and quantified based on the increase in absorbance at 593 nanometers.

Trolox Equivalent Antioxidant Capacity Assay

The TEAC assay is based on the addition of peroxidase to 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) which creates the radical cation $ABTS^+$ [296]. When antioxidants are present in the solution, the absorbance of $ABTS^+$ is reduced to a proportional amount related to the antioxidant capacity of the sample.

Thiobarbituric Acids Reactive Substances Assay

The TBARS assay is used to measure lipid peroxidation in the plasma [276]. Lipid peroxidation occurs with a ROS removes a hydrogen atom from a PUFA yielding lipid hydroperoxide and lipid aldehyde. One of the most common lipid aldehydes is malondialdehyde (MDA). Since ROS have short half-lives, they cannot be measured in the plasma, thus the stable lipid aldehydes are used for measurement. TBARS assays react MDA with thiobarbituric acid to produce a pink compound that can be quantified colorimetrically.

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CHAPTER 3

PECAN-ENRICHED DIETS ALTER CHOLESTEROL PROFILES AND TRIGLYCERIDES IN ADULTS AT-RISK FOR CARDIOVASCULAR DISEASE IN A RANDOMIZED, CONTROLLED TRIAL[1]

[1]Guarneiri, L.L., C.M. Paton, and J.A. Cooper. *J Nutr*, 2021. 151(10): p. 3091-3101. Reprinted here with permission of the publisher.

Abstract

Background: Research indicates that tree nuts are cardio-protective, but studies on pecan consumption are limited. Objective: The study examined the impact of daily pecan consumption on blood lipids and glycemia measures in adults at higher risk for cardiovascular disease (CVD). Methods: This was a randomized, controlled trial (clinicaltrials.gov: NCT04376632) where 56 adults (BMI > 28 kg/m2 or hypercholesterolemia) were randomized into one of three treatments for an 8-week intervention: two pecan groups and a nut-free control group (n=18). The ADD group (n=16) consumed pecans (68g) as part of a free-living diet, and the SUB group (n=18) substituted the pecans (68g) for isocaloric foods from their habitual diet. At baseline and 8 weeks, a high-fat meal (17% of daily energy needs) was consumed with 4h postprandial blood draws to determine changes in blood lipids (primary outcome) and markers of glycemia (secondary outcome). Fifty-two participants completed the trial and were in the final analyses of within and between group changes. Results: There was a significant reduction from baseline to 8-weeks in fasting total cholesterol (TC) (204±8.76 to 195±8.12; 205±8.06 to 195±6.94mg/dL), low-density lipoprotein cholesterol (143±8.09 to 129±7.71; 144±6.60 to 135±6.16mg/dL), triglycerides (TG) (139±12.1 to 125±14.6; 133±10.7 to 120±10.3mg/dL), TC:high-density lipoprotein cholesterol (HDL-c) ratio (3.92±0.206 to 3.58±0.175; 4.08±0.167 to 3.79±0.151), non-HDL cholesterol (151±8.24 to 140±7.95; 155±6.87 vs 143±6.00mg/dL), and apolipoprotein-B (99.1±5.96 to 93.0±5.35; 104±3.43 vs 97.1±3.11mg/dL) in the ADD and SUB groups, respectively ($p<0.05$ for all), with no changes in control from baseline to 8 weeks. In the ADD group, there was a reduction in postprandial TGs ($p<0.01$), while the SUB group showed a reduction in postprandial glucose ($p<0.05$). No changes were found for fasting glucose or fasting

and postprandial insulin. Conclusion: Daily pecan consumption, regardless of diet instructions, improves fasting and postprandial blood lipids in adults that are at-risk for CVD.

Introduction

Cardiovascular disease (CVD) is the leading cause of death for adults in the U.S. with one in every four deaths attributed to CVD [1]. Risk factors include increased adiposity, elevated blood pressure, smoking, insulin resistance, and dyslipidemia [2], which is characterized as elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), triglycerides (TG), or low levels of high-density lipoprotein cholesterol (HDL-c) [3]. Common interventions for mitigating dyslipidemia include modifying dietary intake and/or exercise patterns [4]. One dietary strategy that has been shown to be effective for improving lipid metabolism [5, 6] and reducing CVD risk [7, 8] is to replace saturated (SFA) and trans-fatty acids with monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) [9].

Tree nuts are rich sources of dietary MUFAs and PUFAs, as well as other vitamins, minerals, and phytonutrients [10], and have been shown to decrease blood lipids, lipid peroxidation, and overall CVD risk [11-16] while also improving lipid metabolism [17, 18]. Although there is substantial health-related research available on tree nut consumption, most studies are on walnuts [13, 14, 19], almonds [15, 20, 21] or pistachios [22-24]. To date, only four studies have examined the impact of pecan consumption on chronic disease risk in adults [5, 25-27]. Since pecans are a rich source of MUFAs, PUFAs, fiber, vitamin E, and polyphenols, and are low in SFAs [28-30], it is plausible that pecan consumption could provide some cardio-protective effects, especially as it relates to lipid metabolism.

We have previously shown that acute pecan consumption suppresses postprandial TGs in healthy young men [5]. Furthermore, two longer-term studies of 4-8 weeks showed that dietary pecan supplementation results in decreased TC, LDL-c and fasting TGs in healthy adults [26, 27]. However, a more recent study that investigated the impact of a smaller daily dose of pecans

(~42.5g/d) in a metabolically at-risk population (overweight with central adiposity) did not show reductions in blood lipids [25]. It is unclear if the lack of change in blood lipids was due to the lower dose of pecans or the at-risk population. Finally, no study has investigated whether the method or instructions for nut incorporation into the diet impacts blood lipid outcomes.

To address the discrepancies and questions listed above, the objective of this study was to examine the impact of daily pecan consumption (with and without dietary/isocaloric substitution instructions) for an 8-week period on blood lipids (the primary outcomes) and markers of glycemia (secondary outcomes) in adults with hypercholesterolemia or at higher risk for CVD ($BMI \geq 28 kg/m^2$). We hypothesized that daily pecan consumption, regardless of the method of incorporation into the diet, would result in improvements in blood lipids and markers of glycemia, and that the responses of the control group would be non-significant.

Methods

Study Design

This study was a single-blind, randomized, parallel controlled trial involving an 8-week intervention conducted at the University of Georgia. The single-blind categorization of this study is in reference to the participants' masking to the dietary substitution instructions that were provided to each pecan group. Participants were not blinded to whether they received a pecan intervention or were in the control group. Data collection occurred August 2018-December 2020, when the goal of 16 subjects/group was obtained. The study protocol included a screening visit and 3 testing visits. Subjects were randomly assigned (balanced blocks stratified by age, sex, and body mass index (BMI)) to one of three groups: a "no nut" control, or one of two pecan groups (ADD or SUB). Subjects in ADD and SUB consumed 68g of pecans/d for 8 weeks; however, dietary instructions for the incorporation of pecans into the diet differed. For ADD,

pecans were consumed as part of a free-living diet, while in SUB, participants received counseling at baseline on how to substitute pecans for isocaloric foods from their habitual diet. This study was approved by the Institutional Review Board for human subjects, and informed written consent was obtained from each participant prior to testing.

Participants

Sixty-nine sedentary men and women between the ages of 30 and 75y with high cholesterol or a BMI of ≥28kg/m^2 were assessed for eligibility. Inclusion based on "high cholesterol" levels was defined as either "borderline high/undesirable" in two blood lipid categories or "high" in TC or LDL-c (**Supplementary Table 3.5)**. To rule out individuals with familial hypercholesterolemia, participants with LDL-c levels greater than the 95th percentile or HDL-c levels lower than the 20th percentile were excluded. Other exclusion criteria included habitual nut consumption (56g/week), nut allergies, special diets (i.e. ketogenic diet, intermittent fasting), excessive alcohol use (42g alcohol/d (men) or 28g alcohol/d (women)), tobacco or nicotine use, exercise >3h/week, weight loss or gain > 5% of body weight in the past 3 months, plans to begin a weight loss or exercise regimen, history of medical events or medication use affecting digestion, absorption, or metabolism, gastrointestinal surgery, and chronic or metabolic diseases. Individuals taking medications that could affect blood lipid or glycemic outcomes were also excluded. Finally, individuals with the following biomarkers were excluded: fasting glucose >126mg/dL, fasting TG >350mg/dL, and blood pressure >180/120mmHg. Eligibility based on blood lipids and glucose were determined from fasting blood samples at the screening visit.

Protocol

Screening Visit

Individuals arrived at the lab following an 8-12h overnight fast and 24h without exercise or alcohol. A fasting blood draw for a lipid panel and glucose measurement was obtained, and anthropometrics and resting metabolic rate (RMR) were measured as previously described [31-33]. Participants' RMR was multiplied by an average activity factor of 1.65 to estimate daily energy needs [34]. Alcohol consumption habits were assessed by the Alcohol Use Disorders Identification Test (AUDIT) [35]. If individuals qualified for the study, subjects were randomized to one of three groups by a researcher that was not involved in data collection or analysis. An allocation ratio of 1:1:1, a permuted block design (balanced for age, sex, and BMI), and a random number generator were used to randomize participants.

Pre-Diet Intervention Visit (V1)

Subjects completed a two-day food diary containing one weekend day and one week day [36] between the screening visit and the pre-diet intervention visit. One of the food diaries took place the day before V1. In addition, the night before V1, participants consumed a lead-in dinner meal and snack (provided by research personnel) that contains 50% of total energy from carbohydrate, 15% of energy from protein, and 35% of energy from fat.

For V1, participants arrived following an 8-12h overnight fast and 24h without exercise or alcohol. Anthropometrics and RMR were measured. Baseline stress levels were obtained through the Perceived Stress Scale (PSS) [37], and physical activity was assessed by calculating Total Metabolic Equivalent Task (MET) minutes/week with the International Physical Activity Questionnaire (IPAQ) [38].

Following the questionnaires, an intravenous (IV) catheter was inserted for the fasting blood draw, and the line was kept patent with saline. Participants then consumed a SFA-rich breakfast shake within 10min. This high-fat meal provided 17% of total daily energy needs based on the RMR measurements from the screening visit and was made from an original milk chocolate ready-to-drink shake (Ensure, Abbott Nutrition, Abbott Laboratories, Inc., Columbus, Ohio, USA), unsalted butter, red palm oil, coconut oil, soy lecithin granules, and powdered chocolate drink mix. The nutrient breakdown of this test meal is provided in **Table 3.1.** One hundred eighteen ml of water was used to rinse out the container and then ingested to ensure the entire liquid meal was consumed. A sensory questionnaire using a 9-point hedonic scale was administered after meal consumption to assess the sensory modalities (appearance, taste, texture and aroma and overall acceptance) of the breakfast shake [39, 40]. Following the SFA meal, blood draws occurred at 30, 60, 90, 120, 150, 180, 210, and 240min. One hundred eighteen ml of water was provided once/h postprandially. After 4h, the IV catheter was removed.

8-Week Dietary Intervention

The day after V1, all participants began the 8 week intervention. Written diet instructions were provided to all participants. Participants in control were instructed to avoid all forms of nuts and to consume $\leq$64g of nut butter/week. Participants in ADD were provided with 68g (~0.5 cup or 2.25 ounces) portions of pecans to consume as part of their free-living diet with no additional diet instructions. Participants in SUB were instructed to substitute the 475 kilocalories provided by the 68g of pecans for foods habitually consumed in their free-living diet [28]. Trained research personnel guided the participants on how to make appropriate energy substitutions based on their previously completed food diaries. For example, if the participant habitually consumed snacks throughout the day, the research personnel highlighted the energy content of

the snacks and asked the participant if it was feasible to replace the habitual snacks with the provided pecans. The guidance provided was individualized based on each participant's dietary intake. **Supplemental Supplementary Table 3.6** shows the complete nutrition information for the 68g portion of pecans. A 4-week supply of pecans (in 68g portions) was provided to participants in ADD and SUB at V1 and V2. Like the control group, the pecan groups were instructed to avoid all other nuts and limit nut butter to ≤64g/week (2 servings). In addition, they were instructed to eat pecans in their raw form (no roasting, cooking, or baking). Subjects were permitted to add the pecans to other foods. Likewise, all subjects were instructed to avoid consuming >42g alcohol/d (men) or >28g alcohol/d (women) and were asked not to make any other changes to their diet or activity levels. Participants were unaware of the diet instructions that were provided to other groups to prevent unintentional or intentional changes in behavior.

Weekly Responsibilities

Subjects in ADD and SUB logged their intake of pecans on a daily nut compliance document, which was submitted to research staff weekly. Poor compliance was categorized as consumption of <75% of pecans throughout the 8-week intervention. All participants completed a food diary once per week alternating between weekdays and weekend days. Daily nutrient intakes based on food diaries were assessed using The Food Processor SQL software (version 10.12.0). The nutrients from the two baseline food diaries and then the food diaries from weeks 1-8 were averaged before analysis. The IPAQ was completed electronically during weeks 2 and 6.

Mid-Diet Intervention Visit (V2)

After 4 weeks of the intervention, participants arrived at the lab following another 8-12h overnight fast and 24h without exercise or alcohol. The same dinner meal and snack that were

consumed before V1 were consumed the night before V2. The same anthropometrics, PSS, IPAQ, and fasting blood draw from V1 were repeated, exactly as stated above.

Post-Diet Intervention Visit (V3)

After 8 weeks of the intervention, participants arrived for V3 under the same pre-visit conditions as V1 and V2. Participants completed the exact same study procedures and measurements from V1, including anthropometrics questionnaires, and SFA meal.

Sample Analysis

During all 3 testing visits, a portion of the fasting blood sample was drawn into a serum separator clot activator vacutainer and held at room temperature for 30min before centrifugation for 15min at 3,000g at 4°C. The serum from the serum separator clot activator vacutainer was transferred into a transport tube and kept at 4°C until detailed blood lipid panel analyses was completed (Quest Diagnositics, Chantilly, VA, USA). This lipid panel was a primary outcome and included TC, TG, LDL-c, HDL-c, low-density lipoprotein (LDL) particle number, LDL size, high-density lipoprotein (HDL) size, total apolipoprotein-B (apoB), and lipoprotein(a).

The rest of the fasting blood sample and all postprandial blood samples were drawn into an EDTA vacutainer, immediately placed on ice, and then centrifuged under the same conditions. The plasma was aliquoted and stored at -80°C until analysis. Sample analysis of primary outcomes included TGs, non-esterified fatty acids (NEFA), while glucose and insulin were secondary outcomes. Fasting sample analysis of γ-tocopherol served as a marker of biological compliance [41]. Plasma TG and NEFA were measured by enzyme-based calorimetric assays (Wako Chemicals USA, Inc., Richmond, VA). Plasma glucose and insulin were measured using a colorimetric glucose oxidase/peroxidase method (glucose oxidase: G2133, peroxidase: P8250;

Sigma Aldrich, St. Louis, MO, USA) and radioimmunoassay (RIA) (MilliporeSigma, Darmstadt, Germany), respectively. Plasma γ-tocopherol was measured by high performance liquid chromatography (HPLC) (Eurofin Craft Technologies, Wilson, NC, USA).

Statistical Analyses

SAS version 9.2 statistical package (SAS Institute Inc, Cary, NC, USA) was used for statistical analyses. All values were reported as mean±SEM unless otherwise noted. Statistical significance was set at $p \leq 0.05$. A sample size of 48 (16/group) was estimated to detect a significant change in LDL-c using G*power 3.19.7 assuming at least 80% power and an α of 0.05 based on the previous pecan study conducted by Morgan et al. [26]. An unpaired t-test was used to assess differences in nut compliance between the two pecan groups. For time course data, change from baseline was calculated (baseline value subtracted from each postprandial time point), then a three-way (treatment, visit, time) repeated measures ANOVA was used to test for within group differences. In addition, the change in iAUC from pre- to post-intervention within each group was calculated for these outcomes for between group comparisons. A one-way ANOVA was used to test for differences at baseline and across the intervention between groups, and a two-way repeated measures ANOVA was used to test for differences within groups from pre- to post-intervention for anthropometrics, perceived stress, total MET minutes, dietary intake, and fasting biochemical outcomes. When significance was found, post hoc analyses were done using least square means with no multiple testing adjustment. Continuous variables were examined for normality using the Shapiro-Wilk test, and an appropriate transformation was applied to non-normal data before analysis.

RESULTS

Subjects

Fifty-six subjects were randomly assigned to an intervention (n=20 control, n=17 ADD, and n=19 SUB); however, four participants did not start or complete the intervention and were not included in final analyses (**Figure 3.1)**. Therefore, fifty-two subjects completed the intervention (n=12 women and n=6 men for control, n=11 women and n=5 men for ADD, and n=13 women and n=5 men for SUB) and were included in the per protocol analyses of primary and secondary outcomes. Five of those 52 subjects did not complete the meal challenge, so only their fasting data was included (control=2, ADD=1, SUB=2). Participant characteristics at baseline are presented in **Table 3.2**. There were no differences between groups at baseline for anthropometric or blood lipids. Additionally, there were no differences between or within groups from pre- to post-intervention for body weight, BMI, waist circumference, hip circumference, systolic blood pressure, or diastolic blood pressure **(Supplementary Table 3.7).** Finally, the change in γ-tocopherol from baseline to 8-weeks within ADD and SUB was significantly greater vs. control (0.41±0.14 and 0.24±0.08 vs -0.06±0.14μg/mL, respectively; $p \leq 0.01$) indicating compliance in the two pecan groups.

On average, participants in ADD and SUB consumed 95±1% and 94±2% of pecans provided, respectively, and compliance was not different between groups. No participant reported poor compliance, and there was no report of intake of nuts in the control group according to food diaries. Based on analysis of the weekly food diaries, as expected due to the daily pecan consumption, grams of total fat ($p<0.0001$ and $p=0.0003$), MUFA ($p<0.0001$ for both), PUFA ($p<0.0001$ and $p=0.005$), and dietary fiber ($p<0.0001$ and $p=0.005$) increased significantly in ADD and SUB, respectively **(Table 3.3)**. In addition, within ADD, there was an

increase in protein intake (p=0.02) and a trend for an increase in energy intake (p=0.07). Regarding micronutrients, copper and magnesium increased significantly within ADD (0.38±0.05 to 1.30±0.11mg and 114±11.3 to 205±15.7mg, respectively; p<0.0001 for both) and SUB (0.54±0.12mg to 1.21±0.07 and 117±13.3 to 185±13.2mg, respectively; p<0.0001 and 0.001), but there were no other changes for fat or water soluble vitamins or minerals. There was a trend for an increase in MUFA in control (p=0.07), but no significant changes within this group. In addition, there were no changes in physical activity (total MET minutes) from baseline throughout the intervention in any of the three groups. There was also no change in sensory ratings from pre- to post-intervention visits for the SFA shake within each group (overall acceptability: control: 6±0 to 6±0.0; ADD: 7±0 to 6±1; SUB: 6±0 to 6±0, respectively; p=0.23). Finally, ratings of stress did not differ from pre- to post-intervention within each group (control: 14±1 to 14±2; ADD: 13±1 to 12±1; SUB: 16±1 to14±1, respectively; p=0.67).

Change in Fasting Biochemical Markers between Groups

The change from pre to post intervention for fasting TC, LDL-c, HDL-c, apoB, TG, NEFA, non-HDL cholesterol, and TC:HDL-c ratio are displayed in **Figure 3.2**. From baseline to 8 weeks, the decreases in TC (p=0.02 for both), LDL-c (p=0.0004 and p=0.004), apoB (p=0.003 and p=0.001), TG (p=0.02 and p=0.01), non-HDL cholesterol (p=0.003 for both), and TC:HDL-c ratio (p<0.001 for both) were greater in ADD and SUB vs control, respectively. There were no differences between pecan groups. Furthermore, the reduction in fasting NEFA from pre to post intervention was greater in ADD vs control (p=0.01), while SUB was not different from either group.

Many of the aforementioned differences between groups across the 8 weeks were already different at the mid-way point of the intervention. Specifically, from baseline to 4 weeks, the

decreases in LDL-c (p=0.004 and 0.05) and TC:HDL-c ratio (p=0.003 and 0.01) were greater in ADD and SUB vs control, respectively. Furthermore, the change in HDL-c was higher in SUB vs control (p=0.01), and there was a trend in ADD vs control (p=0.09). Similarly, the change in TG was lower in ADD vs control (p=0.007), and there was a trend for a greater decrease in SUB vs control (p=0.09). There were no differences between any group for the change in LDL particle number, LDL size, LDL medium, HDL large, LDL peak size, lipoprotein(a), glucose, and insulin from baseline to 4 or 8 weeks.

Fasting Biochemical Markers

Fasting blood lipids at baseline, 4 weeks, and 8 weeks are displayed in **Table 3.4**. For TC, there was a reduction from baseline to 4 and 8 weeks for ADD (p=0.05 and p=0.03, respectively) and a reduction at 8 weeks for SUB (p=0.02). Similarly, there was a reduction in LDL-c from baseline to 4 and 8 weeks within ADD (p=0.0002 and p=0.002, respectively) and a reduction at 8 weeks within SUB (p=0.02). There were no differences in TC or LDL-c within control.

For HDL-c, there was an increase from baseline to 4 weeks for ADD (p=0.03) and SUB (p=0.001) but not at 8 weeks. Furthermore, the TC:HDL-c ratio was reduced at 4 and 8 weeks in ADD (p=0.001 and p=0.008, respectively) and SUB (p=0.02 for both), and there was a trend for an increase in TC:HDL-c ratio at 8 weeks in control (p=0.08). There was also a reduction at 4 and 8 weeks for non-HDL cholesterol in ADD (p=0.01 for both) and at 8 weeks in SUB (p=0.009). There were no differences in non-HDL cholesterol within control. Finally, for total apoB, there was a reduction at 8 weeks in ADD (p=0.04) and SUB (p=0.02), and an increase at 8 weeks in control (p=0.05). LDL particle number, LDL small, LDL medium, LDL peak size, and lipoprotein(a) did not change within any treatment group across the intervention.

For TG, there was a reduction from baseline to 8 weeks in ADD (p=0.009) and SUB (p=0.05) and an increase in control (p=0.04). In addition, there was a decrease in fasting NEFA in ADD from baseline to 8 weeks (p=0.003), and a decrease in SUB at 4 weeks (p=0.01), but not at 8 weeks. Finally, analysis of glycemic measures revealed no change within any group across the intervention for insulin or glucose (**Table 3.4**).

Postprandial Biochemical Markers

The meal responses for TG, NEFA, insulin, and glucose are presented in **Figure 3.3**. Postprandial TGs were suppressed at post-intervention vs baseline in ADD ($p \leq 0.05$) (**Figure 3.3B**) but not in SUB or control. Furthermore, postprandial glucose was suppressed at post-intervention vs baseline in SUB ($p \leq 0.01$) **(Figure 3.4F)** but not in ADD or control. In addition, the meal response for NEFA **(Figure 3.3D-F)** and insulin **(Figure 3.4A-C)** did not differ from pre- to post-intervention for any of the three groups (**Figures 3.3B-D**). Finally, the change in meal response from V1 to V3 was not significantly different between groups for TG (change in iAUC: control: -13.0±44.2 vs ADD: -36.8±29.5 vs SUB: 1.18±28.9mg/dL; p=0.77), NEFA (control: -0.05±0.22 vs ADD: 0.27±0.14 vs SUB: 0.15±0.11mEq/L; p=0.36), insulin (control: 0.10±6.89 vs ADD: 5.77±5.99 vs SUB: 1.53±3.32mg/dL; p=0.75), or glucose (control: -7.96±24.4 vs ADD: 10.9±8.70 vs SUB: -36.7±22.0mg/dL; p=0.125).

Discussion

For the first time in a population at-risk for CVD, we have shown that daily pecan consumption (68g) for 8 weeks, with or without dietary isocaloric substitution instructions, resulted in significant improvements in fasting TC, LDL-c, TG, HDL-c, TC:HDL-c ratio, non-HDL cholesterol, and apoB. In addition, there were improvements in fasting NEFA and postprandial glucose or TGs in at least one of the two pecan groups. Based on the energy intake

data, the dietary substitution instructions were effective since there no change in energy intake within SUB while there was a trend for an increase in ADD. Contrary, to our hypothesis, there were no changes in fasting LDL particle number, LDL particle size, lipoprotein(a), or fasting or postprandial insulin or glucose. There were no changes in body weight, physical activity, or stress between or within groups, and the changes in self-reported food intake were as expected in the pecan groups. Moreover, the increase in γ-tocopherol was significantly greater in both pecan groups vs control, confirming compliance with the pecan interventions, thus the improvements in blood lipids are likely attributable to the daily consumption of pecans.

It is of equal importance to study physiologic/clinical significance along with statistical significance. One way to do this is by examining the magnitude of change due to an intervention. We found that pecan consumption lowered TC by 4.7% and 4.9% and LDL-c by and 9.5% and 6.4% in ADD and SUB, respectively. These findings are clinically meaningful because a 1.0% reduction in LDL-c is associated with a 1.2-2.0% reduction in the risk of coronary artery disease (CAD) [42, 43]. The similar reduction of blood lipids in ADD and SUB is also a novel finding, and indicates that dietary substitution instructions are not required when recommending daily pecan consumption for patients with dyslipidemia. Additionally, when compared to other types of interventions, our results show a larger degree of success. A meta-analysis of 51 exercise interventions reported that, on average, the reduction in TC and LDL-c was 1.0% and 5.0%, respectively [44], while another meta-analysis reported no benefit of weight loss exercise interventions focusing on weight loss on blood lipids [45]. It has also been shown that exercise and weight loss are often time-consuming and are difficult to adhere to long-term [46, 47]. Therefore, the addition of pecans to the diet, or substituting foods in the typical diet with pecans, not only produced a greater and more consistent reduction in TC and LDL-c compared to

exercise and weight loss interventions [44-47], but may also be a more sustainable approach for long-term health.

Since there are so few previous studies on daily pecan consumption, there is limited data to compare our results to [25, 27, 48]. Previously, beneficial results from daily pecan consumption were only observed in healthy populations following 4-8 weeks of daily pecan consumption [27, 48]. McKay et al. [25] found no change in TC or LDL-c following 4 weeks of pecan consumption (~42.5g/d) in overweight or obese adults. Since our study was also in an overweight and obese population, it is possible that the smaller dosage of pecans in the McKay study was not enough to elicit improvements in blood lipids. A previous meta-analysis concluded that tree nut doses of $\geq$60g/d are necessary to observe a reduction in LDL-c [49]. Therefore, cardioprotective dietary recommendations should include pecans in doses of at least 68g/d.

Although this study was not designed to be mechanistic, previous work and knowledge, as well as the analysis of the food diaries, suggests two nutrients in the pecans may elicit the observed reduction in TC and LDL-c following pecan consumption. The first is the fatty acid composition of the pecans. The dose of pecans in this study contained 24.9g MUFA and 13.6g PUFA [28, 50], and this fatty acid profile was reflected in the nutrient analysis from the food diaries where significant increases in MUFA and PUFA were observed in both nut groups. Previous research has shown that diets rich in unsaturated fats produce favorable effects on cholesterol [7, 51, 52]. Those clinical trials are supported by mechanistic research, which indicates that SFAs increase LDL-c by downregulating LDL receptors and increasing LDL formation, while unsaturated fatty acids have the opposite effect [7, 53, 54]. The second is the high fiber content of pecans since moderate and high fiber intake is associated with decreased CVD risk due to the reduction of LDL-c [55, 56]. The dose of pecans provided 6.5g/d of dietary

fiber, which corresponded to significant increases of 8.1g and 5.3g/d of fiber in the ADD and SUB groups' overall diet during the intervention, respectively. While the details of this complex mechanism still need to be elucidated, one can conclude that the higher daily fiber intakes, along with the favorable fatty acid profile, from the pecans likely contributed to the observed LDL-c reduction in the present study.

Another meaningful finding from this study was the significant reduction in apoB within each pecan group. Although never previously reported with pecan consumption alone, the reduction in apoB observed in this study is consistent with previous findings in other tree nuts [49]. ApoB is a large protein found on atherogenic lipoproteins such as chylomicron remnants, LDL-c, very low density lipoprotein (VLDL), intermediate-density lipoprotein, and lipoprotein(a) [57]. One might be surprised that the reduction in apoB was not accompanied by a reduction in LDL particle number. However, since apoB is found on numerous lipoproteins, it's possible that the reduction in apoB occurred due to the reduction of another lipoprotein. Emerging research indicates that apoB is more useful in predicting atherosclerotic CVD risk than LDL-c [58, 59]. A meta-analysis concluded that a 10mg/dL decrease in apoB reduces the risk of coronary heart disease and overall CVD risk by 9% and 6%, respectively [60]. The magnitude of reduction in apoB that occurred in the pecan groups corresponds to a 5-6% reduced risk of CHD and 3-4% reduced risk of CVD, further illustrating the cardioprotective benefits of pecan consumption.

We previously reported that acute consumption of a muffin containing pecans resulted in lower postprandial TG responses in males compared to a muffin made with butter [5]. However, no study had ever investigated the postprandial TG and glucose responses to a high-fat meal following a long-term tree nut intervention. For the first time, we showed a reduction in

postprandial TG and glucose responses following a high SFA meal in at least one of the pecan groups. Importantly, this effect occurred with a test meal that was absent of pecans. This outcome is clinically meaningful because elevated postprandial TG and glucose are an independent risk factor for CVD [61-63]. Since Americans consume more SFAs on the weekend compared to weekdays [64], habitual pecan consumption may provide a cardioprotective benefit when the occasional high SFA meal is consumed. It is unclear why this effect was observed in only one pecan group for each outcome (TG for ADD and glucose for SUB), especially since there were no significant differences in the self-reported intake of any nutrient between the two pecan groups.

This study was not without limitations. As previously mentioned, measurements of dietary intake, physical activity, and stress were all self-reported, which may contain some degree of under- or over-reporting. In addition, significant increases in TG and apoB were observed within the control group. Although the change within the control group was unexpected, it is also not uncommon, as evidenced by significant increases in TC within the control group of a previous pecan trial by Morgan et al. [26]. We can only speculate why this effect was observed. The self-reported dietary intake data does not provide a logical answer, since no changes in nutrient intake, such as carbohydrate content, occurred during the intervention; however, we know that "no treatment" control groups are at-risk of expectation bias in which the expectation of no benefit from the study leads to a less favorable outcome [65]. Furthermore, we only controlled the dinner meal the evening before each testing visit rather than a including a multi-day lead-in diet. This design was intentional to isolate the effects of adding pecans to one's habitual diet. Another limitation is the generalizability of results since we used a relatively high dose of pecans and a shorter duration. We can only speculate on the effectiveness

of a smaller dose over a longer duration, but it is helpful to consider that 42.5g (1.5oz) within a low-saturated fat diet is recommended by the FDA as a qualified health claim [66]. Finally, this study was not powered or designed to detect differences between sexes or races.

In conclusion, daily pecan (68g) consumption reduced fasting blood lipids, including TC, LDL-c, apoB, postprandial TG, and postprandial glucose in adults with high cholesterol or those at a greater risk for CVD (BMI$\geq$28kg/m^2). The results of this study are clinically meaningful because the magnitude of reduction in LDL-c in the pecan groups (6.4-9.5%) could correspond to a 6.5-11.4% reduction in the risk for CAD. Furthermore, the reduction in postprandial TG and glucose provides additional protection in the fed state. This study shows that a simple and cost-effective method to reduce risk factors of CVD in susceptible populations is achievable. Future studies should investigate the impact of pecan consumption for a longer duration at a lower dose in a variety of populations.

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Table 3.1 Nutrient breakdown for the high saturated fat meal challenge

Composition	SFA-rich meal
Percentage of total energy from	
Protein	5.0
Carbohydrate	25.0
Fat	69.5
Percentage of energy from fatty acids	
SFA	46.9
MUFA	15.7
PUFA	6.9

HF=high fat; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid, SFA saturated fatty acid

Table 3.2 Characteristics at baseline of adults at-risk for cardiovascular disease in pecan or nut-free groups

	Control (n=18)	ADD (n=16)	SUB (n=18)
Female (%)	66	69	72
Age (y)	50 ± 16	49 ± 11	46 ± 10
Height (cm)	166.8 ± 11.7	167.6 ± 7.9	166.4 ± 10.1
Weight (kg)	87.9 ± 28.5	84.9 ± 13.7	90.3 ± 20.6
Body Mass Index (kg/m^2)	31.0 ±7.1	30.2 ± 4.1	32.5 ± 6.9
Waist Circumference (cm)	112.6 ± 15.2	111.9 ± 9.1	115.0 ± 13.1
Hip Circumference (cm)	97.7 ± 18.0	96.4 ± 8.9	99.5 ± 15.9
Systolic Blood Pressure (mmHG)	124 ± 14	128 ± 16	153 ± 17
Diastolic Blood Pressure (mmHG)	81 ± 10	85 ± 15	83 ± 11
Body Fat Percentage (%)	32.1 ± 6.7	30.7 ± 6.6	33.1 ± 7.1
Total Cholesterol (mg/dL)	202 ± 44.6	204 ± 33.9	205 ± 33.2
LDL Cholesterol (mg/dL)	141 ± 43.5	143 ± 31.3	144 ± 27.2
HDL Cholesterol (mg/dL)	50.6 ± 15.6	52.9 ± 8.00	51.2 ± 11.0
Triglycerides (mg/dL)	131 ± 39.1	139 ± 46.8	133 ± 44.0
Non-esterified Fatty Acids (mEq/L)	0.458 ± 0.214	0.449 ± 0.182	0.436 ± 0.165
Glucose (mg/dL)	101 ± 19.9	97.2 ± 12.3	95.4 ± 14.3
Insulin (µU/mL)	16.1 ± 6.67	14.2 ± 7.39	20.1 ± 8.13
Total MET (minutes/week)	1,183 ± 257	1,312 ± 254	1,301 ± 408
Perceived Stress Scale	14 ± 1	13 ± 1	16 ± 1

All values are mean ± SD. There were no significant differences between groups at baseline for any outcome. All outcomes were measured in serum, except for non-esterified fatty acids, glucose, and insulin, which were measured in plasma. ADD=consumed pecans as part of a free-living diet; LDL=Low-Density Lipoprotein; HDL=High-Density Lipoprotein; MET=Metabolic Equivalent Task; SUB=substituted pecans for isocaloric foods from their habitual diet.

Table 3.3 Daily nutrient intake of adults at-risk for cardiovascular disease at baseline and throughout an 8-week pecan or nut-free diet

	Control		ADD		SUB	
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
Energy (kcal)	1917 ± 156	1977 ± 174	1850 ± 136	2329 ± 170[#]	2066 ± 136	2201 ± 101
Kcal from carbohydrate (%)	48.3 ± 1.25	46.9 ± 1.45	47.6 ± 2.70	36.7 ± 1.46***	48.1 ± 1.74	40.5 ± 1.45***
Kcal from protein (%)	15.0 ± 0.66	15.6 ± 0.74	15.4 ± 0.65	13.8 ± 0.58*	14.4 ± 0.69	12.7 ± 0.35*
Kcal from fat (%)	35.9 ± 1.47	36.6 ± 1.02	36.3 ± 2.44	48.3 ± 0.96***	36.1 ± 1.44	45.5 ± 1.53***
Kcal from alcohol (%)	0.90 ± 0.52	0.87 ± 0.39	0.75 ± 0.42	1.11 ± 0.64	1.45 ± 0.79	1.33 ± 0.55
Carbohydrate (g)	228 ± 16.6	236 ± 105	216 ± 18.4	214 ± 17.3	250 ± 20.7	226 ± 15.5
Fiber (g)	16.5 ± 2.15	15.4 ± 1.21	12.4 ± 1.39	20.5 ± 1.52***	12.8 ± 2.30	18.1 ± 1.50**
Protein (g)	69.4 ± 5.07	74.0 ± 4.69	70.2 ± 5.06	81.0 ± 7.40*	73.8 ± 6.50	69.8 ± 3.74
Fat (g)	78.8 ± 8.53	80.3 ± 7.06	76.6 ± 8.45	126 ± 9.98***	83.3 ± 6.36	110 ± 4.84***
MUFA (g)	27.8 ± 3.21	33.1 ± 3.04[#]	31.8 ± 3.66	63.2 ± 4.48***	34.1 ± 2.48	53.0 ± 2.12***
PUFA (g)	19.6 ± 2.90	19.7 ± 2.64	16.8 ± 2.68	32.2 ± 2.20***	21.0 ± 2.86	29.3 ± 1.50**
SFA (g)	30.7 ± 3.53	26.5 ± 2.25	27.3 ± 3.65	29.4 ± 3.75	27.4 ± 2.51	27.0 ± 1.89
Trans-FA (g)	0.71 ± 0.19	0.92 ± 0.17	0.79 ± 0.16	0.83 ± 0.18	0.75 ± 0.19	0.70 ± 0.12
Cholesterol (mg)	233 ± 34.8	253 ± 26.3	302 ± 53.7	307 ± 53.0	275 ± 33.3	206 ± 24.0

All values are mean ± SEM *(N=52)*. * Indicates a significant difference from baseline within a group ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$). [#] Indicates a trend for a change within a group ($p < 0.10$). There were no significant differences between groups at baseline. ADD= consumed pecans as part of a free-living diet; FA=fatty acid; g=gram; kcal=kilocalorie; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; SUB=substituted pecans for isocaloric foods from their habitual diet

Table 3.4 Fasting biochemical markers of adults at-risk for cardiovascular disease at baseline and throughout an 8-week pecan-enriched or nut-free diet

	Control			ADD			SUB		
	Week 0	Week 4	Week 8	Week 0	Week 4	Week 8	Week 0	Week 4	Week 8
TC (mg/dL)	202 ± 43.6	209 ± 44.7	208 ± 47.8	204 ± 33.9	196 ± 35.2*	195 ± 31.4*[a]	205 ± 33.2	206 ± 33.4	195 ± 28.6*[a]
LDL-c (mg/dL)	141 ± 43.5	146 ± 45.0	148 ± 39.3	143 ± 31.3	127 ± 30.6***[a]	129 ± 29.9**[a]	144 ± 27.2	139 ± 25.9[a]	135 ± 25.4*[a]
HDL-c (mg/dL)	50.6 ± 11.5	50.8 ± 12.8	49.1 ± 12.3	52.9 ± 8.00	56.5 ± 7.85*	55.2 ± 6.88	51.2 ± 11.0	56.5 ± 14.9***[a]	52.6 ± 9.70
TG (mg/dL)	131 ± 39.1	163 ±75.0	158 ±83.9*	139 ± 46.8	128 ± 60.4[a]	125 ± 56.5**[a]	133 ± 44.0	138 ± 65.3	138 ± 65.3*[a]
NEFA (mEq/L)	0.46 ± 0.21	0.41 ± 0.19	0.47 ± 0.19	0.45 ± 0.18	0.40 ± 0.17	0.34 ± 0.14**[a]	0.44 ± 0.17	0.35 ± 0.19*	0.40 ± 0.19
TC:HDL-c Ratio	4.14 ± 1.25	4.38 ± 1.50	4.41 ± 1.23	3.92 ± 0.80	3.53 ± 0.68**[a]	3.58 ± 0.68**[a]	4.08 ± 0.69	3.79 ± 0.67*[a]	3.79 ± 0.622*[a]
Non-HDL Chol. (mg/dL)	150 ± 43.1	156 ± 46.3	157 ± 45.5	151 ± 31.9	141 ± 34.8**	140 ± 30.8**[a]	155 ± 27.5	152 ± 23.1	143 ± 24.0**[a]
LDL-P (nmol/L)	1303 ± 391	1314 ± 366	1356 ± 326	1332 ± 294	1249 ± 305	1311 ± 371	1310 ± 248	1313 ± 183	1269 ± 273
LDL Small (nmol/L)	253 ± 125	255 ± 117	266 ± 107	221 ± 74.5	210 ± 89.4	236 ± 123	199 ± 59.9	180 ± 47.8	183 ± 64.0
LDL Medium (nmol/L)	295 ± 117	288 ± 122	300 ± 99.3	314 ± 98.6	289 ± 107	312 ± 123	298 ± 70.6	274 ± 68.3	263 ± 51.3
HDL Large (nmol/L)	4580 ± 787	4890 ± 839	5090 ± 1220	5170 ± 900	5810 ± 1380	5610 ± 911	5210 ± 880	5660 ± 1630	5030 ± 1070
LDL Peak Size (nm)	21.7 ± 0.54	21.7 ± 0.68	21.5 ± 0.48	21.9 ± 0.43	22.0 ± 0.48	21.9 ± 0.44	21.9 ± 0.51	22.0 ± 0.62	22.0 ± 0.55
ApoB (mg/dL)	96.9 ± 25.4	98.5 ± 28.5	103 ± 23.2*	99.1 ± 22.3	96.1 ± 24.0	93.0 ± 20.0*[a]	104 ± 13.7	100 ± 11.0	97.1 ± 12.4*[a]
Lp(a) (nmol/L)	31.9 ± 27.1	34.3 ± 30.4	31.1 ± 29.2	79.3 ± 82.6	78.8 ± 76.7	80.5 ± 74.8	73.1 ± 103	69.1 ± 106	71.2 ± 108
Insulin (µU/mL)	16.1 ± 6.67	17.3 ± 7.86	19.3 ± 12.4	14.2 ± 7.39	14.7 ± 8.18	15.2 ± 7.58	20.1 ± 8.13	23.4 ± 10.5	21.4 ± 9.93
Glucose (mg/dL)	101 ± 19.9	101 ± 17.7	100 ± 20.2	97.2 ± 12.3	94.4 ± 7.43	94.8 ± 10.3	95.4 ± 14.3	97.1 ± 14.0	101 ± 16.0

All values are mean ± SD (control: n=18; ADD: n=16; SUB: n=18). * Indicates a significant difference from baseline within a group ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$). [a] Indicates that the change from baseline to 4 or 8 weeks was significantly different from that of the control group ($p \leq 0.05$). All outcomes were measured in serum, except for non-esterified fatty acids, insulin, and glucose, which were measured in plasma. ADD=consumed pecans as part of a free-living diet; TC=Total Cholesterol; LDL-c=Low-density lipoprotein cholesterol; HDL-c=High-density lipoprotein cholesterol; SUB=substituted pecans for isocaloric foods from their habitual diet; TG=Triglycerides; NEFA=Non-esterified free fatty acid; TC:HDL-c=Total Cholesterol:HDL-c Ratio; LDL-P=Low-density lipoprotein particle number; ApoB=Apolipoprotein B; Lp(a)=Lipoprotein(a).

Supplementary Table 3.5 Blood lipid qualification

	Desirable (mg/dL)	Borderline High/Undesirable (mg/dL)	High/Undesirable (mg/dL)
Total Cholesterol	Less than 200	200-239	240 and higher
LDL Cholesterol	Less than 130	130-159	160 and higher
HDL Cholesterol	50 and higher	40-49	Less than 40
Triglycerides	Less than 150	150-199	200 and higher

HDL=High-density lipoprotein; LDL=Low-density lipoprotein

Supplementary Table 3.6

Nutrient breakdown for pecans (68 grams)

Energy (kcal)	469.9
Carbohydrates (g)	9.4
Total Sugars (g)	2.7
Total Dietary Fiber (g)	6.5
Protein (g)	6.2
Fat (g)	48.9
SFA (g)	4.2
MUFA (g)	27.7
Oleic Acid	27.6
Paullinic Acid	0.1
PUFA (g)	14.7
ALA (ω-3)	0.7
Linoleic Acid (ω-6)	14.0

Kcal= kilocalorie; g=gram; SFA=Saturated Fatty Acid; MUFA=Monounsaturated Fatty Acid; PUFA=Polyunsaturated Fatty Acid; ALA=Alpha-Linolenic Acid.

Supplementary Table 3.7 Anthropometrics of adults at-risk for cardiovascular disease in pecan or nut-free groups

	Control			ADD			SUB		
	Week 0	Week 4	Week 8	Week 0	Week 4	Week 8	Week 0	Week 4	Week 8
BW (kg)	87.9 ± 28.5	88.1 ± 29.4	88.2 ± 28.7	84.9 ± 13.7	85.5 ± 14.0	86.1 ± 14.0	90.3 ± 20.6	90.3 ± 21.0	90.9 ± 21.4
BMI (kg/m^2)	31.0 ± 7.07	31.0 ± 7.32	30.9 ± 7.07	30.2 ± 4.09	30.4 ± 4.28	30.6 ± 4.20	32.5 ± 6.86	32.5 ± 7.05	32.5 ± 7.05
WC (cm)	98.0 ± 18.0	97.0 ± 19.1	97.9 ± 18.3	96.4 ± 8.9	95.5 ± 7.7	97.9 ± 11.5	99.5 ± 15.9	98.9 ± 16.6	99.2 ± 15.5
HC (cm)	113 ± 15.2	111 ± 13.8	111 ± 15.7	112 ± 9.08	112 ± 9.41	111 ± 7.61	115 ± 13.1	115 ± 12.6	116 ± 13.6
SBP (mmHg)	124 ± 14	123 ± 16	127 ± 17	128 ± 16	126 ± 16	131 ± 19	126 ± 17	127 ± 14	131 ± 14
DBP (mmHg)	81 ± 10	78 ± 8	79 ± 9	85 ± 15	85 ± 16	85 ± 14	83 ± 10	82 ± 7	85 ± 12

All values are mean ± SD (control: n=18; ADD: n=16; SUB: n=18). There were no significant differences between or within groups. ADD=consumed pecans as part of a free-living diet; BW=body weight; BMI=body mass index; DBP=Diastolic Blood Pressure; HC=hip circumference; SUB=substituted pecans for isocaloric foods from their habitual diet; SBP=Systolic Blood Pressure; WC=waist circumference.

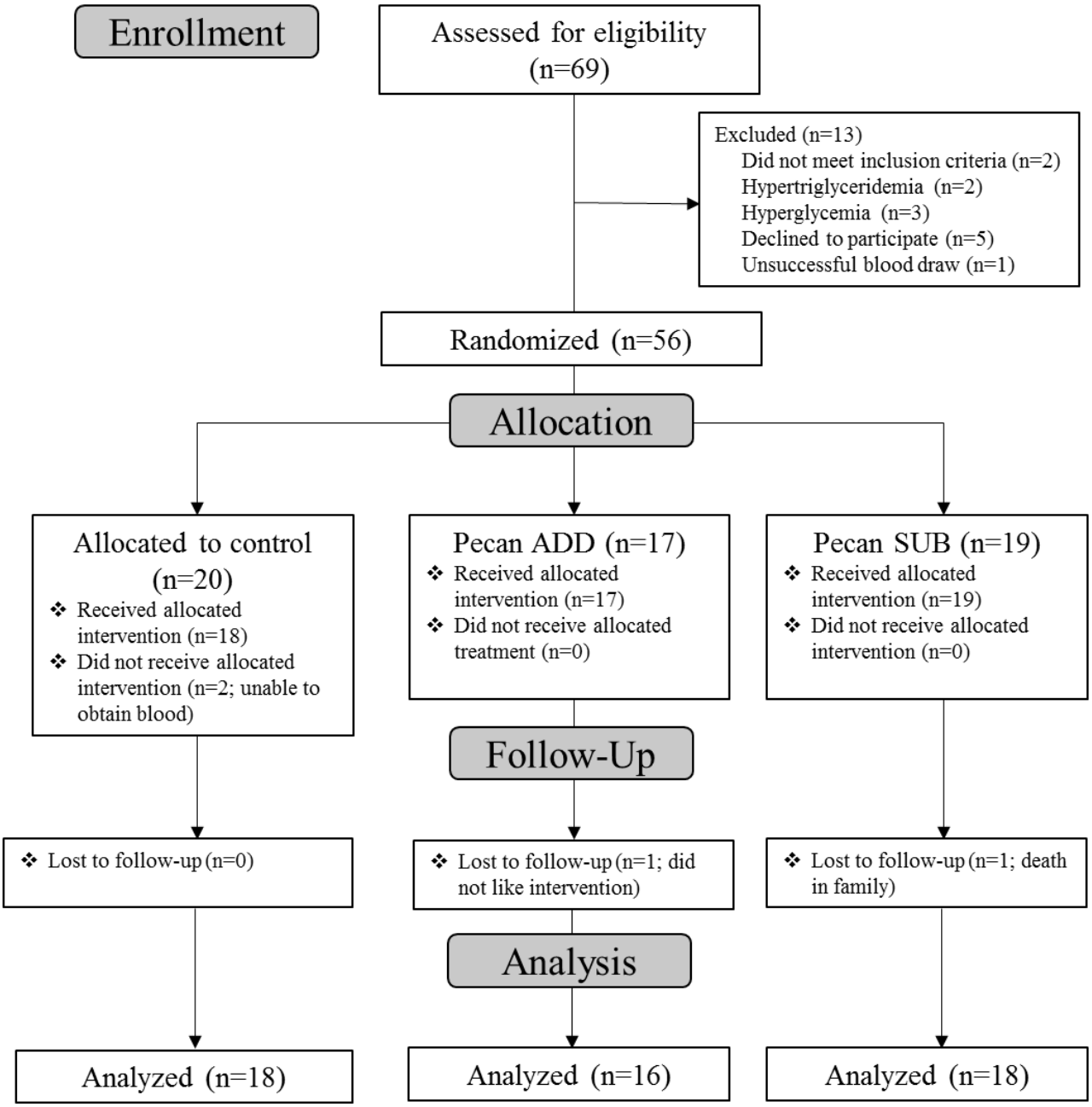

Figure 3.1 Consolidating Standards of Reporting (CONSORT) flow diagram selection of participants.

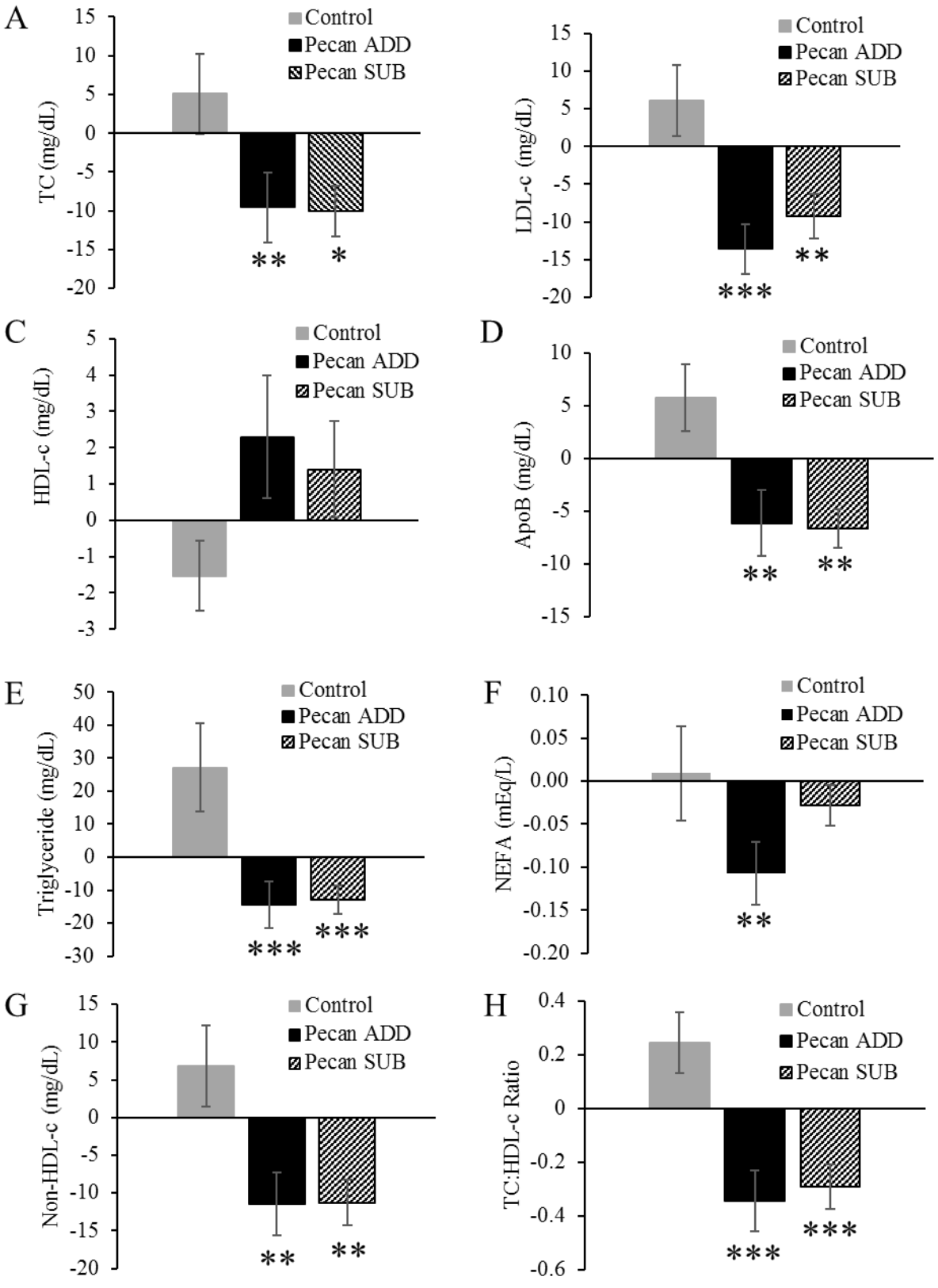
A
TC (mg/dL)
Control
Pecan ADD
Pecan SUB
**
*
B
LDL-c (mg/dL)
Control
Pecan ADD
Pecan SUB
***
**
C
HDL-c (mg/dL)
Control
Pecan ADD
Pecan SUB
D
ApoB (mg/dL)
Control
Pecan ADD
Pecan SUB
**
**
E
Triglyceride (mg/dL)
Control
Pecan ADD
Pecan SUB
***
***
F
NEFA (mEq/L)
Control
Pecan ADD
Pecan SUB
**
G
Non-HDL-c (mg/dL)
Control
Pecan ADD
Pecan SUB
**
**
H
TC:HDL-c Ratio
Control
Pecan ADD
Pecan SUB
***
***

Figure 3.2 Change in serum fasting (A) total cholesterol (TC), (B) low-density lipoprotein cholesterol (LDL-c), (C) high-density lipoprotein cholesterol (HDL-c), (D) apolipoprotein-B (apoB), (E) triglycerides (TG), (F) non-esterified fatty acids (NEFA), (G) non-HDL-c, and (H) TC:HDL-c ratio from pre to post-pecan-enriched diets or nut-free diets in adults at-risk for cardiovascular disease (control: n=18; ADD: n=16; SUB: n=18). Asterisks denote level of statistically significant difference versus the control group (* for $p \leq 0.05$, **for $p \leq 0.01$, and *** for $p \leq 0.001$). All values are presented as mean ± SEM. Pecan ADD=consumed pecans as part of a free-living diet; Pecan SUB=substituted pecans for isocaloric foods from their habitual diet.

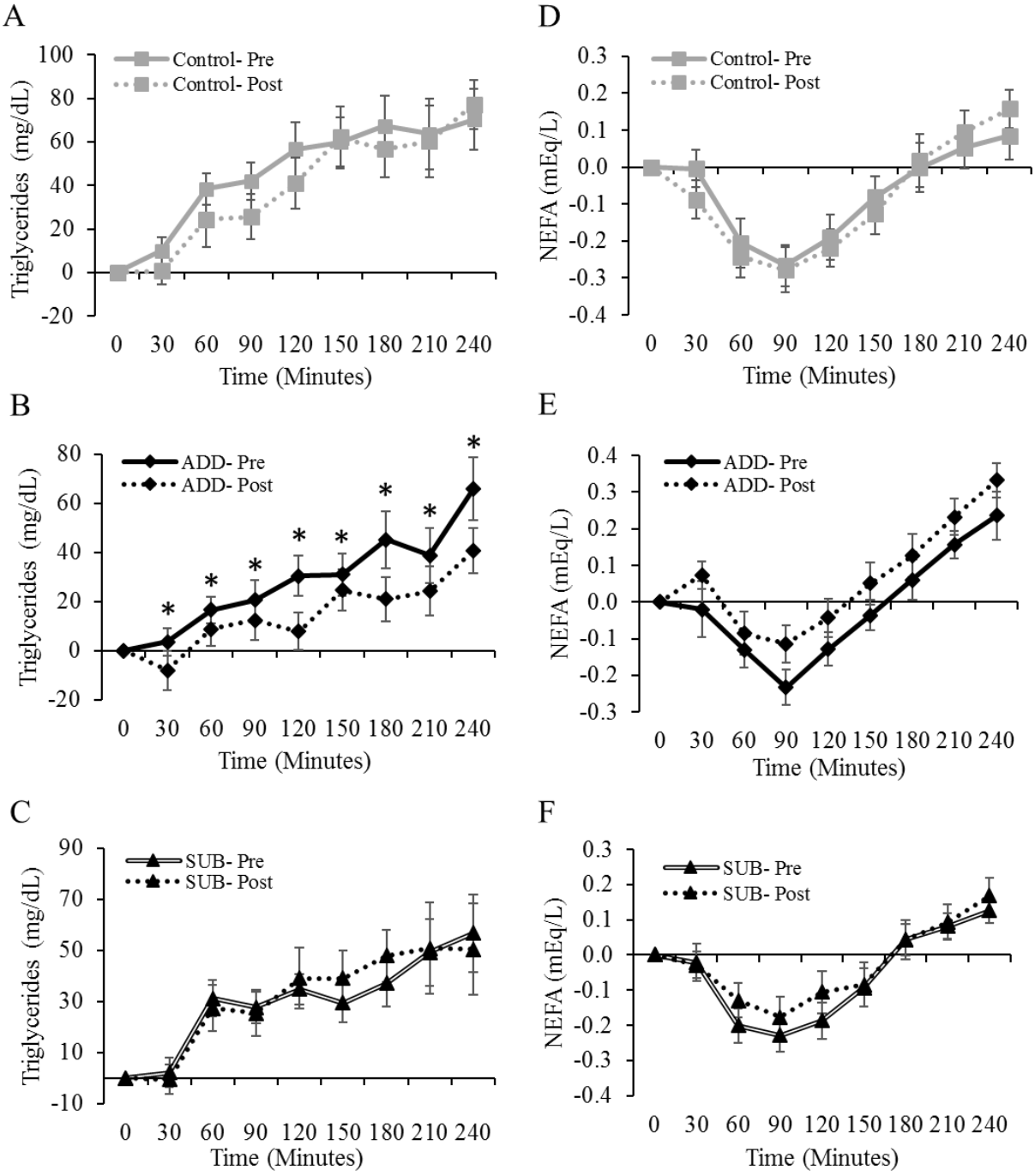
A
Control- Pre
Control- Post
Triglycerides (mg/dL)
Time (Minutes)
D
NEFA (mEq/L)
B
ADD- Pre
ADD- Post
E
C
SUB- Pre
SUB- Post
F

Figure 3.3 Time course for change in plasma triglycerides (TG) (A-C) and non-esterified fatty acids (NEFA) (D-F) for each group before and after pecan-enriched diets or nut-free diets in adults at-risk for cardiovascular disease (control: n=16; ADD: n=15; SUB: n=16). Subjects consumed a high-fat breakfast meal immediately after time 0. * Indicates a significant difference between the pre- and post-intervention meal responses within a group ($p \leq 0.05$). All values are presented as mean ± SEM. ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet.

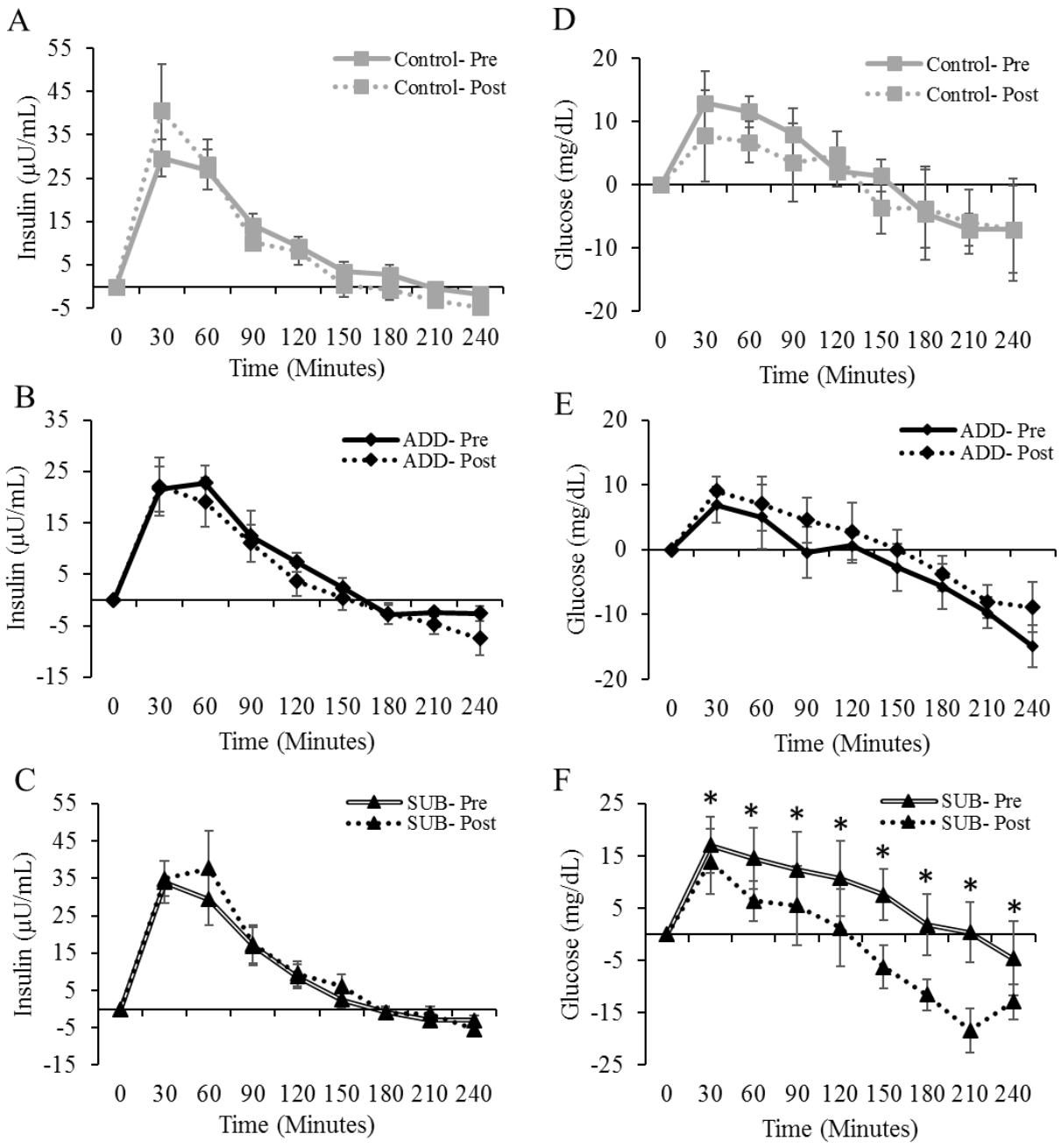
A
Insulin (µU/mL)
55
45
35
25
15
5
-5
Control- Pre
Control- Post
0 30 60 90 120 150 180 210 240
Time (Minutes)
D
Glucose (mg/dL)
20
10
0
-10
-20
Control- Pre
Control- Post
0 30 60 90 120 150 180 210 240
Time (Minutes)
B
Insulin (µU/mL)
35
25
15
5
-5
-15
ADD- Pre
ADD- Post
0 30 60 90 120 150 180 210 240
Time (Minutes)
E
Glucose (mg/dL)
20
10
0
-10
-20
ADD- Pre
ADD- Post
0 30 60 90 120 150 180 210 240
Time (Minutes)
C
Insulin (µU/mL)
55
45
35
25
15
5
-5
-15
SUB- Pre
SUB- Post
0 30 60 90 120 150 180 210 240
Time (Minutes)
F
Glucose (mg/dL)
25
15
5
-5
-15
-25
*
SUB- Pre
SUB- Post
0 30 60 90 120 150 180 210 240
Time (Minutes)

Figure 3.4 Time course for change in plasma insulin (A-C) and glucose (D-F) for each group before and after pecan-enriched diets or nut-free diets in adults at-risk for cardiovascular disease (control: n=16; ADD: n=15; SUB: n=16). Subjects consumed a high-fat breakfast meal immediately after time 0. * Indicates a significant difference between the pre- and post-intervention meal responses within a group ($p \leq 0.01$). All values are presented as mean ± SEM. ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet.

CHAPTER 4

ANGIOPOIETIN-LIKE PROTEIN RESPONSES TO PECAN-ENRICHED DITS VERSUS A NUT EXCLUDED DIET: A RANDOMIZED, CONTROLLED TRIAL[2]

[2]Guarneiri, L.L., C.M. Paton, and J.A. Cooper. Submitted to *J Med Food*, 03/04/2022.

Abstract

Background: Daily pecan consumption improves fasting and postprandial triglycerides, but its effect on angiopoietin-like proteins (ANGPTLs) is unknown. The objective of this study was to investigate the impact of daily pecan consumption for 8 weeks on fasting and postprandial ANGPTL3, -8, and -4. **Methods:** This was an 8-week, randomized, controlled trial with three treatments: two pecan groups and a nut-free control group (n=16). The ADD group (n=15) consumed pecans (68g) as part of a free-living diet, and the SUB group (n=16) substituted the pecans (68g) for isocaloric foods from their habitual diet. Fifty-six participants were randomized but nine participants did not start or complete the intervention and/or testing visits. At pre- and post-intervention, a high saturated fat meal was consumed with 3.5h postprandial blood draws to determine changes in ANGPTL3, -8, and -4. **Results:** There was a significant suppression in postprandial ANGPTL3 from pre- to post-intervention within ADD and SUB (p=0.004 and p=0.002, respectively) but not control (ns). There were no other changes within or between groups for fasting and postprandial outcomes. **Conclusion:** Daily pecan consumption improved postprandial ANGPTL3, which may mediate improvements in lipid metabolism.

Introduction

According to the World Health Organization, more than half of adults have overweight or obesity globally [1]. Increased adiposity and dyslipidemia are associated with greater risk for cardiovascular disease (CVD) [2], and elevated postprandial triglycerides (TGs) are also an independent risk factor for CVD [3]. Since TGs take 6-12h to clear from circulation, individuals that consume 3-5 meals/day are estimated to maintain elevated postprandial TGs for two-thirds of the day [4]. As a result, it is crucial to identify methods to mitigate the detrimental effects of the postprandial period. One plausible method is to incorporate foods rich in unsaturated fatty acids (UFA) and polyphenols into the daily diet. Previous research has shown that consumption of acute meals and/or diets rich in these nutrients such as tree nuts, tart cherries, and omega-3 fatty acid supplements suppress postprandial TGs [5-9].

As research reveals the beneficial effects of foods rich in UFAs and polyphenols, it is important to elucidate the underlying mechanisms. Angiopoietin-like proteins (ANGPTL)-3, -4, and -8 are a family of proteins that regulate lipid metabolism [10]. In the postprandial state, ANGPTL3 and -8 work synergistically to inhibit lipoprotein lipase (LPL) in oxidative tissues such as the heart and muscles [11]. This action reduces the uptake of TG-rich very-low density lipoproteins to increase TG circulation and promote energy storage in the white adipose tissue [12]. Furthermore, ANGPTL4 contributes to the reduction of LPL in adipose tissue during the fasted state [13]. Altogether, the suppression of these proteins promotes the clearance of TG from circulation, resulting in CVD-risk reduction [10].

Pecans are a rich source of UFAs and antioxidants [14, 15]. Our previous study demonstrated that acute consumption of a breakfast meal containing pecans suppressed postprandial TG and ANGPTL3 more than the control meal in healthy men [6, 16]. Furthermore,

we recently showed that postprandial TGs were suppressed following the daily addition of pecans to the free-living diet (ADD) in adults at-risk for CVD [7]. In the group that isocalorically substituted pecans for habitual foods in the diet (SUB), postprandial glucose, but not TGs, were suppressed. Finally, fasting TGs were reduced in both ADD and SUB but increased in the no-nut group (control). It remains to be elucidated whether those changes in TGs and/or glucose were mediated by, or at least associated with, the ANGPTL family. Therefore, our objective was to investigate the impact of daily pecan consumption for 8 weeks on fasting and postprandial ANGPTL3, -4, and -8 in the ADD, SUB, and control groups. We hypothesized that postprandial ANGPTL3 and ANGPTL8 would be suppressed in ADD, but not SUB or control, based on previously observed reductions in postprandial TGs [7]. We also hypothesized that there would be reductions in fasting ANGPTL4 in ADD and SUB, and that the fasting changes within the pecan groups would be significantly different from control.

Methods

Study Design

This study was a single-blind, randomized, parallel controlled trial (clinicaltrials.gov: NCT04376632) involving an 8-week intervention conducted in the Human Nutrition Lab. Data collection occurred from August 2018 to December 2020, when the goal of 18 participants/group was obtained. There was a screening visit and 2 testing visits (pre- and post-diet intervention). Subjects were randomly assigned to one of three groups: a “no nut” control group, or one of two pecan groups (ADD or SUB). Subjects in ADD and SUB each consumed 68g of pecans per day for 8 weeks; however, dietary instructions for the incorporation of pecans into the diet differed. For ADD, pecans were consumed as part of a free-living diet, while in SUB, participants received counseling at the pre-intervention visit on how to substitute

pecans for isocaloric foods from their habitual diet. This study was approved by the Institutional Review Board for human subjects, and informed written consent was obtained from each participant prior to testing.

Participants

Sixty-nine sedentary men and women between the ages of 30 and 75y with high cholesterol or a body mass index (BMI) of ≥28kg/m^2 were assessed for eligibility. Inclusion based on high cholesterol levels has been described previously [7]. Exclusion criteria included habitual nut consumption (>2 servings/week), nut allergies, special diets (i.e. ketogenic diet, intermittent fasting), excessive alcohol use (>3 drinks/d for men or >2 drinks/d for women), tobacco or nicotine use, exercise >3h/week, weight loss or gain >5% of body weight in the past 3 months, plans to begin a weight loss or exercise regimen, history of medical events or medication use affecting digestion, absorption, or metabolism, gastrointestinal surgery, and chronic or metabolic diseases. Individuals taking lipid-lowering medications, fish oil supplements, steroid/hormone therapy, or medications for diabetes mellitus or Attention Deficit Hyperactivity Disorder were also excluded. Finally, individuals with the following biomarkers were excluded: fasting glucose >126mg/dL, fasting TGs >350mg/dL, or blood pressure >180/120mmHg. Eligibility based on blood lipids and glucose were determined from fasting blood samples at the screening visit.

Protocol

Screening Visit

Individuals arrived following an 8-12h overnight fast and 24h without exercise or alcohol. A fasting blood draw for a lipid panel and glucose measurement was obtained, and anthropometrics including height, weight, and blood pressure were measured. Resting metabolic

rate (RMR) (kcal/d) was measured for 30min using indirect calorimetry (TrueOne 2400, Parvo Medics, Sandy, Utah, USA) under standard conditions [17]. The final 20min of respiratory gases were used to calculate RMR using the Weir Equation [18]. Participants' RMR was multiplied by an average U.S. activity factor of 1.65 to estimate daily energy needs [19], which was used for calculating the high-saturated fatty acid (SFA) test meal. In addition, alcohol consumption habits were assessed by the Alcohol Use Disorders Identification Test [20]. If individuals qualified for the study, subjects were randomized to one of the three treatment groups by a researcher who was not involved in data collection or analysis. An allocation ratio of 1:1:1, a permuted block design (balanced for age, sex, and BMI), and a random number generator was used to randomize participants.

Pre-Diet Intervention Visit (V1)

The night before V1, participants consumed a lead-in dinner meal and snack (provided by research personnel) that contained 50% of total energy from carbohydrate, 15% of energy from protein, and 35% of energy from fat. The morning of V1, participants arrived following an 8-12h overnight fast and 24h without exercise or alcohol. Height, weight, and blood pressure were measured. Next, an intravenous catheter was inserted into the antecubital vein for the fasting blood draw, and the line was kept patent with saline. Participants then consumed a SFA-rich breakfast shake within 10min. This high-fat meal provided 17% of total daily energy needs based on the RMR measurements from the screening visit and was made from an original milk chocolate ready-to-drink shake (Ensure, Abbott Nutrition, Abbott Laboratories, Inc., Columbus, Ohio, USA), unsalted butter, red palm oil, coconut oil, soy lecithin granules, and powdered chocolate drink mix. The nutrient breakdown for the test meal is provided in **Table 4.1** Four ounces of water was used to rinse out the container and then ingested to ensure the entire liquid

meal was consumed. Following the SFA meal challenge, blood draws occurred at 30, 90, 150, and 210min postprandially. Four ounces of water was provided at fasting and once/h postprandially.

Dietary Intervention

The day after V1, all participants began the 8-week intervention. Written diet instructions were provided to all participants. Those in the control group were instructed to avoid all forms of nuts and to consume ≤2 servings of nut butter per week. Participants in ADD were provided with 68g (~0.5 cup or 2.25 ounces) portions of pecans to consume as part of their free-living diet with no additional diet instructions. Participants in SUB were instructed to substitute the 4,966 kilojoules (470 kilocalories) provided by the 68g of pecans for foods habitually consumed in their free-living diet. Research personnel guided the participants on how to make appropriate energy substitutions based on foods habitually consumed in their diet. Pecans were provided to participants in ADD and SUB in pre-portioned containers. Similar to the control group, the pecan groups were instructed to avoid all other nuts and to consume ≤2 servings of nut butter per week. In addition, they were instructed to eat the pecans in their raw form (no roasting, cooking, or baking). Subjects were permitted to add the pecans to other foods such as oatmeal, cereal, or salad. Likewise, all subjects were instructed to avoid consuming >3 alcoholic drinks/d (men) or >2/d (women) and were asked not to make any other changes to their diet or activity levels. Participants were unaware of the diet instructions that are provided to other treatment groups.

Weekly Responsibilities

Subjects in ADD and SUB completed a daily nut compliance log that detailed the time of day for pecan consumption. Nut compliance logs were submitted to research staff once per week.

Poor compliance was considered to be consumption of <75% of pecans throughout the 8-week intervention.

Post-Diet Intervention Visit (V2)

After 8 weeks of the diet intervention, participants reported for V2 under the same pre-visit conditions as V1. Participants completed the exact same study procedures and measurements that took place at V1, including the SFA meal challenge.

Sample Analysis

All fasting and postprandial blood samples were drawn into an EDTA vacutainer, immediately placed on ice, and then centrifuged at 3000rpm for 15min at 4°C. The plasma was aliquoted and stored at -80°C until analysis. ANGPTL3, -4, and -8 were all primary outcomes. ANGPTL3 and ANGPTL4 levels were measured using human ANGPTL3 and ANGPTL4 DuoSet enzyme linked immunoassay kits (R&D Systems, Inc. Minneapolis, MN). ANGPTL8 levels were measured using Betatrophin (139-198) enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA).

Statistical Analyses

SAS version 9.2 statistical package (SAS Institute Inc, Cary, NC, USA) was used for all statistical analyses. All values were reported as mean±SD unless otherwise noted. Statistical significance was set at $p \leq 0.05$. A samples size of 54 (18/group) was estimated to detect a large effect size for fasting ANGPTL4 using G*power 3.19.7 assuming at least 80% power and an α of 0.05. For the time course data, change from baseline was calculated (baseline value subtracted from each postprandial time point), then a repeated measures linear mixed model for treatment, visit, and time was used to test for within group differences. In addition, a repeated measures linear mixed model for treatment and visit was used to test for within group differences in

anthropometrics and fasting ANGPTLs. Finally, incremental area under the curve (iAUC) was calculated using the trapezoidal method, and the change in iAUC from pre- to post-intervention was calculated for between group comparisons using a one-way ANOVA. Post hoc analyses were done using least-squares mean. Finally, an unpaired t-test was used to assess differences in nut compliance between the two pecan groups.

Results

Participants

Fifty-six individuals were allocated to an intervention group, but nine participants did not start or complete the intervention and/or testing visits **(Figure 4.1)**. Therefore, forty-seven subjects (n=10 women and n=6 men for control, n=11 women and n=4 men for ADD, and n=12 women and n=4 men for SUB) completed the study and were included in the final per protocol analyses. Participant characteristics at the pre-intervention visit are presented in **Table 4.2**. There were no differences between groups at baseline for anthropometric or ANGPTL outcomes. On average, participants in both the ADD and SUB groups consumed 95% of pecans provided, respectively, and compliance was not different between groups. No participant reported poor compliance (defined as <75% of pecans consumed throughout intervention).

ANGPTL Results

Fasting: Fasting ANGPTL3, -8, and -4 values are presented in **Table 4.3.** There were no changes in ANGPTL3, -8, or -4 from pre- to post-intervention for any group. There were also no differences between groups for fasting values.

Postprandial: The time course for the meal responses for ANGPTL3, -8, and -4 are presented in **Figures 4.2 and 4.3**. There was a significant suppression of postprandial ANGPTL3 from pre- to post-intervention within ADD and SUB (p=0.004 and p=0.002, respectively) but not

control (ns) **(Figures 4.2A-C)**. There were no differences within groups for the ANGPTL8 or ANGPTL4 meal responses **(Figures 4.2D-I).** Likewise, the change in meal response from pre- to post-intervention was not different between groups for ANGPTL3 (change in iAUC: ADD: -85±210 vs SUB: -93±160 vs control: 4±192ng/mL/3.5h; p=0.29), ANGPTL8 (ADD: 1.5±4.0 vs SUB: 0.5±3.6 vs control: 0.9±3.4ng/mL/3.5h; p=0.77), or ANGTPL4 (ADD: 11±158 vs SUB: 10±89 vs control: 22±167ng/mL/3.5h; p=0.95) **(Figure 4.3).**

Discussion

To our knowledge, this is the first study to investigate the effect of chronic pecan consumption on fasting or postprandial ANGPTLs. We have shown that the incorporation of pecans in the diet, regardless of dietary instructions, suppresses postprandial ANGPTL3 in adults at-risk for CVD. Although ANGPTL3 and ANGPTL8 can work synergistically to inhibit LPL during the fed state, ANGPTL3 is able to inhibit LPL on its own [11]. Therefore, the previously observed suppression of postprandial TG in ADD was likely mediated by the observed suppression of ANGPTL3 [7]. This finding also supports previous research on the attenuation of postprandial TG and ANGPTL3 in men following acute pecan consumption [16, 21]. The results from the present study are novel since the test meal did not contain pecans, indicating that habitual pecan consumption provides a protective effect against an occasional meal that is high in SFA.

It was initially surprising that we also observed the suppression of postprandial ANGPTL3 in SUB since we previously reported no change in postprandial TGs within this group [7]. However, recent literature suggests a positive association between ANGPTL3 and insulin resistance [22]. In our previously reported data, postprandial glucose, but not insulin, was suppressed in SUB, suggesting improved insulin sensitivity [7]. Therefore, it is possible that the

suppression of postprandial glucose in SUB was mediated by the decrease in ANGPTL3. Altogether, these findings are clinically meaningful since elevated postprandial TGs and glucose are independent risk factors for CVD [23-25] and many adults spend two-thirds of the day in the fed state [4].

Our postprandial ANGPTL3 findings are in accordance with a previous study by Kavaini et al. in 2019 [8] who reported suppression of postprandial ANGPTL3, ANGPTL8, and TG following a SFA-rich meal after 7 days on a high polyunsaturated fatty acid (PUFA) diet (containing walnuts) in healthy women. Contrary to our hypotheses, we did not observe any changes in fasting or postprandial ANGPTL8 or ANGPTL4 in the present study. This is different than the 2019 study by Kaviani as well as a 2021 study by Kaviani et al [8, 26] that reported improvements in at least two of the three fasting outcomes (ANGPTL3, ANGPTL4, or ANGPTL8) following diets rich in different fatty acids and postprandial ANGPTL8 suppression after the high PUFA diet [8]. There are several differences between the two studies by Kaviani et al. [8, 26] and ours that may explain the divergent results. The two previous studies were 5-7d controlled feeding trials [8, 26], while participants in the current study consumed pecans as part of a free-living diet. Therefore, the shorter durations, highly controlled diets, healthier populations, or fatty acid composition of the diets in the previous studies by Kaviani et al. may have produced the suppression of ANGPTLs that were not observed in the present study.

Since this study was not designed to be mechanistic, we can only speculate on what bioactive(s) in pecans resulted in suppressed postprandial ANGPTL3. Pecans contain more flavonoids than any other tree nut [14], and flavonoids are potent inhibitors of oxidative stress [27, 28] and regulators of lipid metabolism [5, 28, 29]. Previous research demonstrated that oxidative stress modulates the liver X receptor (LXR) [30], which regulates the transcription of

ANGPTL3 in the liver [31]. Furthermore, there is evidence that polyphenolic compounds decrease the expression of LXR and hepatic ANGPTL3 mRNA and protein while upregulating the expression of adipose tissue LPL mRNA to reduce TG circulation [32-34]. Based on this evidence, we theorize that oxidative stress modulates LXR to increase the expression of ANGPTL3 mRNA, and that phenolic compounds inhibit this pathway by decreasing oxidative stress. We recently reported that there was decreased postprandial lipid peroxidation and increased postprandial total antioxidant capacity in ADD and SUB [35], which could explain the observed suppression of ANGPTL3 in these groups. In addition, we know that fatty acid composition affects the regulation of lipid metabolism [36], so it was not surprising that oils of varying fatty acid composition were shown to impact ANGPTLs differently [26]. Therefore, it is also possible that the UFAs in pecans may have contributed to our observed findings. Unsaturated fats, especially PUFAs, also downregulates LXR [37, 38]. Altogether, it is possible that the flavonoid or UFA content of the pecans worked alone or synergistically to downregulate ANGPTL3 expression via LXR.

Previous investigations of the impact of diet on ANGPTLs have observed that sex acts as a biological variate [8, 16]. Therefore, a limitation of this study is that it was not designed or powered to detect differences between sexes. In addition, since restrictions were not placed on the participants' free-living diets, it is possible that the improvement in postprandial ANGPTL3 could be the result of another nutrient in the diet. This is unlikely, however, since we did not observe any changes in ANGPTL3 within the control group. Additionally, the free-living diet of this study also strengthens the generalizability applicability of our findings. Finally, this study employed a relatively high dose of pecans for a short duration, thus we can only speculate on the effectiveness of smaller dose of pecans over different durations.

In conclusion, daily pecan (68g) consumption suppressed postprandial ANGPTL3 in adults at-risk for CVD. The suppression of ANGPTL3 likely mediated the previously observed suppression of postprandial TG and glucose in the ADD and SUB groups, respectively [7]. These findings contribute to the small but growing body of literature on the ANGPTL family of proteins. Future research should further investigate the impact of other tree nuts on ANGPTLs, with a special focus on sex as a biological variate. Finally, since elevated postprandial TGs and glucose are independent risk factors for CVD [23-25], these results provide evidence that simply incorporating pecans into the daily diet can provide a benefit to cardiovascular health.

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Table 4.1 Nutrient breakdown for the high saturated fat meal challenge

Composition	SFA-rich meal
Percentage of total energy from	
Protein	5.0
Carbohydrate	25.0
Fat	69.5
Percentage of energy from fatty acids	
SFA	46.9
MUFA	15.7
PUFA	6.9

Abbreviations: HF=high fat, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid, SFA=saturated fatty acid

Table 4.2 Participant characteristics at the pre-intervention visit

	ADD (n=15)	SUB (n=16)	Control (n=16)
Age (y)	50 ± 11	46 ± 11	49 ± 14
Height (cm)	166.9 ± 7.6	166.4 ± 10.6	168.1 ± 11.7
Weight (kg)	84.6 ± 14.0	92.6 ± 20.2	91.9 ± 27.7
Body Mass Index (kg/m^2)	30.3 ± 4.2	33.4 ± 6.7	31.9 ± 6.8
SBP (mmHg)	127 ± 17	128 ± 18	123 ± 13
DBP (mmHg)	85 ± 15	83 ± 11	80 ± 10
ANGPTL3 (ng/mL)	474 ± 106	485 ± 202	530 ± 265
ANGPTL8 (ng/mL)	8.1 ± 2.9	7.7 ± 1.9	8.3 ± 2.9
ANGPTL4 (ng/mL)	258 ± 200	253 ± 165	258 ± 190

All values are mean ± SD. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet; SBP=systolic blood pressure; DBP=diastolic blood pressure; ANGPTL=angiopoietin-like protein.

Table 4.3 Fasting ANGPTL

	ADD (n=15)		SUB (n=16)		Control (n=16)	
	Pre-Intervention	Post-Intervention	Pre-Intervention	Post-Intervention	Pre-Intervention	Post-Intervention
ANGPTL3 (ng/mL)	474 ± 106	465 ± 112	485 ± 202	515 ± 204	530 ± 265	534 ± 266
ANGPTL8 (ng/mL)	8.1 ± 2.9	7.8 ± 3.2	7.7 ± 1.9	7.8 ± 2.9	8.3 ± 2.9	9.1 ± 3.5
ANGPTL4 (ng/mL)	258 ± 200	239 ± 178	253 ± 165	258 ± 159	258 ± 190	258 ± 227

All values are mean ± SD. There were no significant differences within or between groups. ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet; ANGPTL=angiopoietin-like protein.

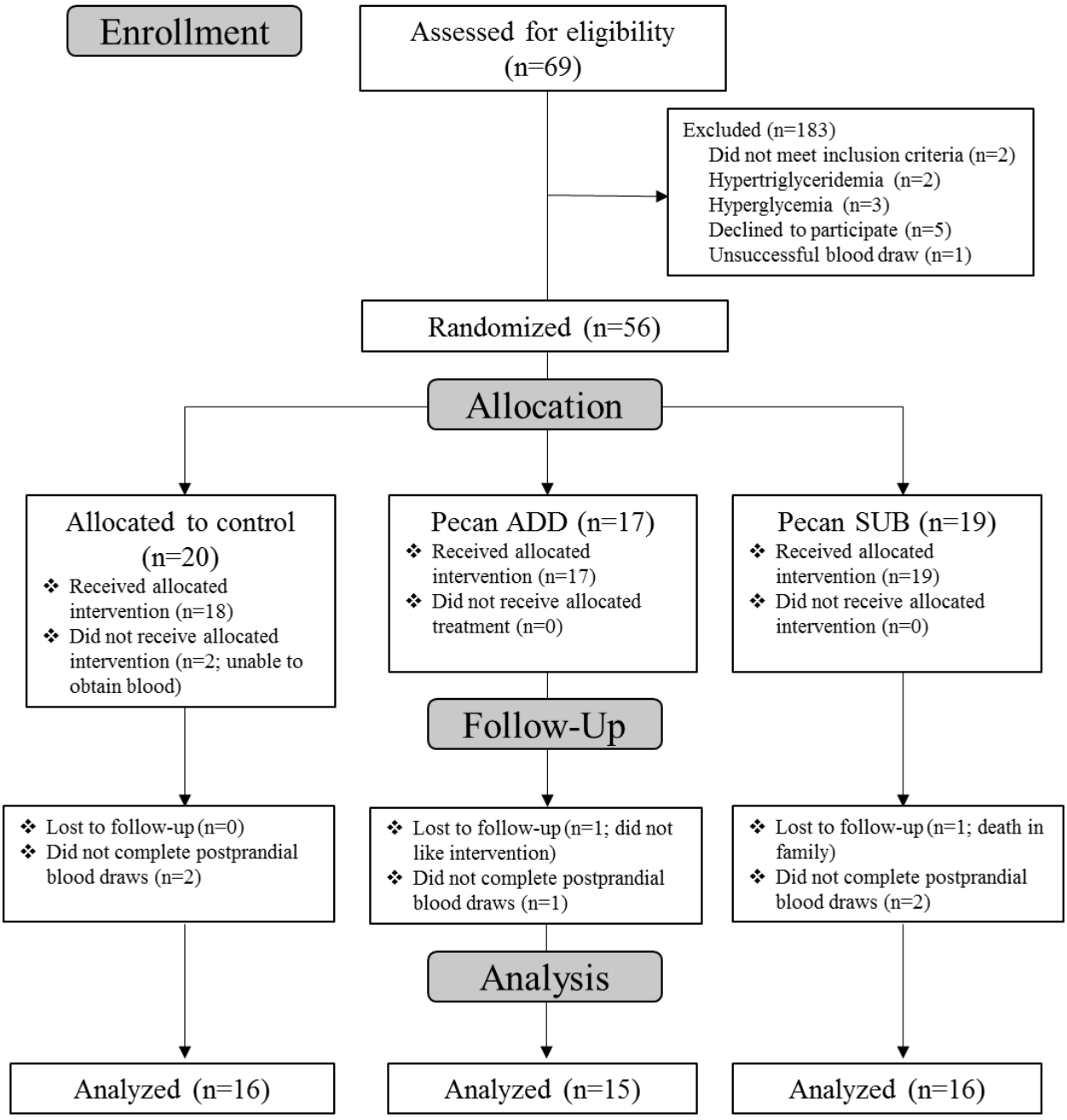

Figure 4.1 Consolidating Standards of Reporting (CONSORT) flow diagram for the selection of participants.

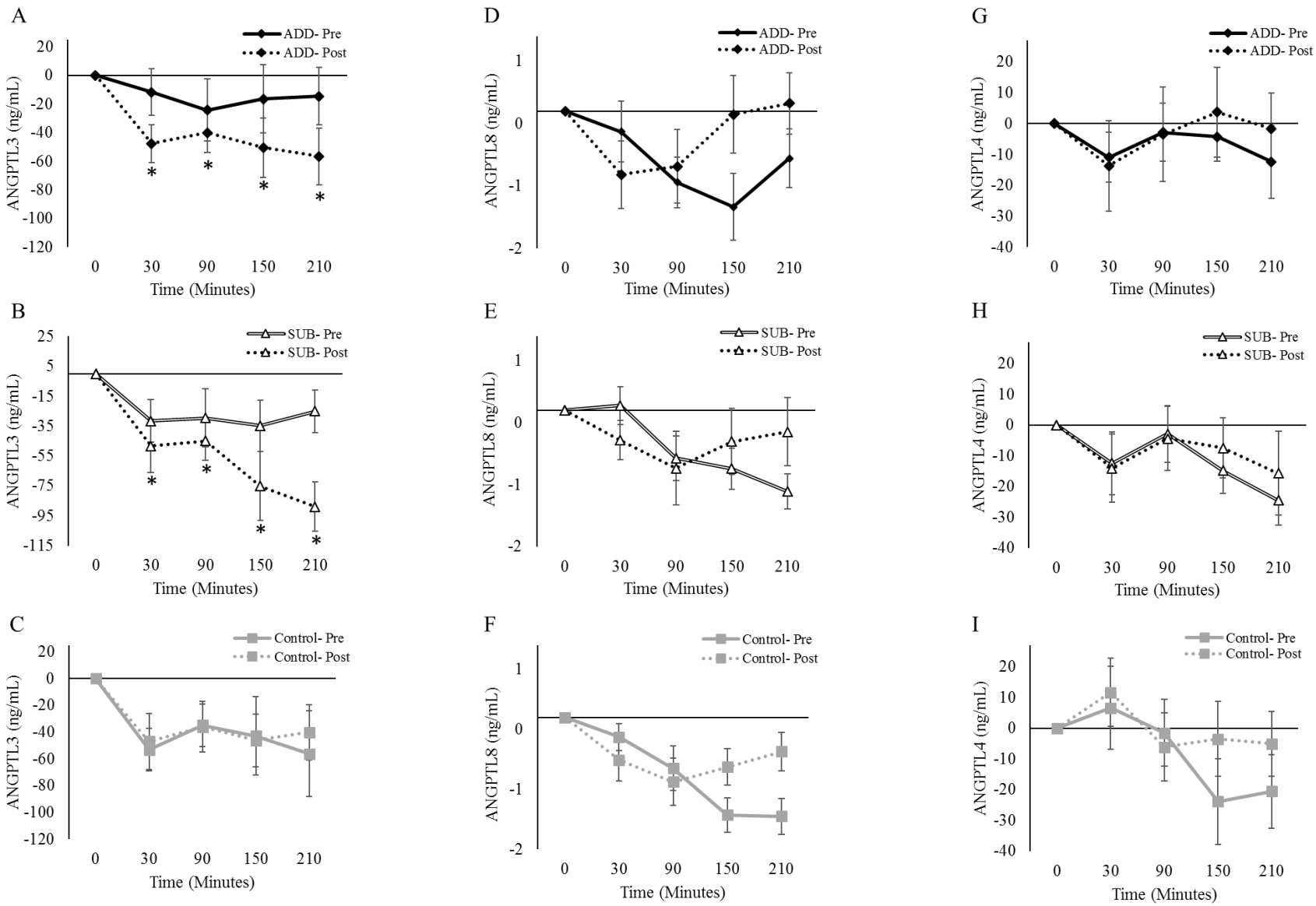
A
ADD- Pre
ADD- Post
ANGPTL3 (ng/mL)
20
0
-20
-40
-60
-80
-100
-120
0
30
90
150
210
Time (Minutes)
*
B
SUB- Pre
SUB- Post
ANGPTL3 (ng/mL)
25
5
-15
-35
-55
-75
-95
-115
C
Control- Pre
Control- Post
D
ANGPTL8 (ng/mL)
1
0
-1
-2
E
F
G
ANGPTL4 (ng/mL)
40
30
20
10
H
I

Figure 4.2 Time course for the change in angiopoietin-like protein (ANGPTL)-3, -8, and -4 meal response in the ADD, SUB, and control groups at pre- and post-intervention (Figures 4.2A-C, D-F, and G-I, respectively). There was a significant suppression of ANGPTL3 from pre- to post-intervention within the ADD and SUB groups ($p=0.004$ and $p=0.002$, respectively). There were no other differences between or within groups for the ANGPTL meal responses. All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet.

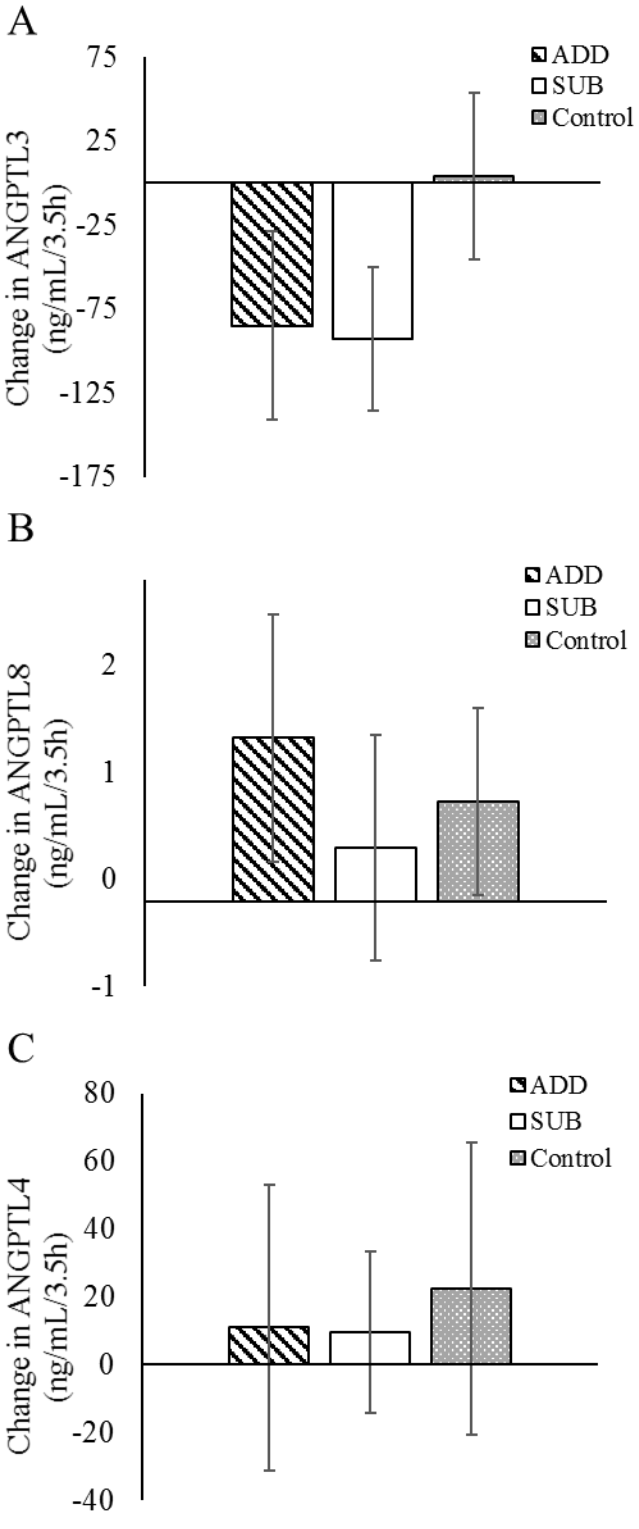
A
75
25
-25
-75
-125
-175
Change in ANGPTL3 (ng/mL/3.5h)
ADD
SUB
Control
B
2
1
0
-1
Change in ANGPTL8 (ng/mL/3.5h)
ADD
SUB
Control
C
80
60
40
20
0
-20
-40
Change in ANGPTL4 (ng/mL/3.5h)
ADD
SUB
Control

Figure 4.3 The change in incremental Area Under the Curve (iAUC) for angiopoietin-like protein (ANGPTL)-3, -8, and -4 from pre- to post-intervention in the ADD, SUB, and control groups (Figures 4.3A-C, respectively). There were no differences between groups. All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet.

CHAPTER 5

APPETITE RESPONSES TO PECAN-ENRICHED DIETS[3]

[3]Guarneiri, L.L., C.M. Paton, and J.A. Cooper. *Appetite*, 2022. 173: 106003. Reprinted here with permission of the publisher.

Abstract

Research suggests that tree nuts improve satiety during an acute meal, but the effects of daily consumption are less clear. The purpose of this study was to examine the impact of daily pecan consumption on markers of appetite in adults at-risk for cardiovascular disease (CVD). This was an 8-week, randomized, controlled trial with three treatments: two pecan groups and a nut-free control group (n=16). The ADD group (n=15) consumed pecans (68g) as part of a free-living diet, and the SUB group (n=16) substituted the pecans (68g) for isocaloric foods from their diet. At pre- and post-intervention, a high-fat meal was consumed with 3.5h postprandial blood draws and visual appetite scales (VAS) to determine changes in cholecystokinin (CCK), peptide YY (PYY), ghrelin, and subjective appetite. Participants also completed VAS questionnaires once/h for the next 5h and recorded dietary intake. Although no differences between groups ($p>0.05$), there was an increase in postprandial CCK and PYY and suppression of postprandial ghrelin within ADD ($p<0.05$) from pre- to post-intervention. Across the entire day, the decreases in prospective consumption and desire to eat were greater in ADD vs SUB (-79±41 vs 11±26mm/9h; $p=0.05$) and ADD vs control (-64±39 vs 23±29mm/9h; $p=0.05$), respectively. There was also a non-significant tendency for a greater decrease in overall appetite in ADD vs control (-67±46 vs 20±27mm/9h; $p=0.06$). Within ADD, overall appetite, prospective consumption, and desire to eat decreased, and fullness increased from pre- to post-intervention ($p<0.05$ for all). There were no changes in self-reported energy intake on test days or other changes within or between groups. In conclusion, adding pecans to the daily diet improves subjective and physiological markers of postprandial appetite in adults that are at-risk for CVD.

Introduction

According to the World Health Organization, the prevalence of global obesity has nearly tripled since 1975 [1]. Since obesity is associated with increased risk for chronic diseases [2], it is essential to identify practices to improve weight management. One promising avenue is to enhance appetite regulation [3]. Tree nuts are associated with weight maintenance despite their high energy density [4, 5], an effect that may be partially mediated through improvements in appetite regulation [6]. Previous research indicates that tree nuts have low bioavailability due to their hard structure and high fiber content. In fact, the metabolizable energy of tree nuts is 16-32% lower than predicted by Atwater factors [7-9]. Furthermore, consumption of tree nuts requires substantial oral processing, which generates satiety signals [6]. Finally, it is possible that certain components of a given tree nut, such as the dietary fatty acid composition, may play a role on appetite responses following ingestion [10-12] Therefore, incorporating tree nuts into the daily diet could be a simple and cost-effective method for improving appetite control, and by extension, weight management.

There are fundamental methods for assessing appetite that encompass physiologic, subjective, or applied measurements. One method is to measure gastrointestinal hormones that are thought to regulate physiological appetite, including cholecystokinin (CCK), peptide YY (PYY), and ghrelin [13]. Ghrelin, the only known orexigenic gut hormone, is released from the stomach before a meal and is proposed to activate orexigenic neurons in the hypothalamus to promote food intake [14]. Conversely, PYY is released from the distal small intestine and proximal colon postprandially and is hypothesized to stimulate satiety by inhibiting orexigenic neurons and triggering anorexigenic neurons in the hypothalamus [15]. Finally, CCK is excreted from the proximal intestine in response to a meal, increasing satiety and reducing meal size by

suppressing orexigenic neurons [16]. The second method involves the assessment of subjective ratings of appetite, usually through visual analog scale (VAS) questionnaires. The final method is designed to be an applied measure where total energy intake (EI) and/or macronutrient intake is determined either through an ad libitum buffet meal or food records [17-19].

Despite the strong evidence associating tree nut consumption with weight maintenance [4, 5], very few studies have investigated the impact of meals or diets containing tree nuts on measures of appetite. Acute meal challenges containing black walnuts, English walnuts, almonds, and pecans increased glucagon-like peptide 1, another satiety hormone, and/or suppressed subjective appetite more than the control meals that were matched for energy and macronutrients but not the fatty acid distribution [20-23]. For longer-term studies incorporating tree nuts, results are mixed. We showed that a 7d highly-controlled PUFA-rich diet containing walnuts decreased fasting ghrelin and increased fasting and postprandial PYY, but there were no changes in VAS scores [12]. Furthermore, two 4-week studies of daily pistachio or almond intake without additional diet instructions reported did not improve subjective appetite [24, 25].

Preliminary research suggests that tree nuts improve subjective and physiological markers of appetite, especially during an acute meal, but more research on longer-term nut intake is needed. In particular, the investigation of pecans in a longer-term setting is warranted due to promising acute appetite responses to this tree nut and its favorable fatty acid profile and fiber content [22, 26]. In addition, no study has investigated whether the method or instructions for incorporation of the tree nut into the diet impacts appetite responses. Therefore, the purpose of this study was to investigate the impact of daily pecan consumption (with and without dietary/isocaloric substitution instructions) for an 8-week period on fasting and postprandial markers of appetite in adults with hypercholesterolemia or at higher risk for CVD (body mass

index (BMI) ≥28kg/m^2). We hypothesized that daily pecan consumption, regardless of the method of incorporation into the diet, would improve fasting and postprandial CCK, PYY, ghrelin, and subjective markers of appetite, and that the responses would be different than that of the control group.

Methods

Study Design

This was a single-blind, randomized, parallel controlled trial (clinicaltrials.gov: NCT04376632) involving an 8-week intervention. The protocol included a screening visit and 2 testing visits (pre- and post-intervention). Subjects were randomly assigned (balanced blocks stratified by age, sex, and BMI to one of three groups: a "no nut" control group, or one of two pecan groups (ADD or SUB). Subjects in the ADD and SUB groups each consumed 68g of pecans per day for 8 weeks; however, dietary instructions for the incorporation of pecans differed. For the ADD group, pecans were consumed as part of a free-living diet, while in the SUB group, participants received counseling at baseline on how to substitute pecans for isocaloric foods from their habitual diet. This study was approved by the Institutional Review Board for human subjects at the University of Georgia (STUDY00005985), and informed written consent was obtained from each participant prior to testing.

Participants

Sixty-nine sedentary men and women between the ages of 30 and 75y with high cholesterol or a BMI of ≥28kg/m^2 were assessed for eligibility. Inclusion criteria based on high cholesterol levels has been described previously [27]. Exclusion criteria included habitual nut consumption (>2 servings/week), nut allergies, special diets (i.e. ketogenic diet, intermittent fasting), excessive alcohol use (>3 drinks/d for men or >2 drinks/d for women), tobacco or

nicotine use, exercise >3h/week, weight change >5% of body weight in the past 3 months, history of medical events or medication use affecting digestion, absorption, or metabolism, gastrointestinal surgery, and chronic or metabolic diseases. Individuals taking lipid-lowering medications, fish oil supplements, steroid/hormone therapy, or medications for diabetes mellitus or Attention Deficit Hyperactivity Disorder were also excluded. Finally, individuals with elevated fasting glucose, triglycerides, or blood pressure were also excluded as previously described [27]. Eligibility based on blood lipids and glucose were determined from fasting blood samples at the screening visit. Subjects were recruited through flyers, paid advertisements in newspapers and on social media, campus emails, and word of mouth.

Protocol

Screening Visit

Individuals arrived at the Human Nutrition Lab (HNL) following an 8-12h overnight fast and 24h without exercise or alcohol. A fasting blood draw for a lipid panel and glucose measurement and anthropometrics were obtained. Next, resting metabolic rate (RMR) was measured for 30min using indirect calorimetry (TrueOne 2400, Parvo Medics, Sandy, Utah, USA) under standard conditions [28]. The final 20min of respiratory gases were used to calculate RMR using the Weir Equation [29]. Participants' RMR was multiplied by an average U.S. activity factor of 1.65 to estimate daily energy needs [30], which was used for determination of the high-fat test meals. In addition, alcohol consumption habits were assessed by the Alcohol Use Disorders Identification Test [31]. If individuals qualified for the study, subjects were randomized to one of the three treatment groups. An allocation ratio of 1:1:1, a permuted block design (balanced for age, sex, and BMI), and a random number generator was used to randomize participants.

Pre-Intervention Visit (V1)

Subjects completed a two-day food diary containing one weekend day and one week day [32] between the screening visit and the pre-diet intervention visit. One of the food diaries took place the day before V1. In addition, the night before V1, participants consumed a lead-in dinner meal and snack (provided by research personnel) that contained 50% of total energy from carbohydrate, 15% protein, and 35% fat. For V1, participants arrived at the HNL following an 8-12h overnight fast and 24h without exercise or alcohol. Height, weight, waist-hip circumference, and blood pressure were measured.

Following anthropometrics, participants completed a 100mm VAS to measure appetite (hunger, fullness, prospective consumption, desire to eat, thirst, and nausea) [19]. An IV catheter was placed in the antecubital space of the participant and a fasting blood draw was collected. Next, participants consumed a SFA-rich, high-fat breakfast shake within 10 minutes. The shake was comprised of an original milk chocolate ready-to-drink shake (Ensure; Abbott Nutrition, Abbott Laboratories), unsalted butter, red palm oil, coconut oil, soy lecithin granules, and powdered chocolate drink mix. The meal provided 17% of total daily energy with 70% of total energy from fat, 25% of energy from carbohydrate, and 5% of energy from protein. The fatty acid distribution was 47%, 16%, and 7% of total energy from SFA, MUFA, and PUFA, respectively. Four ounces of water was used to rinse out the container and then ingested to ensure the entire liquid meal was consumed. A high-SFA meal (devoid of pecans) was intentionally chosen since the impact of an acute pecan-containing meal on appetite has already been studied [22]. This study design allowed for the investigation of whether habitual consumption of pecans provides appetitive benefits or “protection” from consuming the occasional less healthy (high SFA) meal. Immediately following the SFA meal, a validated

sensory questionnaire was administered to assess sensory modalities of the breakfast shake [33, 34]. A modified 9-point hedonic scale was used with '1' indicating 'dislike extremely' and '9' indicating 'like extremely'. Then, VAS questionnaires were administered, and blood draws collected, every 30min for 3.5h postprandially. Four ounces of water was provided once/h. After the 210min postprandial time point, the IV catheter was removed and participants were instructed to eat a self-selected lunch within 1h of leaving the lab. In addition, participants were provided with VAS questionnaires to complete once/h for the next 5h starting at 300min (5h) post-SFA meal. Participants also completed a food diary to record all food and drink consumed for the rest of the day.

8-Week Dietary Intervention

The day after V1, all participants began the 8-week intervention. Written diet instructions were provided to all participants. Those in the control group were instructed to avoid all forms of nuts and to consume ≤2 servings (64g) of nut butter per week. Participants in the ADD group were provided with 68g (~0.5 cup or 2.25 ounces) portions of pecans to consume each day as part of their free-living diet with no additional dietary instructions. Participants in the SUB group were instructed to substitute the 470 kilocalories provided by the 68g of pecans for foods habitually consumed in their free-living diet. Trained research personnel guided the participants on how to make appropriate energy substitutions based on their previously completed food diaries. For example, if the participant habitually consumed snacks throughout the day, the research personnel highlighted the energy content of the snacks and asked the participant if it was feasible to replace the habitual snacks with the provided pecans. If participants did not consume snacks, research personnel asked the participant if it was feasible to reduce portion sizes at meals to accommodate the addition of the pecans. The guidance provided was

individualized based on each participant's dietary intake. Similar to the control group, the pecan groups were instructed to avoid all other nuts and to consume ≤2 servings of nut butter/wk. In addition, they were instructed to eat the pecans in their raw form (no roasting, cooking, or baking) but could add them to other foods.

Subjects in the ADD and SUB groups also completed a daily nut compliance log that detailed the time of day for pecan consumption. Nut compliance logs were submitted to research staff once per week. Poor compliance was categorized as consumption of <75% of pecans throughout the 8-week intervention. All subjects were instructed to avoid consuming >3 drinks/d for men or >2 drinks/d for women and were asked not to make any other changes to their diet or activity levels. Participants were unaware of the diet instructions that were provided to other treatment groups to prevent unintentional or intentional changes in behavior.

Post-Intervention Visit (V2)

After 8 weeks of the diet intervention, participants reported for V2 under the same conditions as V1. Participants completed the exact same study procedures and measurements that took place at V1, including the SFA meal.

Sample Analyses

All fasting and postprandial blood samples were drawn into an EDTA vacutainer, immediately placed on ice, and then centrifuged at 4°C. The plasma was aliquoted and stored at -80°C until analysis. Total PYY and active ghrelin were measured using radioimmunoassays (Millipore, Billerica, MA, USA). CCK octapeptide was measured using an extraction-free enzyme immunoassay (Phoenix Pharmaceuticals, Burlingame, CA, USA). Each participant's total number of samples were run within the same assay.

Statistical Analyses

SAS version 9.2 statistical package (SAS Institute Inc, Cary, NC, USA) was used for statistical analyses. The Food Processor SQL software (version 10.12.0) was used to assess all nutrient intake data. All hypotheses and analytic plans were pre-specified. All values were reported as mean±SEM unless otherwise noted. Statistical significance was set at $p \leq 0.05$. We chose to use PYY for our appetite hormone sample size calculation given that previous work has shown strong PYY responses to high-SFA meals [12, 35]. A samples size of 45 (15 per group) was estimated using G*power 3.19.7 assuming at least 80% power and an α of 0.05. This calculation was based on previous research from our lab that demonstrated a Cohen's d effect of 0.242 for postprandial PYY [12]. This effect size represents a 7% difference in postprandial PYY responses between the intervention and control groups. For VAS measurements, an 8-10% difference in the response between a control and treatment is considered clinically relevant [36]. Therefore, we also calculated a Cohen's d effect of 0.303, which represented an 10% difference in postprandial hunger between the control and intervention groups in the study by Stevenson et al. [12]. The required sample size was 30 (10 per group), thus we sought to recruit 45 participants based on the sample size calculation for PYY.

For the VAS data, overall appetite was calculated at each time point using the following equation: [desire to eat + hunger + (100 – fullness) + prospective consumption]/4 [37, 38]. All VAS analyses were first conducted for the full day (baseline through 540min). In addition, it was determined a priori that VAS data would also be analyzed for the time in the lab (baseline through 210min) and the time at home/away from the lab (210 through 540min) separately to determine which time period drove any appetite responses that were observed across the full day. It was also determined a priori that all nutrient intake data would be analyzed for the full day, as

well as separated out by consumption in the lab (i.e. the SFA meal) and consumption at home/away from the lab.

An unpaired t-test was used to assess differences in nut compliance between the two pecan groups. For time course data, a repeated measures linear mixed model for treatment, visit, and time was used to test for within group differences. In addition, the change in area under the curve (AUC) from pre- to post-intervention within each group was calculated to compare changes in meal responses across the intervention between groups. For anthropometrics, dietary intake, and fasting measures of appetite, a repeated measures linear mixed model for treatment and visit was used to test for differences within groups from pre- to post-intervention. Also for these outcomes, change from pre- to post-intervention was calculated, and a one-way analysis of variance (ANOVA) was used to test for differences between groups for change data and at baseline. Post hoc analyses were done using least square means with no multiple testing adjustment.

Results

Participants

Fifty-six individuals were allocated to an intervention group, but nine participants did not start or complete the intervention and/or testing visits. Therefore, forty-seven subjects (n=10 women and n=6 men for control, n=11 women and n=4 men for ADD, and n=12 women and n=4 men for SUB) completed the study and were included in the final analyses. Participant anthropometrics at baseline were previously reported [39]. The average age at baseline in the ADD, SUB and control groups was 50±11, 46±11, and 49±14, respectively. The average BMI was 30.3±4.2, 33.4±6.7, and 31.9±6.8, respectively. There were no differences between groups at baseline for anthropometric or appetite outcomes **(Table 1).** Additionally, there were no

differences between or within groups from pre- to post-intervention for body weight, BMI, waist circumference, hip circumference, or blood pressure (data not shown). Participants in both the ADD and SUB groups consumed 95% of pecans provided, and compliance was not different between groups [39].

Physiological Responses

There were no differences within or between groups for fasting CCK, PYY, or ghrelin **(Table 5.1).** The meal responses for CCK, PYY, and ghrelin are presented in **Figure 5.1**. The change in meal response from pre- to post-intervention was not significantly different between groups for CCK (change in AUC: ADD: 0.08±0.07 vs SUB: 0.02±0.06 vs control: 0.04±0.05ng/mL/3.5h; ns), PYY (ADD: 22±23 vs SUB -2±20 vs control: 5±14pg/mL/3.5h; ns), or ghrelin (ADD: -17±8 vs SUB: 3.2±10 vs control: -17±9pg/mL/3.5h; ns) **(Figure 5.1 D, 1H, 1L,** respectively**)**. However, within group analyses showed a significant elevation of CCK and PYY from pre- to post-intervention within ADD (p=0.02 and p=0.05, respectively) but not control or SUB (ns) **(Figure 5.1 A-C, E-G)**. In addition, there was a significant suppression of postprandial ghrelin within ADD and control (p=0.008 and 0.003, respectively) but not SUB (ns) **(Figure 5.1 I-K)**.

Subjective Responses

There were no differences between or within groups for subjective appetite at fasting **(Table 5.1)**. The meal responses and change in AUC from pre- to post-intervention for the subjective appetite responses using VAS are presented in **Figures 5.2-5.5**. For overall appetite, there was a non-significant tendency for a greater decrease in AUC for ADD vs control for the full day (-67±46 vs 20±27mm/9h; p=0.06) and at home (-48±37 vs 21±23mm/5h; p=0.07) but not the lab (ns) **(Figure 5.2D)**. Within ADD, there was also a suppression from pre- to post-

intervention for the full day and at home (p=0.006 and p=0.04) and a trend for suppression in the lab (p=0.06) **(Figure 5.2A).** There were no other differences within or between groups for overall appetite **(Figure 5.2).**

When looking at each of the questions that made up the overall appetite score, there were some similar patterns. For hunger, the decrease in the AUC from pre- to post-intervention was greater in ADD vs control for home (-61±36 vs 20±21mm/5h; p=0.04) **(Figure 5.5A)**. Within ADD, there was also a suppression for home (p=0.009) and a trend for suppression for the full day (p=0.07) with no changes occurring in the lab (ns) **(Figure 5.3A).** There were no other differences within or between groups for hunger **(Figure 5.3B, 5.3C, 5.5A)**. For fullness, there were no differences between groups **(Figure 5.5B)**. However, there was a significant elevation from pre- to post-intervention within ADD for the full day (p=0.05) but not for lab or home independently (ns) **(Figure 5.3D).** There were no other differences within groups **(Figure 5.3E, 5.3F)**.

For prospective consumption, the decrease in AUC from pre- to post-intervention was greater for ADD vs SUB for the full day (-80±41 vs 11±26mm/5h; p=0.05) and lab (-29±14 vs 7±11mm/3.5h; 0.03) but not home (-48±34 vs -1±23 mm/5h; ns) **(Figure 5.5C)**. There was also a trend for a greater decrease in the AUC for ADD vs control groups for the full day (-80±41 vs 3±29mm/9h; p=0.07) **(Figure 5.5C)**. Within ADD, there was a significant suppression from pre- to post-intervention for the full day and lab (p=0.0001 for both) and a trend for suppression at home (p=0.07) **(Figure 5.4A)**. There were no other differences within **(Figure 5.4B, 5.4C)** or between groups **(Figure 5.5C)** for prospective consumption. Finally, for desire to eat, the decrease in AUC from pre- to post-intervention was greater for ADD vs control for the full day (-64±39 vs 23±29mm/9h; p=0.05) **(Figure 5.5D)**. There was also a trend for a greater decrease in

ADD vs control at home (-47±33 vs 19±22mm/5h; p=0.06) **(Figure 5.5D)**. Within ADD, there was a suppression from pre- to post-intervention for the full day (p=0.03) and a trend for suppression at home (p=0.08) but no change at the lab (ns) **(Figure 5.4D).** There were no other differences within **(Figure 5.4E, 5.4F)** or between groups **(Figure 5.5D)**. There were no differences between or within groups for thirst or nausea (data not shown). In addition, there were no differences within or between groups for sensory ratings for the SFA shake (data previously reported) [27].

Nutrient Intake

The daily nutrient intake from the pre- and post-intervention visits based on the nutrient analysis of the food diary and provided SFA meal are presented in **Table 5.2.** There were no differences within or between groups for EI (kcal) during the full day, the time at home, or in the lab. For the full day and home, there was a trend for a decrease in intake of SFA within SUB (p=0.06), and this decrease was significantly greater than the change within control (p=0.03). There was also a trend for a greater increase in protein intake in SUB vs ADD from pre- to post-intervention for the full day and home (p=0.08). There were no other differences within of between groups for the daily intake of energy, macronutrients, fatty acids, dietary fiber, or cholesterol for the full day or time at home. Since the SFA meal was identical at the pre- and post-intervention visits, the changes in nutrient intake were driven by the time at home.

Discussion

For the first time, we have shown that adding pecans to the diet resulted in differences between groups for subjective, but not physiologic, postprandial appetite. There were also notable within group changes. Specifically, we observed improvements in postprandial CCK, PYY, ghrelin, and subjective appetite measures in the ADD group. There were, however, no

changes in postprandial appetite within the SUB group, indicating that the presence or absence of isocaloric substitution instructions may be an important consideration for physiologic appetite regulation. The improvements in postprandial ratings of hunger and desire to eat for the full day or time spent at home for ADD vs control also supports improved appetite in the pecan group not receiving substitution instructions. Altogether, the observed within and between group improvements for both subjective and physiological outcomes suggests strong agreement between the two types of measures and highlights the potency of the effect of adding pecans to the diet on appetite since many studies show improvements for either hormones or VAS, but not both [11, 40, 41]. Finally, the aforementioned appetite changes occurred despite no changes in body weight from baseline to 8 weeks, within or between groups.

The postprandial improvements within the ADD group from pre- to post-intervention are profound given the absence of nuts within the test meal. Most studies assessing postprandial appetite in response to tree nut consumption compare test meals with and without the nut of interest [20, 22, 24, 25]. Our test meal was a high SFA shake, which allows us to determine the impact of chronic vs. acute pecan consumption on appetite. This design is unique and allows for a strong practical application where we can determine whether chronic pecan consumption can protect energy balance or improve appetite when the occasional unhealthy meal (such as one high in SFA) is consumed.

One previous study employed a similar design involving a 7d PUFA-rich diet containing walnuts and a high SFA test shake [12]. Similar to the present study, they reported improvements in postprandial PYY following high SFA meal consumption [12]. The previous study did not find improvements in postprandial ghrelin or VAS; however, they measured total ghrelin whereas we measured active (acylated) ghrelin, the biologically active form that stimulates

appetite [42]. Therefore, measuring total (non-acylated and acylated) ghrelin in the previous study may have masked changes that were occurring in the biologically active form. We also measured VAS for 5h after leaving the lab allowing for a more complete picture of subjective appetite across the day. As a result, we observed improvements in overall appetite, desire to eat, and fullness during the full day, which were strongly driven by the time at home. The lack of post-visit VAS questionnaires in the previous study [12] may have inhibited their ability to detect differences in appetite. It is equally possible that pecan consumption for 8 weeks simply elicited a stronger effect on appetite than a 7d walnut-enriched diet. Only two other studies have investigated the effect of long-term tree nut consumption on appetite, but these studies administered VAS questionnaires on days that the respective tree nuts were consumed [24, 25] making it difficult to compare our results to due to the major differences in methodology.

The improvements in postprandial hormones and subjective markers of appetite within ADD could be clinically meaningful for energy balance but more research is needed to confirm. It is accepted that an 8-10% difference in the response between a control and treatment is considered clinically relevant for VAS [36]. In the present study, the change in overall appetite across the full day was 22.7% different between ADD and control, suggesting a clinically meaningful magnitude of change. To our knowledge, there is not a reference range for the change in appetite hormones that is considered clinically relevant, but previous research demonstrates that postprandial increases in CCK and PYY, and decreases in ghrelin, are associated with decreased EI [43-45]. Furthermore, VAS ratings of hunger are associated with increased food intake during a test meal, and the opposite effect was found for ratings of fullness [46]. These studies suggest that improvements in physiological and subjective appetite may decrease EI and subsequently promote weight maintenance in the long-term. However, we did

not observe decreases in EI from the pre- to post-intervention test day based on the self-report food diaries. This null finding may reflect the subjective nature of food diaries, which is vulnerable to under- and over-reporting [47, 48] and are likely not sensitive enough to detect small changes in EI. It is possible that reduced EI would have occurred if an objective measure in a controlled setting (ad libitum buffet) was incorporated, which has been done in past studies [43-46].

Aside from the strong improvements across nearly every measure of appetite within the ADD group, a second interesting, and surprising, outcome was the lack of change within the SUB group. Biomarker analysis did confirm high compliance for pecan consumption in both groups [39], so it is unclear why the appetite results diverged between the two pecan groups. The most likely explanation is the different practices for pecan incorporation into the two diets. It is possible that isocaloric substitution within the SUB group led to equal exchanges in nutrients with respect to their known effect on appetite. Another possible explanation is the different changes in SFA intake (trend for a decrease within SUB but not ADD). There is some evidence that meals and short-term diets rich in SFA promote satiety and lower EI more than USFA-rich meals/diet [35, 41, 49]. Since the intake of SFA decreased from 45 to 37g at the pre- to post-intervention days with no changes in subjective appetite, it is possible that the daily pecan consumption actually provided a protective effect on appetite in the SUB group.

Although the changes within the ADD group were profound, there were no differences between groups for appetite hormones. Therefore, those results should be interpreted with some degree of caution. Furthermore, we know that self-report food diaries are vulnerable to under- and over-reporting and lack sensitivity [47, 48], so the use of food diaries as a measure of nutrient intake is a limitation of this study. Another limitation is that the potential effect of

hormonal fluctuations due to the menstrual cycle of the premenopausal female participants on appetite outcomes was not accounted for [50]. In addition, we used a relatively high dose of pecans because previous research in almonds and pistachios suggested that 43-56 g/d for 4 weeks was not enough to observe changes in subjective appetite [24, 25]. Furthermore, Del Gobbo et al. reported that ≥60 g/d produces stronger changes in other biochemical markers [51]. However, more research is needed to determine the effect of smaller doses for longer durations. Finally, this study was not designed to evaluate the effects of sex, race, or BMI, so future work in this area is warranted.

In conclusion, despite no differences between groups for appetite hormones, adding pecans to the daily diet for 8 weeks improved postprandial CCK, PYY, ghrelin, and subjective appetite. Additionally, the change in many measures of postprandial subjective appetite was more suppressed in ADD vs control during the full day or the time spent at home. These findings occurred on days in which pecans were not consumed, indicating that daily pecan consumption may provide a longer-term or chronic protective effect on post-meal appetite. Furthermore, the results of this study may inform weight management strategies and provide justification for why tree nut consumption is not associated with weight gain, despite their high energy density [4, 5]. Future studies should investigate the impact of different methods for pecan incorporation into the diet, specifically which types of foods or nutrients are being removed/substituted, through specific food group substitutions as well as measured ad libitum EI. Furthermore, since weight loss interventions often result in unfavorable changes in appetite [52], more research is needed to determine if tree nut consumption provides a protective effect on appetite in the context of a weight loss intervention.

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Table 5.1 Fasting physiological and subjective markers of appetite

	Control (n=16)		ADD (n=15)		SUB (n=16)	
	Pre-Intervention	Post-Intervention	Pre-Intervention	Post-Intervention	Pre-Intervention	Post-Intervention
CCK (ng/mL)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.0
PYY (pg/mL)	91.1 ± 9.4	90.6 ± 8.9	103.3 ± 6.5	110.1 ± 7.1	99.0 ± 9.9	98.8 ± 6.8
Ghrelin (pg/mL)	106.1 ± 16.0	102.9 ± 14.3	109.9 ± 10.2	106.2 ± 12.6	92.1 ± 8.9	95.1 ± 9.8
Appetite (mm)	49 ± 5	46 ± 6	54 ± 5	57 ± 6	54 ± 6	50 ± 5
Hunger (mm)	35 ± 6	38 ± 8	40 ± 6	47 ± 8	46 ± 6	45 ± 7
Fullness (mm)	25 ± 5	35 ± 8	18 ± 5	19 ± 5	27 ± 7	31 ± 6
Pros. Cons. (mm)	49 ± 7	46 ± 7	52 ± 7	54 ± 7	46 ± 6	44 ± 6
Desire to Eat (mm)	36 ± 7	36 ± 7	43 ± 7	45 ± 8	51 ± 8	43 ± 7

All values are mean ± SEM. There were no significant differences within or between groups. ADD=consumed pecans as part of a free-living diet; CCK=cholecystokinin; PYY=peptide YY; Pros. Cons.=Prospective Consumption; SUB=substituted pecans for isocaloric foods from their habitual diet.

Table 5.2 Nutrient intake on the day of the pre- and post-intervention visits

	Control (n=16)		ADD (n=15)		SUB (n=16)	
	Pre-Intervention	Post-Intervention	Pre-Intervention	Post-Intervention	Pre-Intervention	Post-Intervention
Total energy intake (kcal/d)	2253 ± 212	2330 ± 247	2154 ± 266	1963 ± 156	2142 ± 194	2116 ± 175
Energy (kcal) in lab	496 ± 29	496 ± 29	451 ± 18	451 ± 18	471 ± 27	471 ± 27
Energy (kcal) at home	1782 ± 203	1859 ± 240	1718 ± 260	1528 ± 155	1685 ± 174	1660 ± 155
Carbohydrate (g/d)	240 ± 24	225 ± 30	216 ± 32	206 ± 20	206 ± 21	210 ± 28
Dietary Fiber (g/d)	13 ± 3	12 ± 2	15 ± 3	13 ± 3	14 ± 2	12 ± 2
Protein (g/d)	65 ± 7	73 ± 9	73 ± 7	65 ± 9^	61 ± 7	73 ± 7
Fat (g/d)	113 ± 13	121 ± 13	108 ± 14	95 ± 7	116 ± 12	104 ± 10
MUFA (g/d)	48 ± 6	50 ± 5	44 ± 6	39 ± 4	45 ± 5	21 ± 2
PUFA (g/d)	22 ± 3	22 ± 3	21 ± 6	18 ±3	24 ± 3	9 ± 1
SFA (g/d)	42 ± 5	47 ± 6	41 ± 4	37 ± 2	45 ± 5	37 ± 3* #
Trans FA (g/d)	1.5 ± 0.4	1.7 ± 0.4	1.3 ± 0.2	1.3 ± 0.3	1.5 ± 0.2	1.2 ± 0.3
Cholesterol (mg/d)	219 ± 35	226 ± 35	195 ± 23	177 ± 26	205 ± 36	206 ± 27

All values are mean ± SEM. There were no significant differences between groups at baseline. * Indicates the decrease from pre- to post-intervention visit was different from control ($p \leq 0.05$). ^ Indicates a trend for greater intake from pre- to post-intervention compared to SUB ($p=0.08$). # Indicates a trend for a decrease within the SUB group ($p=0.06$). ADD= consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet; FA=fatty acid; g=gram; kcal=kilocalorie; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; SFA=saturated fatty acid.

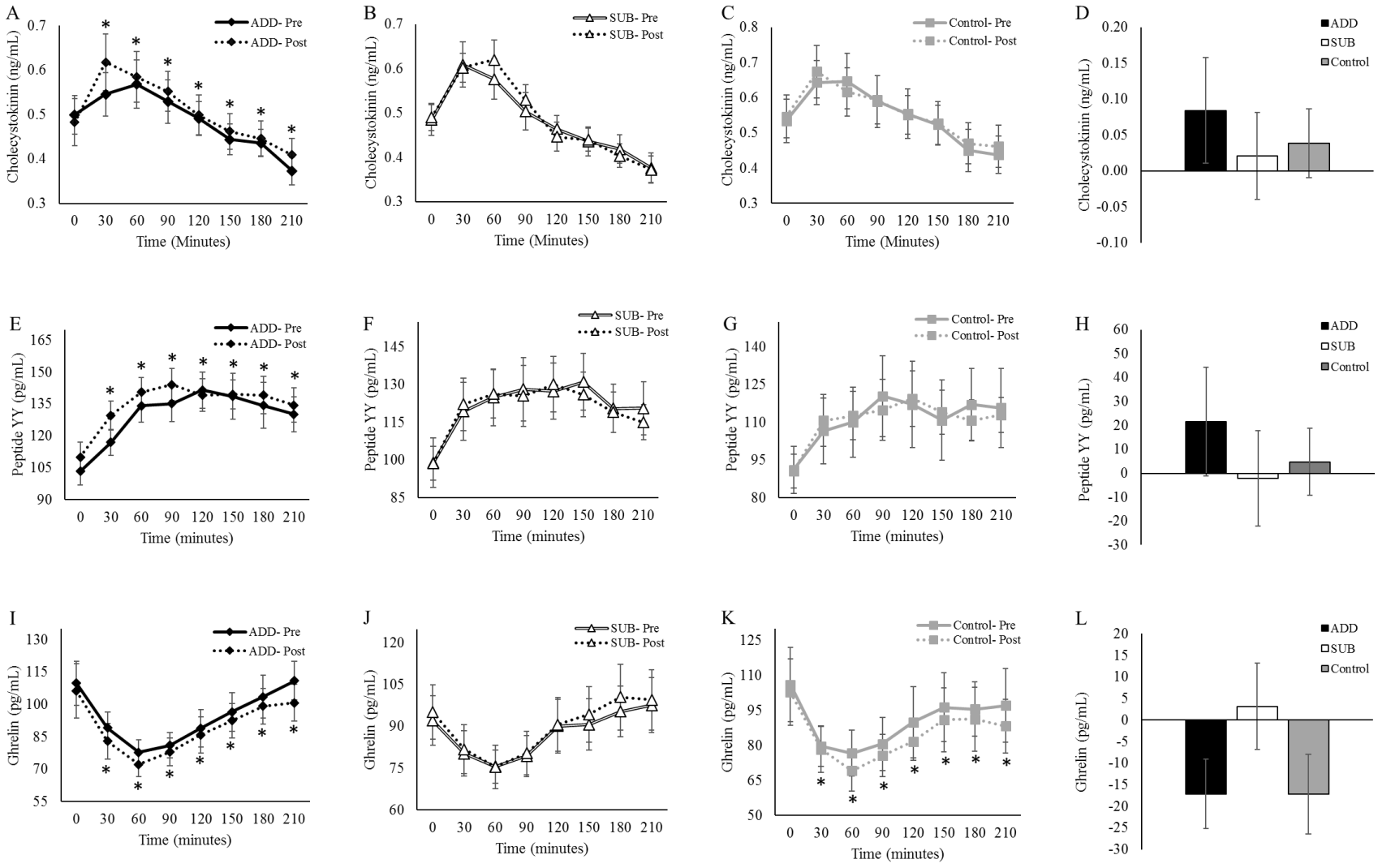
A
Cholecystokinin (ng/mL)
ADD- Pre
ADD- Post
Time (Minutes)
B
Cholecystokinin (ng/mL)
SUB- Pre
SUB- Post
Time (Minutes)
C
Cholecystokinin (ng/mL)
Control- Pre
Control- Post
Time (Minutes)
D
Cholecystokinin (ng/mL)
ADD
SUB
Control
E
Peptide YY (pg/mL)
ADD- Pre
ADD- Post
Time (minutes)
F
Peptide YY (pg/mL)
SUB- Pre
SUB- Post
Time (minutes)
G
Peptide YY (pg/mL)
Control- Pre
Control- Post
Time (minutes)
H
Peptide YY (pg/mL)
ADD
SUB
Control
I
Ghrelin (pg/mL)
ADD- Pre
ADD- Post
Time (minutes)
J
Ghrelin (pg/mL)
SUB- Pre
SUB- Post
Time (minutes)
K
Ghrelin (pg/mL)
Control- Pre
Control- Post
Time (minutes)
L
Ghrelin (pg/mL)
ADD
SUB
Control

Figure 5.1 Time course for cholecystokinin (CCK), peptide YY (PYY), and ghrelin in the ADD, SUB, and control groups at the pre- and post-intervention (8 weeks) visits (Figure 5.1A-C, E-G, and I-K, respectively), and the change in area under the curve (Figure 5.1D, 5.1H, 5.1L). Subjects consumed a high-saturated fat meal immediately after time 0. * Denotes the significant decrease within a group ($p<0.05$) from pre- to post-intervention. All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet.

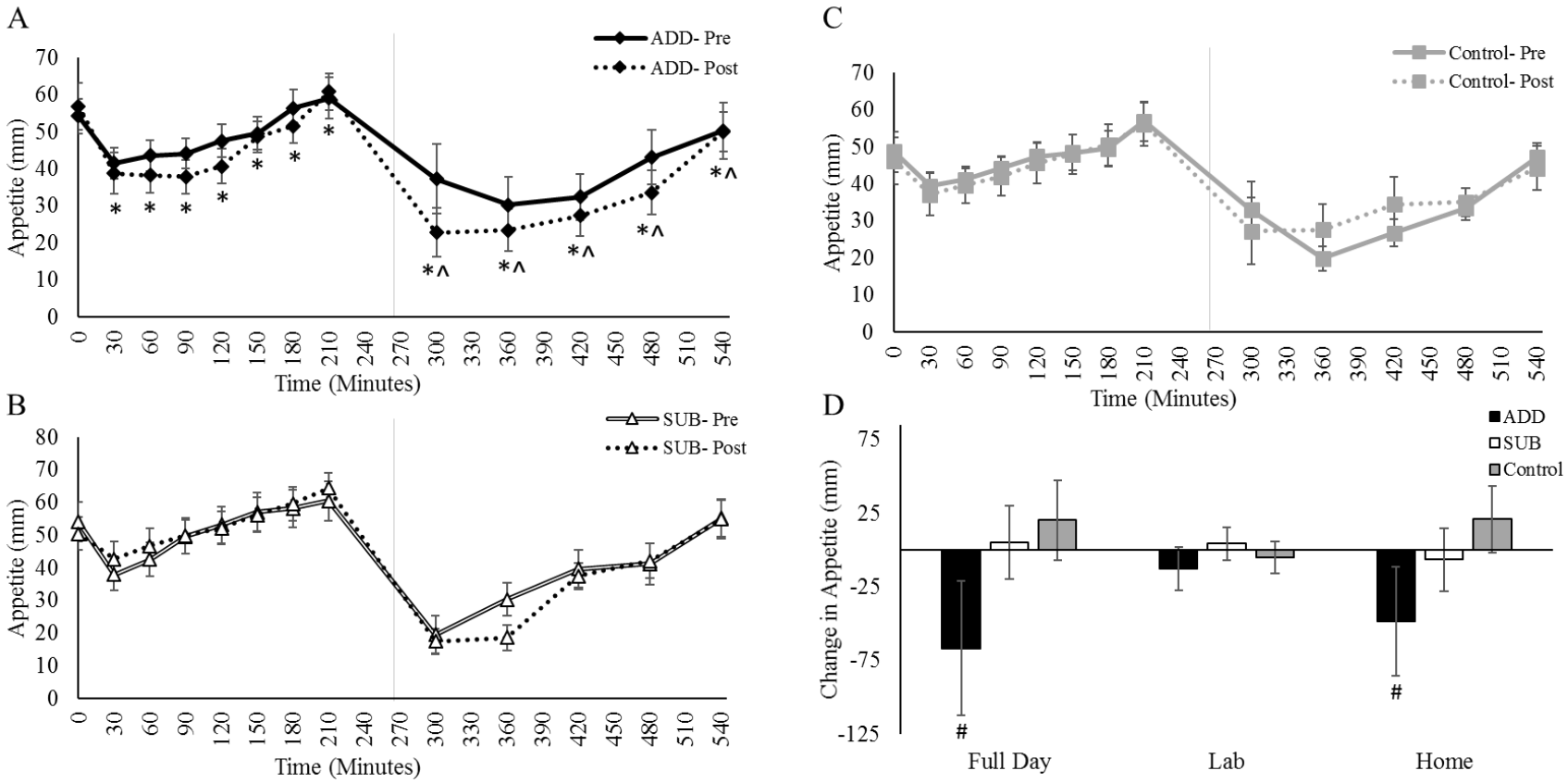
A
ADD- Pre
ADD- Post
Appetite (mm)
Time (Minutes)
B
SUB- Pre
SUB- Post
Appetite (mm)
Time (Minutes)
C
Control- Pre
Control- Post
Appetite (mm)
Time (Minutes)
D
ADD
SUB
Control
Change in Appetite (mm)
Full Day
Lab
Home

Figure 5.2 Time course for the pre- to post-intervention (8 weeks) visits for overall appetite in the ADD, SUB, and control groups (Figures 2A-C, respectively), and the change in area under the curve (Figure 2D). Subjects consumed a high-saturated fat meal immediately after time 0. The vertical gray line represents the free-living meal that was consumed approximately 1h after leaving the lab. * Denotes a significant decrease from pre- to post-intervention within a group for the full day ($p<0.05$). ^ Denotes a significant decrease within a group for the time at home ($p<0.05$). # Denotes a trend for a difference for ADD vs. control ($p<0.10$). All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet.

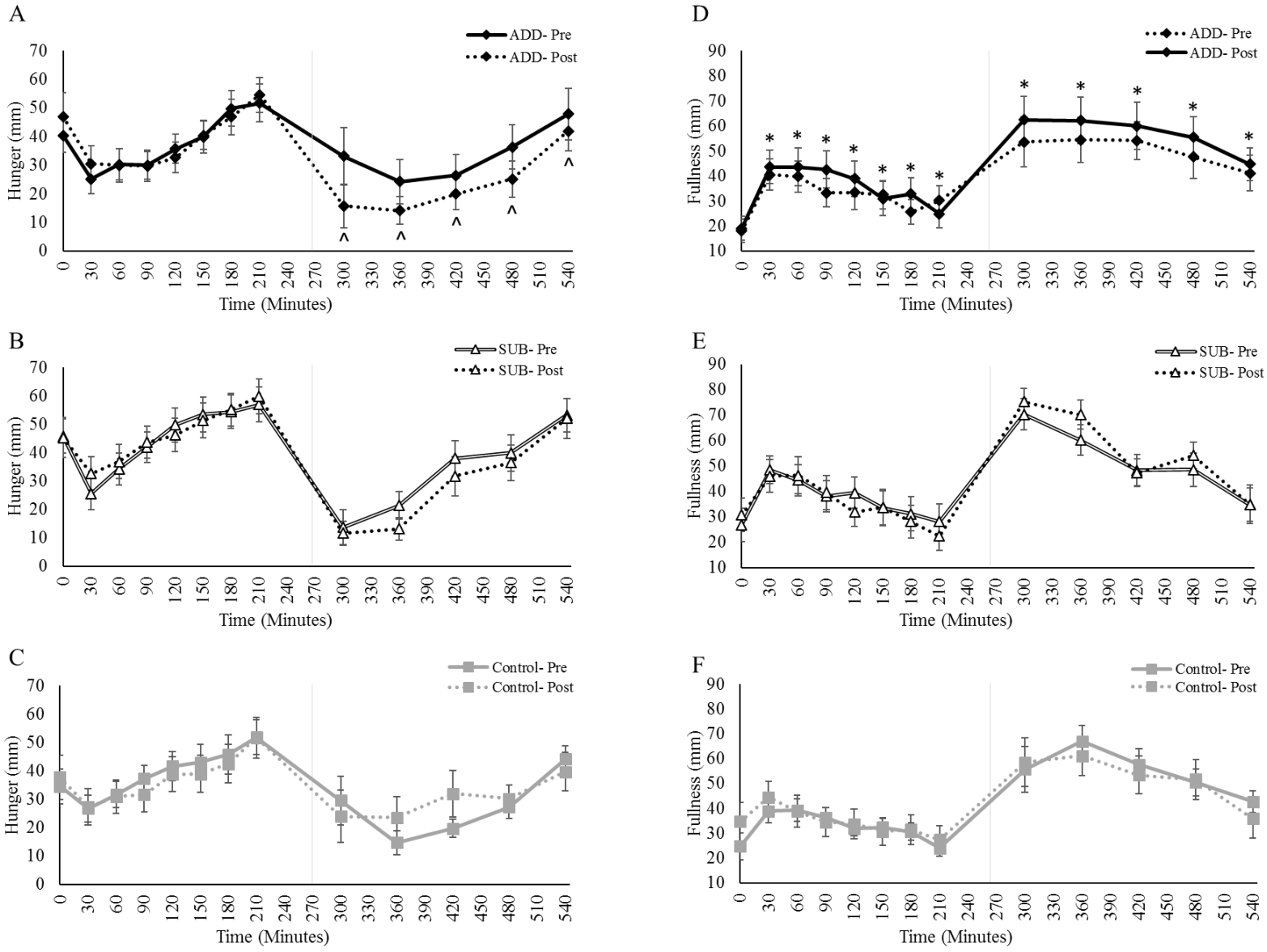
A
ADD- Pre
ADD- Post
Hunger (mm)
Time (Minutes)
B
SUB- Pre
SUB- Post
Hunger (mm)
Time (Minutes)
C
Control- Pre
Control- Post
Hunger (mm)
Time (Minutes)
D
ADD- Pre
ADD- Post
Fullness (mm)
Time (Minutes)
E
SUB- Pre
SUB- Post
Fullness (mm)
Time (Minutes)
F
Control- Pre
Control- Post
Fullness (mm)
Time (Minutes)

Figure 5.3 Time course for subjective hunger and fullness ratings in the ADD, SUB, and control groups at the pre- and post-intervention (8 weeks) visits (Figures 5.3A-C and D-F, respectively). Subjects consumed a high-saturated fat meal immediately after time 0. The vertical gray line represents the free-living meal that was consumed approximately 1h after leaving the lab. * Denotes a change within a group from pre- to post-intervention for the full day ($p<0.05$). ^ Denotes a change within a group for the time at home ($p<0.05$). All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet.

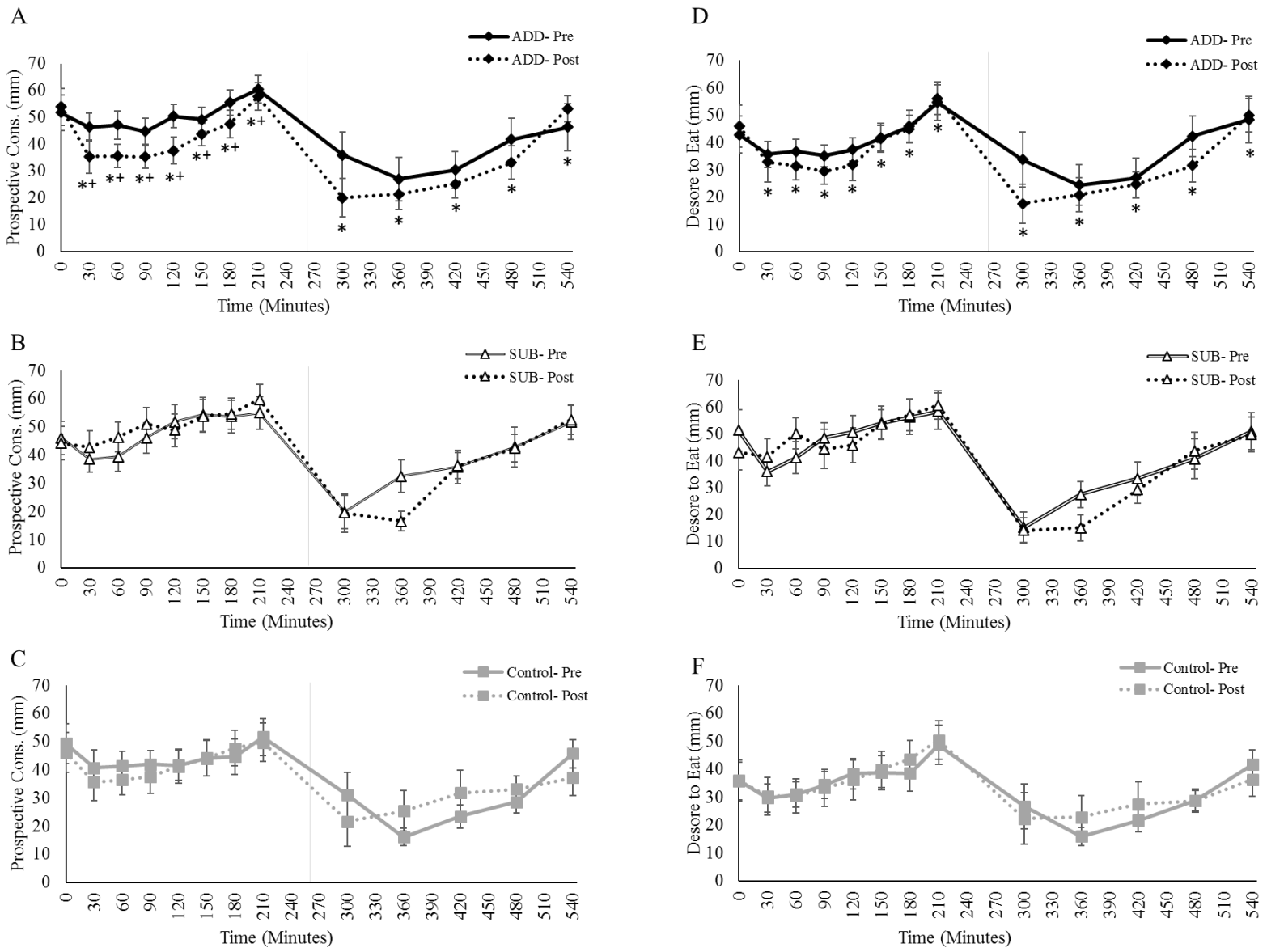
A
ADD- Pre
ADD- Post
Prospective Cons. (mm)
Time (Minutes)
B
SUB- Pre
SUB- Post
Prospective Cons. (mm)
Time (Minutes)
C
Control- Pre
Control- Post
Prospective Cons. (mm)
Time (Minutes)
D
ADD- Pre
ADD- Post
Desore to Eat (mm)
Time (Minutes)
E
SUB- Pre
SUB- Post
Desore to Eat (mm)
Time (Minutes)
F
Control- Pre
Control- Post
Desore to Eat (mm)
Time (Minutes)

Figure 5.4 Time course for subjective ratings of prospective consumption and desire to eat in the ADD, SUB, and control groups at the pre- and post-intervention (8 weeks) visits (Figures 5.4A-C and D-F, respectively). Subjects consumed a high-saturated fat meal immediately after time 0. The vertical gray line represents the free-living meal that was consumed approximately 1h after leaving the lab. * Denotes a change within a group from pre- to post-intervention for the full day ($p<0.05$). + Denotes a change within a group for the lab ($p<0.05$). All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; Prospective Cons.=Prospective Consumption; SUB=substituted pecans for isocaloric foods from their habitual diet.

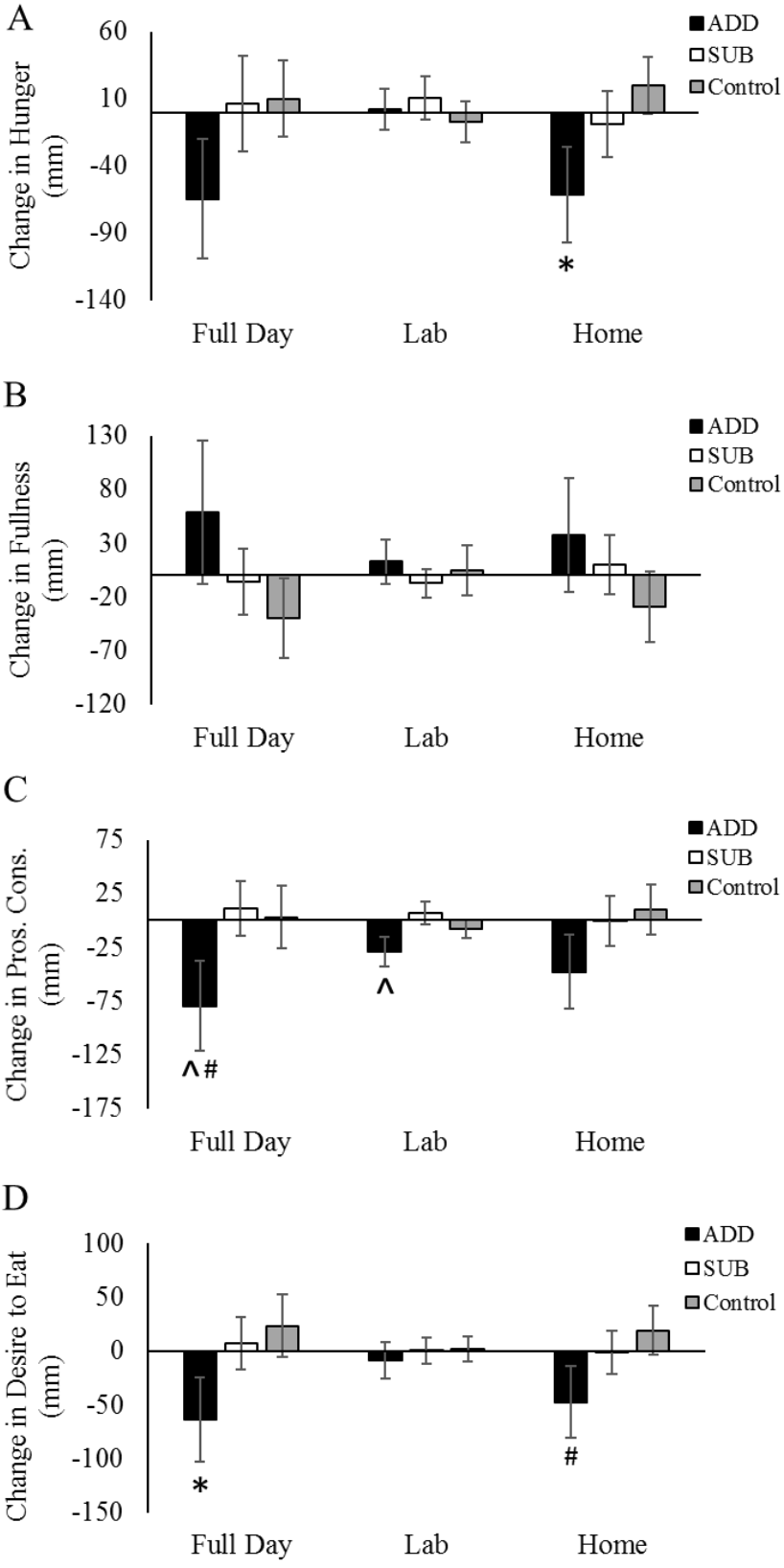
A
Change in Hunger (mm)
60
10
-40
-90
-140
*
ADD
SUB
Control
Full Day
Lab
Home
B
Change in Fullness (mm)
130
80
30
-20
-70
-120
ADD
SUB
Control
Full Day
Lab
Home
C
Change in Pros. Cons. (mm)
75
25
-25
-75
-125
-175
^#
^
ADD
SUB
Control
Full Day
Lab
Home
D
Change in Desire to Eat (mm)
100
50
0
-50
-100
-150
*
#
ADD
SUB
Control
Full Day
Lab
Home

Figure 5.5 Change in area under the curve for (A) hunger, (B) fullness, (C) prospective consumption, and (D) desire to eat in the ADD, SUB, and control groups from the pre to post-intervention (8 weeks) visits. * Denotes a difference between ADD vs. control ($p<0.05$). ^ Denotes a difference between ADD vs. SUB ($p<0.05$). # Denotes a trend for a difference between ADD vs. control ($p<0.10$). All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; Pros. Cons.=Prospective Consumption; SUB=substituted pecans for isocaloric foods from their habitual diet.

CHAPTER 6

PECAN-ENRICHED DIETS INCREASE ENERGY EXPENDITURE AND FAT OXIDATION IN ADULTS AT-RISK FOR CARDIOVASCULAR DISEASE IN A RANDOMIZED, CONTROLLED TRIAL[4]

[4]Guarneiri, L.L., C.M. Paton, and J.A. Cooper. *JHND*, 2021. p. 1-12.
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Abstract

Background: Research indicates that diets enriched with unsaturated fatty acids improve energy metabolism, but studies on tree nuts, which are a rich source of those fats, are limited. **Objective:** To examine the impact of daily pecan consumption for 8 weeks on energy metabolism in adults with hypercholesterolemia or at higher risk for cardiovascular disease (CVD) (BMI $\geq$ 28 kg/m^2). **Methods:** For this randomized, controlled trial, fifty-six sedentary adults were randomized into one of three treatments for an 8-week intervention: two pecan groups and a nut-free control group (n=18). The ADD group (n=16) consumed pecans as part of a free-living diet, while the SUB group (n=18) substituted the pecans for isocaloric foods from their habitual diet. At baseline and 8 weeks, a high saturated fat meal was consumed along with indirect calorimetry measurements at fasting and for 4h postprandially to determine changes in resting metabolic rate (RMR), diet induced thermogenesis (DIT), and substrate utilization (primary outcomes). Forty-seven participants completed the trial and were included in analyses. **Results:** In the SUB group, there was an increase in fasting RMR (1,607±117 to 1,701±114kcal/d; p=0.01) and fasting fat oxidation (0.83±0.08 vs 0.99±0.08g/20min; p=0.009) and a decrease in fasting respiratory exchange ratio (RER) (0.85±0.01 vs 0.83±0.01; p=0.05) from pre- to post-intervention. In the ADD group, there was an increase in postprandial DIT (p<0.001). There were no changes within the control group or between groups for any outcome measure. **Conclusion:** Daily consumption of pecans may increase select measures of EE and fat oxidation in adults at-risk for CVD.

Introduction

Greater than forty percent of adults have obesity in the United States [1]. As a result, many Americans are at an elevated risk for co-morbidities such as type 2 diabetes mellitus, hypertension, joint problems, and cardiovascular disease [2]. Regrettably, weight loss efforts are often not sustainable [3], demonstrating the crucial need to promote weight maintenance. On average, individuals only gain 0.5-1 kg/y, but a consistent increase in body weight can lead to obesity overtime [4]. Therefore, it is plausible that small adjustments to energy intake or energy expenditure (EE) may mitigate the energy imbalances that are fueling annual weight gain, and by extension, obesity.

Resting metabolic rate (RMR) and diet-induced thermogenesis (DIT) are two components of EE that are crucial regulators of energy balance [5] as suppression of either one has been associated with obesity [6]. Furthermore, substrate utilization governs macronutrient balance, and suppressed fat oxidation leads to weight gain [7]. Previous research indicates that long-term intake of unsaturated fatty acids (USFA) induces greater EE and fat oxidation [8]. More specifically, diets with high polyunsaturated fatty acid (PUFA)/saturated fatty acid (SFA) ratios resulted in higher RMR and postprandial fat oxidation compared to diets with low PUFA/SFA ratios, especially in populations with obesity [9, 10]. Furthermore, diets rich in monounsaturated fatty acids (MUFA) show increased RMR, DIT, and estimated daily EE compared to diets rich in SFA [11, 12]. Therefore, the simple incorporation of foods that are rich in USFA to the daily diet could improve EE and macronutrient balance and subsequently promote weight maintenance.

Tree nuts are rich sources of USFA [13], which may explain why they are associated with weight maintenance despite their high energy density [14, 15]. Previous studies report that acute meal challenges containing walnuts have higher fat oxidation and DIT compared to control

meals [16, 17]. Furthermore, we have previously shown significant improvements in postprandial fat oxidation following a 7d PUFA-rich diet (containing walnuts) [18]. Finally, studies of long-term intake of peanut or peanut oil led to a 5-11% increase in RMR [19, 20]. However, not all nut studies have shown metabolic improvements as 2.5 to 6 months of daily almond consumption did not improve markers of energy metabolism [21, 22]. Therefore, this preliminary research indicates that tree nuts and peanuts may increase RMR, DIT, and fat oxidation, but more research is needed to confirm this notion, particularly in pecans where beneficial effects have been observed on blood lipid profiles but no metabolic research exists [23, 24].

It is also important to note that the two studies with null findings in almonds did not provide any type of dietary instructions to participants [21, 22] while the study with peanuts that improved RMR provided instructions regarding substitution of habitual fat calories to accommodate the increased fat intake from the peanuts [19]. Therefore, the type of dietary instructions, or lack thereof, for nut incorporation may be important for inducing beneficial changes in energy metabolism, but no study has directly assessed this. The purpose of this study was to examine the impact of daily pecan consumption (with and without dietary/isocaloric substitution instructions) for an 8-week period on fasting and postprandial energy metabolism in adults with hypercholesterolemia or at higher risk for CVD (body mass index (BMI) $\geq$28kg/m^2). We hypothesized that daily pecan consumption, regardless of the method of incorporation into the diet, would improve fasting and postprandial EE and substrate utilization, and that the responses of the control group would be non-significant.

Methods

This was a single-blind, randomized, parallel controlled trial (clinicaltrials.gov: NCT04376632) involving an 8-week intervention conducted at the University of Georgia. Recruitment began in August 2018 and final testing was completed in December 2020 when participant testing was completed to address all primary outcomes. The protocol included a screening visit and 2 testing visits (pre- and post-diet intervention). Subjects were randomly assigned (balanced blocks stratified by age, sex, and body mass index (BMI)) to one of three groups: a "no nut" control group, or one of two pecan groups (ADD or SUB). Subjects in the ADD and SUB groups both consumed 68g of pecans per day for 8 weeks; however, dietary instructions for the incorporation of pecans differed. For the ADD group, pecans were consumed as part of a free-living diet, while in the SUB group, participants received counseling at baseline on how to substitute pecans for isocaloric foods from their habitual diet. This study was approved by the Institutional Review Board for human subjects, and informed written consent was obtained from each participant prior to testing.

Study design and participants

Sixty-nine sedentary men and women between the ages of 30 and 75y with high cholesterol or a BMI of ≥28kg/m^2 were assessed for eligibility. Inclusion criteria based on "high cholesterol" levels has been described previously [24]. To rule out individuals with familial hypercholesterolemia, participants with LDL-c levels > 95th percentile or HDL-c levels < 20th percentile were excluded. Other exclusion criteria included habitual nut consumption (>2 servings/week), nut allergies, special diets (i.e. ketogenic diet, intermittent fasting), excessive alcohol use (>3 drinks/d for men or >2 drinks/d for women), tobacco or nicotine use, exercise >3h/week, weight change >5% of body weight in the past 3 months, history of medical events or

medication use affecting digestion, absorption, or metabolism, gastrointestinal surgery, and chronic or metabolic diseases. Individuals taking lipid-lowering medications, fish oil supplements, steroid/hormone therapy, or medications for diabetes mellitus or Attention Deficit Hyperactivity Disorder were also excluded. Finally, individuals with the following biomarkers were excluded: fasting glucose >126mg/dL, fasting TG >350mg/dL, and blood pressure >180/120mmHg. Eligibility based on blood lipids and glucose were determined from fasting blood samples at the screening visit. Subjects were recruited through flyers, paid advertisements in newspapers and on social media, campus emails, and word of mouth.

Protocol

Screening Visit

Individuals arrived at the Human Nutrition Laboratory (HNL) following an 8-12h overnight fast and 24h without exercise or alcohol. A fasting blood draw for a lipid panel and glucose measurement and anthropometrics were obtained. Next, resting metabolic rate (RMR) were measured for 30min using indirect calorimetry (TrueOne 2400, Parvo Medics, Sandy, Utah, USA) under standard conditions [25]. The final 20min of respiratory gases were used to calculate RMR using the Weir Equation [26]. Participants' RMR was multiplied by an average U.S. activity factor of 1.65 to estimate daily energy needs [27], which was only used for determination of the test meals. In addition, alcohol consumption habits were assessed by the Alcohol Use Disorders Identification Test [28]. If individuals qualified for the study, subjects were randomized to one of the three treatment groups by a researcher that was not involved in data collection or analysis. An allocation ratio of 1:1:1, a permuted block design (balanced for age, sex, and BMI), and a random number generator were used to randomize participants.

Pre-Diet Intervention Visit (V1)

Subjects completed a two-day food diary containing one weekend day and one week day [29] between the screening visit and the pre-diet intervention visit. One of the food diaries took place the day before V1. In addition, the night before V1, participants consumed a lead-in dinner meal and snack (provided by research personnel) that contains 50% of total energy from carbohydrate, 15% protein, and 35% fat. For V1, participants arrived at the HNL following an 8-12h overnight fast and 24h without exercise or alcohol. Height, weight, waist-hip circumference, blood pressure, and RMR were measured.

Following anthropometrics, participants then consumed a SFA-rich, high-fat breakfast shake within 10min. This meal provided 17% of total daily energy needs and was made from an original milk chocolate ready-to-drink shake (Ensure, Abbott Nutrition, Abbott Laboratories, Inc., Columbus, Ohio, USA) with added fat sources as previous described [30]. The nutrient breakdown of this test meal is provided in **Table 6.1.** Four ounces of water was used to rinse out the container and then ingested to ensure the entire liquid meal was consumed. Following the SFA meal challenge, indirect calorimetry was measured intermittently for 3.5h. For every 30-minute time segment, there was 20min of data collection followed by a 10min break. Four ounces of water was provided once/h postprandially.

8-Week Dietary Intervention

The day after V1, all participants began the 8-week intervention. Written diet instructions were provided to all participants. Participants in the control group were instructed to avoid all forms of nuts and to consume $\leq$2 servings (64g) of nut butter per week. Participants in the ADD group were provided with 68g (~0.5 cup or 2.25 ounces) portions of pecans to consume as part of their free-living diet with no additional diet instructions. This dose was chosen based on

previous research that demonstrated therapeutic benefits of 68g/d of pecans in healthy subjects [31]. Participants in the SUB group were instructed to substitute the 470 kilocalories provided by the 68g of pecans for foods habitually consumed in their free-living diet. A trained researcher that previously completed 1,200 hours of supervised dietetics practice guided the participants on how to make appropriate energy substitutions based on their previously completed food diaries. For example, if the participant habitually consumed snacks throughout the day, the research personnel highlighted the energy content of the snacks and asked the participant if it was feasible to replace the habitual snacks with the provided pecans. The guidance provided was individualized based on each participant's dietary intake. Similar to the control group, the pecan groups were instructed to avoid all other nuts and to consume $\leq$2 servings of nut butter/wk. In addition, they were instructed to eat the pecans in their raw form (no roasting, cooking, or baking) but could add them to other foods. All subjects were instructed to avoid consuming >3 drinks/d for men or >2 drinks/d for women and were asked not to make any other changes to their diet or activity levels. Participants were unaware of the diet instructions that were provided to other treatment groups to prevent unintentional or intentional changes in behavior.

Weekly Responsibilities

Subjects in the ADD and SUB groups completed a daily nut compliance log that detailed the time of day for pecan consumption. Nut compliance logs were submitted to research staff once per week. Poor compliance was categorized as consumption of <75% of pecans throughout the 8-week intervention. All participants completed a food diary once per week alternating between weekdays and weekend days. Daily nutrient intakes based on food diaries were assessed using The Food Processor SQL software (version 10.12.0). The nutrients from the two baseline food diaries and then the food diaries from weeks 1-8 were averaged before analysis.

Post-Diet Intervention Visit (V2)

After 8 weeks of the diet intervention, participants reported for V2 under the same conditions as V1. Participants completed the exact same study procedures and measurements that took place at V1, including the SFA meal challenge.

Calculations

The final 20min of the 30min fasted indirect calorimetry measurement at the screening visit and both testing visits was used to calculate RMR using the Weir Equation [26]. For the SFA meal challenges at V1 and V2, the final 15min from each 20min postprandial measurement was used to calculate EE [26] and respiratory exchange ratio (RER). DIT was calculated by subtracting postprandial EE from the baseline EE. The RER was calculated by dividing the O_2 consumption by the CO_2 production. Macronutrient utilization was calculated with the equations developed by Frayn et al [32]. Finally, methanol burns were conducted for the development of correction factors that were applied to the collected data each day [33]. The average correction factors were 99.5% and 98.6% for O_2 and CO_2, respectively.

Statistical Analyses

SAS version 9.2 statistical package (SAS Institute Inc, Cary, NC, USA) was used for statistical analyses. All values were reported as mean±SEM unless otherwise noted. Statistical significance was set at $p \leq 0.05$. A samples size of 36 (12 per group) was estimated to detect a significant change in postprandial fat oxidation using G*power 3.19.7 assuming at least 80% power and an α of 0.05 based on the 0.2g/15min increase in postprandial fat oxidation observed in a previous study conducted by Stevenson et al [18]. An unpaired t-test was used to assess differences in nut compliance between the two pecan groups. For primary outcomes, within

group analyses were conducted followed by between group analyses and finally post hoc analyses (when appropriate). For time course data, change from baseline was calculated (baseline value subtracted from each postprandial time point), then a three-way (treatment, visit, time) repeated measures ANOVA was used to test for within group differences. In addition, the change in incremental area under the curve (iAUC) from pre- to post-intervention within each group was calculated for time course data to compare between groups. A one-way ANOVA was used to test for differences at baseline and between groups, and a two-way repeated measures ANOVA was used to test for differences within groups from pre- to post-intervention for anthropometrics, dietary intake, and fasting measures of metabolism. Post hoc analyses were completed using least square means with no multiple testing adjustment.

Results

Participants

Fifty-six individuals were allocated to an intervention group, but nine participants did not start or complete the intervention and/or testing visits **(Figure 6.1)**. Therefore, forty-seven subjects (n=10 women and n=6 men for control, n=11 women and n=4 men for ADD, and n=12 women and n=4 men for SUB) completed the study and were included in the final per protocol analyses. Participant characteristics at baseline are presented in **Table 6.2**. There were no differences between groups at baseline for anthropometric or metabolic variables. Additionally, there were no differences between or within groups from pre- to post- intervention for body weight, BMI, waist circumference, hip circumference, systolic blood pressure, or diastolic blood pressure (data not shown).

On average, participants in both the ADD and SUB groups consumed 95% of pecans provided, and compliance was not different between groups. There was no documentation of nut

intake on the food diaries of the control group. Further, no participant reported poor compliance (defined as <75% of pecans consumed throughout intervention). Based on the analysis of weekly food diaries, as expected due to the daily pecan consumption, total fat (78.1±8.9 to 124.8±10.7 and 85.8±6.9 to 110.7±4.8g; $p<0.0001$ and $p=0.001$), MUFA (32.2±3.9 to 62.4±4.7 and 35.0±2.7 to 53.2±2.1g; $p<0.0001$ for both), PUFA (17.4±2.8 to 32.3±2.3 and 21.9±3.2 to 29.7±1.6g; $p<0.0001$ and $p=0.01$), and dietary fiber (12.8±1.5 to 20.5±1.6 and 14.0±2.4 to 18.0±1.7g; $p=0.0002$ and $p=0.04$) increased significantly within the ADD and SUB groups, respectively. In addition, there was a trend for an increase in protein and energy intake within the ADD group (70.8±5.4 to 80.5± 7.9g and 1861±145 to 2309±181; $p=0.07$ and $p=0.13$, respectively) but not within the SUB or control groups. Regarding micronutrients, copper and magnesium increased within ADD (0.4±0.6 to 1.3±0.1 and 115.3±12.1 to 203.9±16.8mg; $p<0.0001$ and $p=0.0002$) and SUB (0.6±0.1 to 1.2±0.1 and 118±15.0 to 179.7±4.3mg; $p<0.0001$ and $p=0.006$) groups, but there were no other changes for fat- or water-soluble vitamins or minerals. Within the control group, there was an increase in MUFA (27.6±3.5 to 34.0±3.3g; $p=0.05$) but no other changes.

The increase in total fat intake in the food diary analysis (46.7±6.5 and 25.0±7.2 vs 4.1±8.4g; $p<.001$), MUFA (33.3±3.6 and 21.9±2.4 vs 0.2±2.7g; $p<0.0001$), copper (0.9±0.1 and 0.6±0.2 vs -0.1±0.1mg; $p<0.0001$), and magnesium (88.6±19.3 and 61.8±18.1 vs -3.4±27.7mg; $p=0.01$) from baseline to throughout the intervention were significantly greater in the ADD and SUB groups vs the control group as expected. Furthermore, the increase in energy intake (467±134 vs 65±119 and 117±184kcal; $p=0.04$) and PUFA (16.0±2.5 vs 7.8±2.7 and 1.2±3.7g; $p=0.01$) from baseline to throughout the intervention were significantly greater in the ADD vs the SUB and control groups. Finally, there was a trend for a greater increase in protein intake (9.7±4.0 vs -5.3±4.9g; $p=0.07$) from baseline to throughout the intervention in the ADD vs SUB

group. There were no significant differences between groups at baseline for intake of energy, macronutrients, or fatty acid composition.

Fasting Metabolism Measures

Fasting RMR, RER, fat oxidation, and carbohydrate oxidation are presented in **Figure 6.2**. There was an increase in fasting RMR from pre- to post-intervention in the SUB group (1,607±117 to 1,701±114kcal/d; p=0.01) but not in the ADD (1,583±68 to 1,591±71kcal/d; p=0.84) or control (1,753±118 to 1,763±117kcal/d; p=0.80) groups. Also in the SUB group from pre- to post-intervention, there was a significant decrease in fasting RER (0.85±0.01 to 0.83±0.01; p=0.05) which corresponded to an increase in fasting fat oxidation (0.83±0.08 to 0.99±0.08g/20min; p=0.009) but not carbohydrate oxidation (2.27±0.27 to 2.04±0.30g/20min; p=0.17). There were no changes from pre- to post-intervention in fasting RER (0.82±0.01 to 0.83±0.01 and 0.85±0.02 to 0.85±0.01; p=0.75 and p=0.91), fat oxidation (1.05±0.10 to 1.01±0.09 and 0.88±0.14 to 0.87±0.11g/20min; p=0.76 and 0.52), or carbohydrate oxidation (1.87±0.18 to 1.98±0.27 and 2.19±0.30 to 2.18±0.19g/20min; p=0.60 and p=0.97) within the ADD and control groups, respectively. The change from pre- to post-intervention for RMR (10±34 vs 7±29 vs 94±48kcal/d; p=0.18), fasting RER (0.001±0.014 vs 0.004±0.007 vs -0.021±0.010; p=0.22), fasting fat oxidation (-0.01±0.10 vs -0.04±0.06 vs 0.16±0.06g/20min; p=0.15), and fasting carbohydrate oxidation (-0.01±0.26 vs 0.11±0.10 vs -0.23±0.11; p=0.40) were not different between the control, ADD, and SUB groups, respectively.

Postprandial Metabolism Measures

Time course data for DIT, RER, fat oxidation, and carbohydrate oxidation in response to the high SFA meal challenge are presented in **Figures 6.3 and 6.4**. Postprandial DIT was at the post-intervention vs baseline in the ADD group (p<0.001) but not in the SUB or control groups

(Figure 6.3A-C). Conversely, in the time course data for postprandial substrate utilization there were no changes within any group from pre- to post-intervention for RER (**Figure 6.3D-F**), fat oxidation (**Figure 6.4A-C**), or carbohydrate oxidation (**Figure 6.4D-F**). Finally, the change in meal response from V1 to V3 was not significantly different between groups for DIT (change in iAUC: control: -0.03±0.29 vs ADD: 0.53±0.24 vs –SUB: 0.21±0.43kcals/3.5h; p=0.24), RER (control: 0.00±0.01 vs ADD: 0.54±0.24 vs SUB: –0.21±0.43; p=0.88), fat oxidation (control: 0.05±0.08 vs ADD: 0.01±0.07 vs SUB: -0.01±0.05g/3.5h; p=0.83), or carbohydrate oxidation (control: 0.10±0.20 vs ADD: 0.10±0.16 vs SUB: -0.01±0.11g/3.5h; p=0.69).

Discussion

Although several previous studies have shown beneficial effects on metabolism from meals or diets rich in USFAs [8], the impact of pecan consumption on fasting and postprandial metabolism was largely unknown. For the first time, we have shown that daily pecan consumption (68g) for 8 weeks resulted in significant improvements in fasting and postprandial EE and fasting substrate oxidation (lower RER; higher fat oxidation) in at least one of the two pecan groups. Contrary to our hypothesis, there were no changes in postprandial substrate utilization in either group. The significant changes in metabolism in this study indicate that the type of dietary instructions for pecan consumption, namely isocaloric substitution, may impact energy metabolism outcomes.

The improvement in fasting RMR is comparable to previous work in MUFA-rich diets. Previous studies demonstrated that substituting sources of MUFA (including peanuts) for sources of fat in the diet increased RMR by 5-11% [11, 19]. In the present study, the MUFA-rich pecans increased RMR by 6% (an average of 94kcal/d) in the SUB group only. It is estimated that an excess of 50kcals/d accounts for gradual weight gain in U.S. adults [34], highlighting the clinical

benefit of the observed increase in RMR and its potential protective effect against annual weight gain.

In addition, there was also a 19.1% increase in fasting fat oxidation in the SUB group. There is limited literature to compare our results to, but similar studies observed no change in fasting fat oxidation following a diets rich in MUFA or PUFA, with or without tree nuts [9, 10, 12, 18, 22, 35, 36]. It is possible that the null findings in previous studies are due to the shorter intervention duration (<4 weeks), lack of methanol burns to correct metabolism data (in two studies), healthier populations, source of USFA, or a smaller dose of tree nuts. Only one study investigated the impact of a large dose of almonds as part of a free living diet and reported no changes in substrate utilization [22]. Although almonds and pecans have similar fatty acid profiles, it is possible that the improvements in fasting fat oxidation following pecan, but not almond, consumption are mediated by the flavonoid content of the pecans, which is two-fold greater than almonds [37]. There is evidence to support this as other flavonoid-rich diets have shown increases in fasting fat oxidation [38, 39]. Previous research also indicates that a lower rate of fasting fat oxidation is predictive of weight gain and insulin insensitivity [7, 40, 41]. The 19% increase we observed is physiologically relevant since increases in fat oxidation as low as 17% have been shown to improve insulin sensitivity [42]. Together, this provides strong evidence that the magnitude of change in both fasting RMR and substrate utilization within the SUB group is clinically meaningful for weight management and chronic disease prevention.

In the postprandial state, we observed a 41% increase in DIT within the ADD group. Other studies observed 22-28% higher DIT following high-PUFA and/or walnut-containing meals vs high-SFA meals [43] but no study has shown improvements in DIT following an USFA-enriched diet when the test meal did not contain the USFA or tree nut of interest.

Therefore, the results of our study are novel and indicate that a pecan-enriched diet improves the metabolic response to a high SFA meal devoid of pecans. This outcome is clinically meaningful since Americans consume more SFAs on weekends [17, 44], so eating pecans daily could be protective against those occasional high SFA meals. Conversely, it was unexpected that postprandial substrate utilization did not improve in either pecan group since our previous study with a similar design (including the high-SFA test meal) showed increased postprandial fat oxidation after a 7d high-PUFA diet containing walnuts [18]. Despite the similarities in test meals between the studies, the divergent results may be due to difference in intervention duration, population, or the fatty acid profile of the diet (PUFA-rich walnuts and other foods vs pecans) between the two studies.

Although this study was not designed to be mechanistic, we can speculate how pecans improved energy metabolism. Self-reported intake of MUFA and PUFA increased within both pecan groups. Furthermore, pecans contain the greatest total phenol content of all tree nuts [37]. Previous research highlights the effect of dietary fatty acid subclasses and polyphenols on EE and substrate utilization in humans [18, 38, 39, 45]. Peroxisome proliferator-activated receptor-α (PPAR-α), a transcription factor most active in the fasted state, is responsible for suppressing genes involved in fat metabolism and thermogenesis [46, 47]. Animal model studies demonstrate that both polyphenol-rich foods and USFA increase the PPAR-α mRNA expression [48-51], which may explain the improvements in fasting but not postprandial substrate utilization. With respect to the increased DIT in the ADD group, it may have been mediated by uncoupling protein 1 (UCP1), a key regulator of thermogenesis [52, 53]. An animal model study indicated that a MUFA-rich diet induced greater UCP1 mRNA expression and total-body oxygen consumption (indicating higher EE) compared to diets rich in PUFA and SFA [54]. Therefore,

both the fatty acid profile and phenolic content of the pecans may have mediated the metabolic improvements, but the exact mechanism remains to be elucidated.

Although research in almonds and peanuts indicated that presence of dietary instructions may influence metabolism [19, 21, 22], this was the first study to directly compare the impact of different dietary instruction on metabolic outcomes. It is unclear why DIT improved in the ADD but not SUB group. One explanation for this is the different change in protein intake within pecan groups (trend for greater increase in protein consumption in ADD compared to SUB) since dietary protein is the most thermogenic of the macronutrients [55]. Notably, the protein content of the high-SFA test meal was consistent, but the trend for higher daily protein intake in the ADD group may have influenced the DIT response. In addition, it is possible that the greater increase in energy intake in ADD vs. SUB may have contributed to the increased DIT due to adaptive thermogenesis mechanisms [56], but this phenomenon remains controversial and is typically observed following extreme overfeeding (excess of 1000kcal/d) [57, 58]. Further, there was no change in body weight within this group. Likewise, it is unknown why the improvements in RMR and fasting substrate utilization occurred in the SUB group only. It is possible that the SUB group substituted the pecans for less healthy foods (such as those rich in SFA) in the habitual diet, which could have resulted in improved fasting energy metabolism. Although this did not show up in the analysis of the food diaries, it is well established that self-reported food intake is vulnerable to under and over-reporting [59, 60]. Consistent with our results, Alper & Mattes [19] observed increases in RMR when peanuts were substituted for other sources of fat in the diet, while almond studies without dietary substitution instructions reported no change in RMR [21, 22]. The divergent results in the two pecan groups highlights a limitation of the study—the free-living diet. The beneficial changes in energy metabolism may be due to not only

the daily pecan consumption but other, non-significant changes to the overall diet that could occur. Conversely, the free-living diet is also a strength as it increases the generalizability or applicability of our findings.

Other limitations to the present study include the relatively high dose of pecans for a short duration. Thus, it is unknown if the changes in energy metabolism would persist over a longer duration or with a smaller dose of pecans. Due to our study design, it also remains unknown whether an acute meal containing pecans would impact postprandial metabolism. Despite this limitation, the present study provides valuable evidence that the pecan-enriched diet protects against the detrimental metabolic effects of the occasional high-SFA meal. Furthermore, hormonal fluctuations due to the menstrual cycle of premenopausal female participants could have affected our metabolic outcomes [61] and were not controlled for throughout the study. Finally, this study was not designed to evaluate the effects of sex, race, or BMI, so future work in this area is warranted.

In conclusion, daily pecan consumption increased RMR, improved fasting substrate oxidation (lower RER and higher fat oxidation), and increased DIT in at least one of the two pecan groups. It appears that the presence or absence of dietary substitution instructions may be an important determinant of energy metabolism outcomes. These findings are clinically meaningful because the magnitude of change is large enough to impact long-term weight management and chronic disease prevention. Future studies should investigate the impact of dietary substitution instruction on other tree nuts, the biochemical mechanisms driving metabolic changes with pecan consumption, and different amounts of pecans to determine the lowest effective dose.

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Table 6.1. Nutrient breakdown for the high saturated fat meal challenge

Composition	SFA-rich meal
Percentage of total energy from	
Protein	5.0
Carbohydrate	25.0
Fat	69.5
Percentage of energy from fatty acids	
SFA	46.9
MUFA	15.7
PUFA	6.9

Abbreviations: HF=high fat, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid, SFA=saturated fatty acid

Table 6.2 Participant characteristics at baseline

	Control (n=16)	Pecan ADD (n=15)	Pecan SUB (n=16)
Age (y)	49 ± 14	50 ± 11	46 ± 11
Height (cm)	168.1 ± 11.7	166.9 ± 7.6	166.4 ± 10.6
Weight (kg)	91.9 ± 27.7	84.6 ± 14.0	92.6 ± 20.2
Body Mass Index (kg/m^2)	31.9 ± 6.8	30.3 ± 4.2	33.4 ± 6.7
Waist Circumference (cm)	100.4 ± 16.8	96.6 ± 9.1	101.9 ± 14.8
Hip Circumference (cm)	114.4 ±15.2	112.2 ± 9.3	116.5 ±12.8
Resting Metabolic Rate (kcal/d)	1753 ± 407	1583 ± 246	1607 ± 439
Fasting RER (units/15min)	0.85 ± 0.06	0.82 ± 0.01	0.85 ± 0.01
Fasting fat oxidation (g/15min)	0.88 ± 0.14	1.05 ± 0.10	0.83 ± 0.08
Fasting CHO oxidation (g/15min)	2.19 ± 1.09	1.87 ± 0.55	2.27 ± 1.03

All values are mean ± standard deviation (n=47). There were no differences between groups at baseline (pre-intervention visit; v1). Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet; CHO=carbohydrate; RER=respiratory exchange ratio;

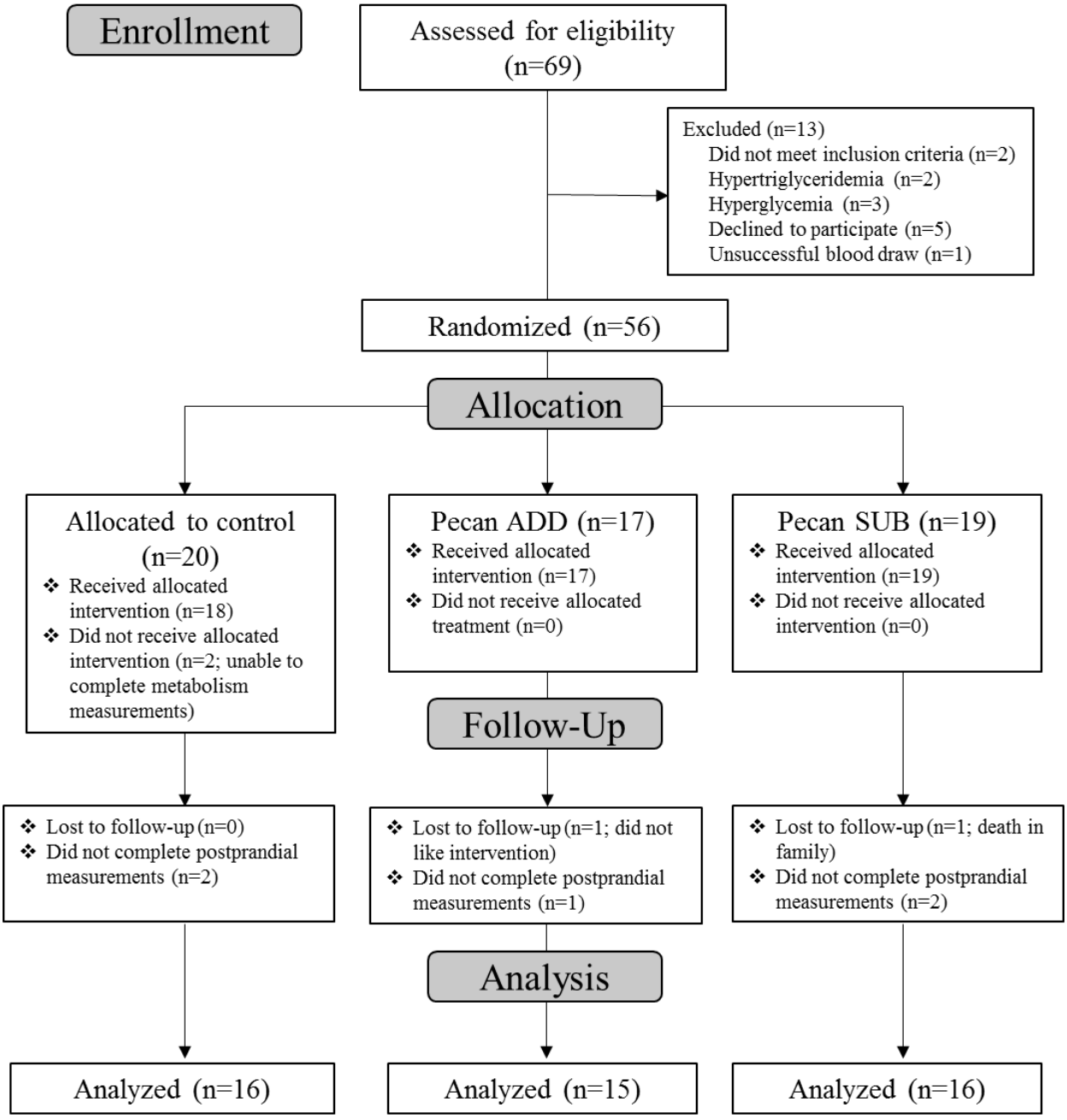

Figure 6.1 Consolidating Standards of Reporting (CONSORT) flow diagram for the selection of participants.

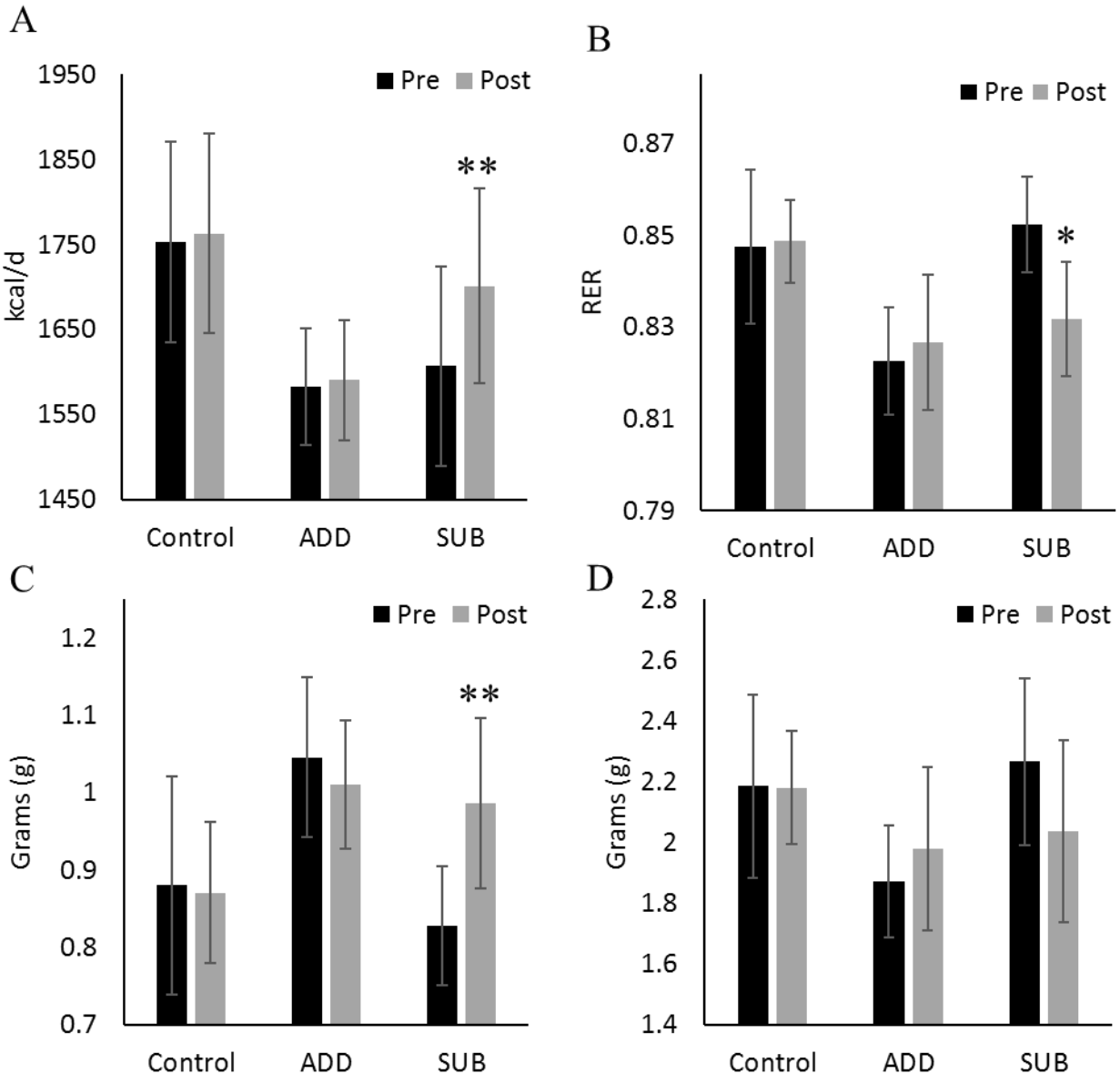
A
1950
1850
1750
1650
1550
1450
kcal/d
Pre
Post
**
Control
ADD
SUB
B
0.87
0.85
0.83
0.81
0.79
RER
Pre
Post
*
Control
ADD
SUB
C
1.2
1.1
1
0.9
0.8
0.7
Grams (g)
Pre
Post
**
Control
ADD
SUB
D
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
Grams (g)
Pre
Post
Control
ADD
SUB

Figure 6.2 (A) Resting metabolic rate (RMR), (B) fasting respiratory exchange ratio (RER), (C) fasting fat oxidation, and (D) fasting carbohydrate oxidation at pre- and post-intervention visits (8 weeks). Within the SUB group, there was a significant increase in RMR and fat oxidation and a decrease in RER from pre- to post-intervention. Asterisks denote level of statistically significant difference versus the control group (* for $p<0.05$, ** for $p<0.01$, and *** for $p<0.001$). All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet; kcals=kilocalorie; RER=respiratory exchange ratio.

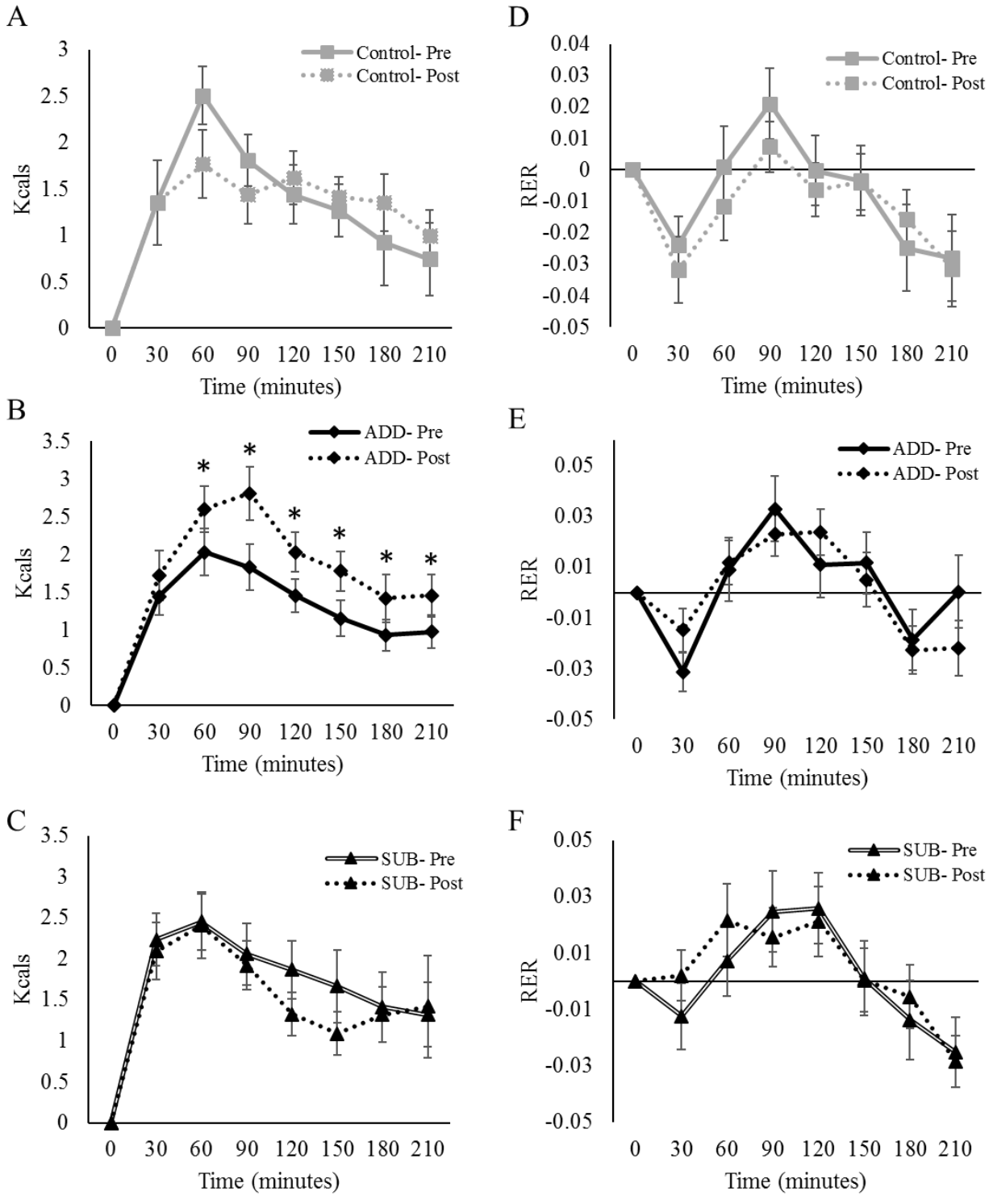
A
Control- Pre
Control- Post
Kcals
Time (minutes)
B
ADD- Pre
ADD- Post
Kcals
Time (minutes)
C
SUB- Pre
SUB- Post
Kcals
Time (minutes)
D
Control- Pre
Control- Post
RER
Time (minutes)
E
ADD- Pre
ADD- Post
RER
Time (minutes)
F
SUB- Pre
SUB- Post
RER
Time (minutes)

Figure 6.3 Time course for change in diet induced thermogenesis (DIT) and respiratory exchange ratio (RER) in the control, ADD, and SUB groups at the pre- and post-intervention (8 weeks) visits in response to the high-saturated fatty acid meal (Figures 6.3A-C and D-F, respectively). There was an increase in DIT at the post- vs. pre-intervention visit within the ADD group ($p<0.001$). All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet; kcals=kilocalorie; RER=respiratory exchange ratio.

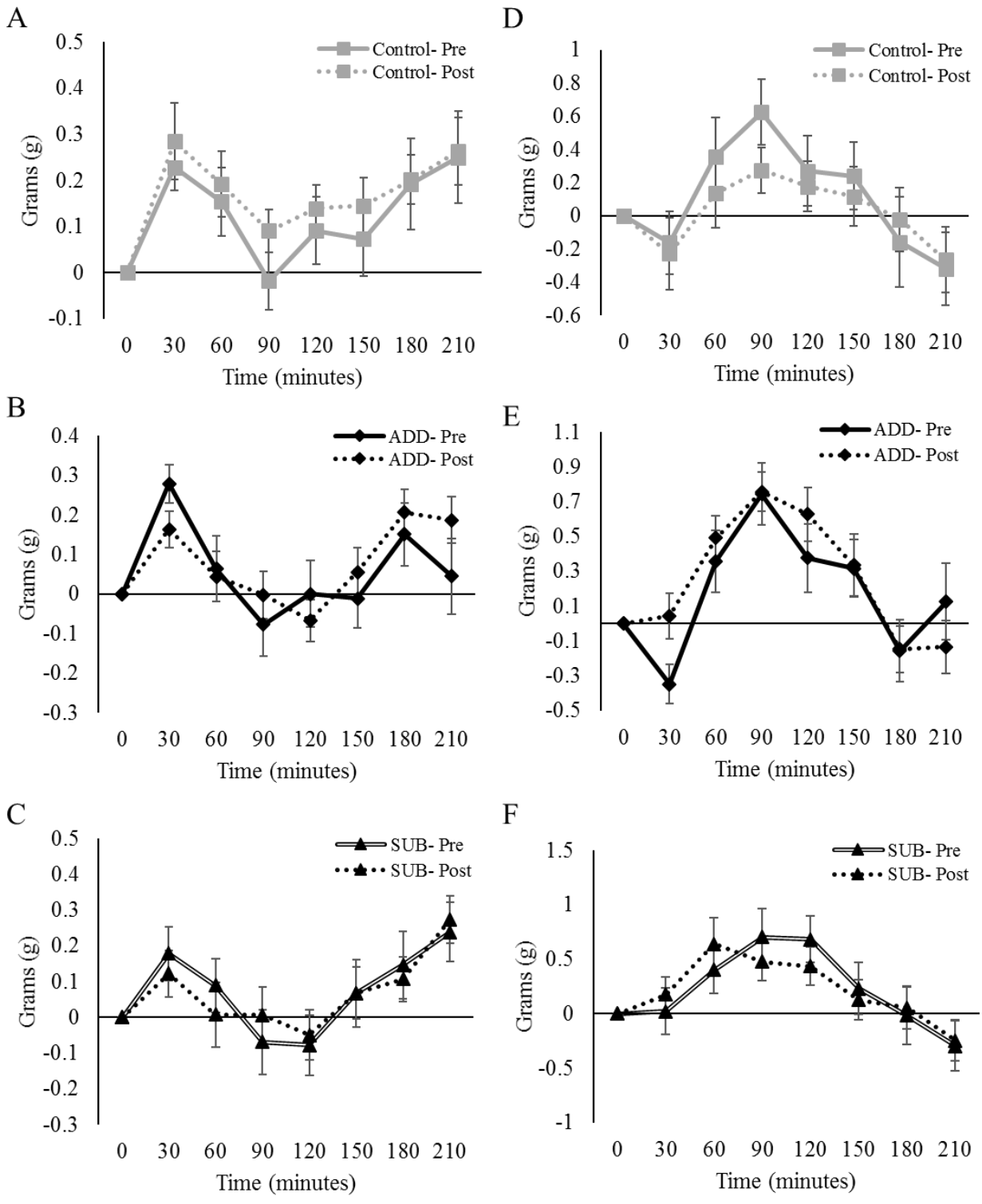
A
Control- Pre
Control- Post
Grams (g)
0.5
0.4
0.3
0.2
0.1
0
-0.1
0 30 60 90 120 150 180 210
Time (minutes)
D
Control- Pre
Control- Post
Grams (g)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
0 30 60 90 120 150 180 210
Time (minutes)
B
ADD- Pre
ADD- Post
Grams (g)
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
0 30 60 90 120 150 180 210
Time (minutes)
E
ADD- Pre
ADD- Post
Grams (g)
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
-0.3
-0.5
0 30 60 90 120 150 180 210
Time (minutes)
C
SUB- Pre
SUB- Post
Grams (g)
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
0 30 60 90 120 150 180 210
Time (minutes)
F
SUB- Pre
SUB- Post
Grams (g)
1.5
1
0.5
0
-0.5
-1
0 30 60 90 120 150 180 210
Time (minutes)

Figure 6.4 Time course for change in fat and carbohydrate oxidation in the control, ADD, and SUB groups at the pre- and post-intervention (8 weeks) visits in response to the high-saturated fatty acid meal (Figures 6.4A-C and D-F, respectively). There were no differences within groups. All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet.

CHAPTER 7

PECAN-ENRICHED DIETS DECREASE POSTPRANDIAL LIPID PEROXIDATION AND INCREASE TOTAL ANTIOXIDANT CAPACITY IN ADULTS AT-RISK FOR CARDIOVASCULAR DISEASE[5]

[5]Guarneiri, L.L., C.M. Paton, and J.A. Cooper. *Nutrition Research,* 2021. 93: p. 69-78. Reprinted here with permission of the publisher.

Abstract

Pecans are a rich source of antioxidants, but the effect of regular consumption on post-meal responses is unknown. The objective of this study was to examine the impact of daily pecan consumption for 8 weeks on fasting and postprandial lipid peroxidation, total antioxidant capacity (TAC), and tocopherols in adults at higher risk for cardiovascular disease (CVD) (hypercholesterolemia or elevated adiposity). We hypothesized that daily pecan consumption would result in increased fasting γ-tocopherols, increased fasting and postprandial TAC, and decreased fasting and postprandial lipid peroxidation. This was a randomized, parallel, controlled trial with 3 treatments: two pecan groups and a nut free control (n=16). The ADD group (n=15) consumed pecans as part of a free-living diet, and the SUB group (n=16) substituted the pecans for isocaloric foods from their habitual diet. At the pre- and post-intervention, a high saturated fat breakfast shake was consumed with postprandial blood draws over 2h. In the ADD and SUB groups, postprandial lipid peroxidation was suppressed (iAUC: 0.9±1.3 to -2.9±2.0 and 4.5±1.7 to 0.7±1.1μM/2h, respectively; $p<0.05$) and TAC was elevated (iAUC: -240.8±110.2 to 130.9±131.7 and -227.6±131.2 to 208.7±145.7μM Trolox Equivalents/2h, respectively; $p<0.01$) from pre- to post-intervention. Furthermore, there was an increase in γ-tocopherol from pre- to post-intervention within the ADD (1.4±0.1 to 1.8±0.1μg/mL; $p<0.001$) and SUB groups (1.8±0.2 to 2.1±0.2μg/mL; $p<0.05$). There were no changes in any variable within the control group. These findings suggest that daily pecan consumption protects against oxidative stress that occurs following a high-fat meal in adults at risk for CVD.

Introduction

Elevated adiposity in adults is characterized by chronic low-grade inflammation and increased oxidative stress (OS), which contributes to the development of obesity-related complications such as metabolic syndrome, type 2 diabetes mellitus, cardiovascular disease (CVD), and some cancers [1]. Furthermore, increasing evidence suggests that the hypertriglyceridemia and hyperglycemia that occur following consumption of foods typical in a Western diet, such as high saturated fatty acid (SFA) meals, also contribute to the production of OS and disease progression [2-4]. It is estimated that most Americans remain in the postprandial state for two-thirds of the day [5], highlighting the crucial need to mitigate the detrimental effects of the postprandial period. One plausible approach is to incorporate bioactive foods rich in antioxidants. Previous research has shown that inclusion of such foods with a high-fat meal can attenuate postprandial OS [6-10]; however, it is less clear if habitual consumption of an antioxidant-rich food protects against the rise in OS from an occasional high SFA meal devoid of antioxidants.

Tree nuts are a rich source of antioxidants, and pecans contain the highest total phenolic content of all tree nuts (predominately flavonoids, proanthocyanidins (PAC), and phenolic acids) [11]. Furthermore, pecans are a rich source of γ-tocopherols (16.6 mg/68 g) [12]. Therefore, it is feasible that a diet rich in pecans may protect against chronic diseases that are associated with elevated OS. Previous research in healthy adults has shown that acute consumption of pecans results in increased γ-tocopherol and total antioxidant capacity (TAC) and decreased lipid peroxidation [13, 14]. Furthermore, a four-week controlled feeding trial containing pecans resulted in increased fasting γ-tocopherol and decreased lipid peroxidation [12]. However, it is unknown if these protective effects of pecan consumption occur in response to a high SFA meal

in a population at risk for CVD. Likewise, since the original four-week study was a controlled feeding trial, it is unknown whether similar beneficial effects will be observed when pecans are simply added to a free-living diet or are incorporated into a diet in which isocaloric substitution instructions for pecans are provided.

The purpose of this study was to investigate the impact of daily pecan consumption (with and without dietary/isocaloric substitution instructions) for 8 weeks on fasting and postprandial TAC and lipid peroxidation and fasting γ-, α-, and δ-tocopherols in adults with elevated risk for CVD. We hypothesized that daily pecan consumption, regardless of the presence or absence of dietary substitution instructions, would result in increased fasting γ-tocopherols, increased fasting and postprandial TAC, and decreased fasting and postprandial lipid peroxidation. We also hypothesized that these improvements would be significantly greater than that of the control group. To test this hypothesis, we conducted a clinical trial comparing the impact of two pecan-enriched diet to a nut-free diet on fasting and postprandial antioxidant status.

Methods

Study Design

This study was a single-blind, randomized, parallel controlled trial (clinicaltrials.gov:NCT04376632) involving an 8-week intervention conducted in the Human Nutrition Laboratory (HNL) at the University of Georgia. There was a screening visit and 3 testing visits (pre-, mid-, and post-diet intervention). Subjects were randomly assigned (balanced blocks stratified by age, sex, and body mass index (BMI)) to one of three groups: a “no nut” control group, or one of two pecan groups (ADD or SUB). Subjects in the ADD and SUB groups each consumed 68 g of pecans per day for 8 weeks; however, dietary instructions for the incorporation of pecans into the diet differed. For the ADD group, pecans were consumed as part

of a free-living diet, while in the SUB group, participants received counseling at the pre-intervention visit on how to substitute pecans for isocaloric foods from their habitual diet. This study was approved by the Institutional Review Board for human subjects (STUDY00005985), and informed written consent was obtained from each participant prior to testing.

Participants

Sixty-nine sedentary men and women between the ages of 30 and 75 y with high cholesterol or a BMI of ≥28 kg/m^2 were assessed for eligibility. Inclusion based on "high cholesterol" levels was defined as either "borderline high/undesirable" in two blood lipid categories or "high" in total cholesterol (TC) or low-density lipoprotein (LDL) (**Table 7.1)**. To rule out individuals with familial hypercholesterolemia, participants with LDL levels greater than the 95th percentile or high density lipoprotein levels lower than the 20th percentile were excluded. Other exclusion criteria included habitual nut consumption (>2 servings/week), nut allergies, special diets (i.e. ketogenic diet, intermittent fasting), excessive alcohol use (>3 drinks/d for men or >2 drinks/d for women), tobacco or nicotine use, exercise >3h/week, weight loss or gain >5% of body weight in the past 3 months, plans to begin a weight loss or exercise regimen, history of medical events or medication use affecting digestion, absorption, or metabolism, gastrointestinal surgery, and chronic or metabolic diseases. Individuals taking lipid-lowering medications, fish oil supplements, steroid/hormone therapy, or medications for diabetes mellitus or Attention Deficit Hyperactivity Disorder were also excluded. Finally, individuals with the following biomarkers were excluded: fasting glucose >126mg/dL, fasting triglycerides (TG) >350mg/dL, or blood pressure >180/120mmHg. Eligibility based on blood lipids and glucose were determined from fasting blood samples at the screening visit. Subjects were recruited through

flyers, paid advertisements in newspapers and on social media, campus emails, and word of mouth.

Protocol

Screening Visit

Individuals arrived at the HNL following an 8-12 h overnight fast and 24 h without exercise or alcohol. A fasting blood draw for a lipid panel and glucose measurement was obtained, and anthropometrics including height, weight, blood pressure and waist and hip circumferences were measured. Resting metabolic rate (RMR) (kcal/d) was measured for 30min using indirect calorimetry (TrueOne 2400, Parvo Medics, Sandy, Utah, USA) under standard conditions [15]. The final 20 min of respiratory gases were used to calculate RMR using the Weir Equation [16]. Participants' RMR was multiplied by an average U.S. activity factor of 1.65 to estimate daily energy needs [17], which was used for calculating the high-SFA test meal. In addition, alcohol consumption habits were assessed by the Alcohol Use Disorders Identification Test to confirm habitual consumption of <3 drinks/d in men and <2 drinks/d in women [18]. If individuals qualified for the study, subjects were randomized to one of the three treatment groups using a random number generator.

Pre-Diet Intervention Visit (V1)

The night before V1, participants consumed a lead-in dinner meal and snack (provided by research personnel) that contained 50% of total energy from carbohydrate, 15% of energy from protein, and 35% of energy from fat. The morning of V1, participants arrived at the HNL following an 8-12h overnight fast and 24h without exercise or alcohol. Height, weight, waist-hip circumference, and blood pressure were measured. Next, an intravenous (IV) catheter was inserted into the antecubital vein for the fasting blood draw, and the line was kept patent with

saline. Participants then consumed a SFA-rich breakfast shake within a 10-minute period. This high-fat meal provided 17% of total daily energy needs based on the RMR measurements from the screening visit and was made from an original milk chocolate ready-to-drink shake (Ensure, Abbott Nutrition, Abbott Laboratories, Inc., Columbus, Ohio, USA), unsalted butter, red palm oil, coconut oil, soy lecithin granules, and powdered chocolate drink mix. The nutrient breakdown for the test meal is provided in **Table 7.2.** Four ounces of water was used to rinse out the container and then ingested to ensure the entire liquid meal was consumed. Following the SFA meal challenge, blood draws occurred at 30, 60, and 120min postprandially. Four ounces of water was provided at fasting and once/h postprandially.

Dietary Intervention

The day after V1, all participants began the 8-week intervention. Written diet instructions were provided to all participants. Those in the control group were instructed to avoid all forms of nuts and to consume ≤2 servings of nut butter per week. Participants in the ADD group were provided with 68 g (~0.5 cup or 2.25 ounces) portions of pecans to consume as part of their free-living diet with no additional diet instructions. Participants in the SUB group were instructed to substitute the 475 kilocalories provided by the 68 g of pecans for foods habitually consumed in their free-living diet. Research personnel guided the participants on how to make appropriate energy substitutions based on foods habitually consumed in their diet. **Supplemental Table 7.5** shows the complete nutrition information for a 68 g portion of pecans. A 4-week supply of pecans (in 68 g portions) was provided to participants in the ADD and SUB groups at V1 and again at V2. Similar to the control group, the pecan groups were instructed to avoid all other nuts and to consume ≤2 servings of nut butter per week. In addition, they were instructed to eat the pecans in their raw form (no roasting, cooking, or baking). Subjects were permitted to add the

pecans to other foods such as oatmeal, cereal, or salad. Likewise, all subjects were instructed to avoid consuming >3 alcoholic drinks/d (men) or >2/d (women) and were asked not to make any other changes to their diet or activity levels. Participants were unaware of the diet instructions that are provided to other treatment groups.

Weekly Responsibilities

Subjects in the ADD and SUB groups completed a daily nut compliance log that detailed the time of day for pecan consumption. Nut compliance logs were submitted to research staff once per week. Poor compliance was considered to be consumption of <75% of pecans throughout the 8-week intervention.

Mid-Diet Intervention Visit (V2)

After 4 weeks of the dietary intervention, participants reported to the HNL following another 8-12h overnight fast and 24h without exercise or alcohol. The same dinner meal and snack that were consumed prior to the V1 were consumed the night before V2. The same anthropometrics and fasting blood draw from V1 were repeated, exactly as stated above.

Post-Diet Intervention Visit (V3)

After 8 weeks of the diet intervention, participants reported to the HNL for V3 under the same pre-visit conditions as V1 and V2. Participants completed the exact same study procedures and measurements that took place at V1, including the anthropometric measurements and SFA meal challenge.

Sample Analysis

During all 3 testing visits, fasting and postprandial blood samples were drawn into an EDTA vacutainer, immediately placed on ice, and then centrifuged at 3000rpm for 15 min at 4°C. The plasma was aliquoted and stored at -80°C until analysis. The primary outcomes, fasting

and postprandial malondialdehyde (MDA) levels, a marker of lipid peroxidation, were measured by the Thiobarbituric Acid Reactive Substances (TBARS) assay (Cayman Chemical Inc., Ann Arbor, MI, USA), and TAC was measured by an Oxygen Radical Absorbance Capacity (ORAC) assay (Zen Bio, Inc., Morrisville, NC). The secondary outcomes, fasting γ-, α-, and δ-tocopherols was measured by high performance liquid chromatography (HPLC) (Eurofin Craft Technologies, Wilson, NC, USA).

Statistical Analyses

SAS version 9.2 statistical package (SAS Institute Inc, Cary, NC, USA) and GraphPad Prism version 8.0 (GraphPad Software, La Jolla, CA) were used for all statistical analyses. All values were reported as mean±SEM unless otherwise noted. Statistical significance was set at $p \leq 0.05$. A samples size of 39 (13 per group) was estimated to detect a large effect size for the TAC meal response using G*power 3.19.7 assuming at least 80% power and an α of 0.05. For the time course data, change from baseline was calculated (baseline value subtracted from each postprandial time point), then a three-way (treatment, visit, time) repeated measures ANOVA was used to test for within group differences. In addition, incremental area under the curve (iAUC) was calculated using the trapezoidal method, and a two-way ANOVA (treatment, visit) was used to test for within group differences in iAUC, anthropometrics, and fasting measures of antioxidant status. Finally, the change in iAUC from pre- to post-intervention was calculated within each group, and a one-way ANOVA was used to test for differences between groups. All iAUC values are presented for the 2h meal response. Post hoc analyses were done using least-squares mean. Finally, an unpaired t-test was used to assess differences in nut compliance between the two pecan groups.

Results

Participants

Fifty-six individuals were allocated to an intervention group, but nine participants did not start or complete the intervention and/or testing visits **(Figure 7.1)**. Therefore, forty-seven subjects (n=10 women and n=6 men for control, n=11 women and n=4 women for ADD, and n=12 women and n=4 men for SUB) completed the study and were included in the final analyses. Participant characteristics at the pre-intervention visit are presented in **Table 7.3**. There were no differences between groups for anthropometric or antioxidant outcomes. On average, participants in both the ADD and SUB groups consumed 95% of pecans provided, respectively, and compliance was not different between groups. No participant reported poor compliance (defined as <75% of pecans consumed throughout intervention).

Postprandial Biochemical Markers

The meal responses for lipid peroxidation and TAC are presented in **Figures 7.2 and 7.3**. For lipid peroxidation, there was a significant treatment by visit interaction ($p \leq 0.01$) (**Figure 7.2A-C**). The significant effect was for suppressed lipid peroxidation from pre- to post-intervention within the ADD and SUB groups (iAUC: 0.9±1.3 to -2.9±2.0 and 4.5±1.7 to 0.7±1.1 µM/2h, respectively; $p \leq 0.05$) but not in the control group (-1.4±1.9 vs 2.2±1.9 µM/2h; ns) **(Figure 7.3A)**. Likewise, the change in iAUC from pre- to post-intervention for lipid peroxidation was significantly more suppressed in ADD and SUB vs control (-3.8±2.4 and -3.5±1.6 vs 3.7±2.2 µM/2h, respectively; $p \leq 0.05$) **(Figure 7.4A)**. There were no differences between the two pecan groups for time course or iAUC data.

For TAC responses to the high SFA meal, there was a significant treatment by visit interaction ($p \leq 0.05$). (**Figure 7.2D-F**). The significant effect was for elevated TAC from pre- to

post-intervention within the ADD and SUB groups (iAUC: -240.8±110.2 to 130.9±131.7 and -227.6±131.2 to 208.7±145.7 µM Trolox Equivalents (TE)/2h, respectively; $p \leq 0.01$) but not in the control group (-160.0±81.7 vs -162.7±132.2 µM TE/2h; ns) **(Figure 7.3B)**. In addition, the change in iAUC from pre- to post- intervention for TAC was significantly greater in SUB vs control (423.2±109.9 vs 14.1±114.4 µM TE/2h; $p \leq 0.05$) **(Figure 7.4B**), and there was a trend for a greater trend for ADD vs control (355.6±159.6 vs 14.1±114.4 µM TE/2h; p=0.06**)**. There were no differences between the ADD and SUB groups for any TAC measure.

Fasting Biochemical Markers

Fasting lipid peroxidation, TAC, and tocopherols at pre-, mid-, and post-intervention are displayed in **Table 7.4**. There was a significant increase in γ-tocopherol from pre- to mid- and post-intervention within the ADD (1.4±0.1 vs 1.8±0.2 and 1.8±0.1 µg/mL; $p \leq 0.001$) and SUB groups (1.8±0.2 vs 2.3±0.3 and 2.1±0.2 µg/mL; $p \leq 0.05$). Similarly, the change in γ-tocopherol from pre- to post-intervention in the ADD and SUB groups was significantly improved vs. control (0.4±0.1 and 0.2±0.1 vs -0.1±0.1 µg/mL; $p \leq 0.01$ and $p \leq 0.05$, respectively). From mid- to post-intervention, there was a trend for a decrease in fasting TAC within the ADD group (1982±119 vs 1748±99 µM TE; p=0.06) and a significant decrease in the SUB group (2183±154 vs 1878±162 µM TE; $p \leq 0.05$); however, there were no differences in fasting TAC within or between groups from pre- to post-intervention. For lipid peroxidation and α-tocopherol, there were no differences within or between groups. Finally, amounts of δ-tocopherol in the plasma were too small (<0.3µg/mL) to be detected with accuracy for all subjects.

Discussion

In support of our hypothesis, we have shown that daily pecan consumption (68 g) for 8 weeks improved both fasting and postprandial measures of antioxidant status. Furthermore, the

magnitude of that improvement was very large with a 154% and 192% increase in postprandial TAC and a 133% and 84% decrease in postprandial lipid peroxidation in the ADD and SUB groups, respectively. These findings are clinically meaningful because postprandial rises in lipids following a high-fat meal are associated with increased OS and chronic disease risk [19, 20]. Therefore, the simple addition of antioxidant-rich pecans to the daily diet can prevent some of the detrimental effects of consuming the occasional high SFA meal. Contrary to our hypothesis, there were no changes in fasting lipid peroxidation or TAC. However, there were also 17-29% increases in fasting γ-tocopherol in the pecan groups. Coupled with the TAC and lipid peroxidation improvements, daily pecan consumption offered protection in both fasted and fed states. It is also notable that these findings occurred regardless of the type of dietary substitution instructions for eating pecans, which makes the results generalizable to many settings in the population.

Previous research has shown that acute consumption of tree nuts alone [13, 14, 21], as well as high-fat meal consumption in conjunction with antioxidant-rich foods [6-10], results in improved postprandial antioxidant status and/or reduced postprandial OS. Despite the beneficial effects of consuming antioxidant-rich foods acutely, most studies investigating the impact of long-term tree nut consumption (1 to 6 months) report no changes in fasting antioxidant measures [22-25] and never test its effects on an unhealthy meal. To our knowledge, we are the first group to investigate the impact of a longer-term, tree nut-enriched diet on the postprandial response to a high-fat meal devoid of antioxidants. This unique study design truly allows us to show that eating some healthy foods regularly, in this case pecans, can help protect against the detrimental effects of occasionally eating an unhealthy (high SFA) meal. A few other groups have employed a similar study design using different antioxidant-rich foods, such as

anthocyanin-rich mulberry fruit jelly, for 1-4 weeks and showed reduced markers of postprandial OS following consumption of moderate- to high-fat meals without antioxidants [26-28]. These studies are consistent with our results giving strength to the idea that antioxidant-rich foods can offset postprandial OS responses from less healthy meals.

Pecans contain more total phenols compared to all other tree nuts with 1371 mg gallic acid equivalents (GAE)/68 g compared to the next highest tree nuts which are pistachios (1127 mg GAE) and English walnuts (1058 mg GAE) [11]. Furthermore, the daily dose of pecans in the present study provided 23 mg of total flavonoid (predominately flavan-3-ols and anthocyanins), 336 mg PAC, and 1,395 mg of phenolic acid [11]. Previous research demonstrates that PAC and phenolic acids directly quench free radicals through divergent mechanisms [29-31] while flavan-3-ols interact with membrane surfaces to limit the access of lipid soluble oxidants to the membrane, an indirect method of reducing membrane oxidation [32]. Therefore, the increase in postprandial TAC and decrease in postprandial lipid peroxidation is likely the product of the complex antioxidant defenses provided by the pecans and cannot be credited to a single phytonutrient. Consuming foods that contain antioxidants with diverse mechanisms produces a synergistic effect that optimizes antioxidant protection [26], highlighting the benefit of a whole foods approach.

In addition to the benefits that were observed in the postprandial state, the pecan-enriched diets, regardless of dietary substitution instructions, increased fasting γ-tocopherol. Previous epidemiological research on the health benefit of γ-tocopherol remains controversial [33-35]. However, clinical trial data reports that γ-tocopherol supplementation results in decreased LDL [36], inflammation [37-39], and platelet aggregation [36] and increased vascular function [37]. Both α- and γ-tocopherol prevent lipid peroxidation through chain-breaking mechanisms, but γ-

tocopherol may be a more potent inhibitor of peroxynitrate [40], a common oxidant that interacts with proteins, lipids, and DNA to generate an OS and inflammatory response [41]. Therefore, the increase in fasting γ-tocopherol following daily pecan consumption in the present study may have mediated the beneficial effects that were observed postprandially.

Although there were numerous strengths to the study, we did have some limitations. Since this was not a controlled feeding trial, the antioxidant content of the overall diet was not controlled. By design, restrictions were not placed on participants' free-living diets in an effort to increase the generalizability of the study findings. Due to this design, it is possible that some of the observed benefits in the study could be the results of another phytonutrient present in the participants' free-living diet. However, for that to be true, it would have had to occur in both pecan groups but not in control since our control group did not show improvements in any outcome measure. Another limitation is the relatively high dose of pecans for a short duration; thus, we can only speculate on the effectiveness of a smaller dose of pecans over a longer duration. Finally, this study was not powered or designed to investigate the effect of sex or race.

In conclusion, daily pecan (68 g) consumption increased postprandial TAC and fasting γ-tocopherol and reduced postprandial lipid peroxidation in adults at risk for CVD. The present study contributes to the growing body of evidence that suggests habitual intake of antioxidant-rich foods may provide protection during the postprandial period, even when antioxidants are not present in the meal consumed. These findings are clinically meaningful because the typical American spends two-thirds of the day in the postprandial state and often consumes meals that are high in SFA [4, 5]. Future studies should investigate the impact of the daily consumption of other tree nuts on the response to a high SFA meal, as well as a different amount of pecans to determine the lowest effective dose.

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Table 7.1 Blood lipid qualification

	Desirable (mg/dL)	Borderline High/Undesirable (mg/dL)	High/Undesirable (mg/dL)
Total Cholesterol	Less than 200	200-239	240 and higher
Low-Density Lipoprotein	Less than 130	130-159	160 and higher
High-Density Lipoprotein	50 and higher	40-49	Less than 40
Triglycerides	Less than 150	150-199	200 and higher

Table 7.2 Nutrient breakdown for the high saturated fat meal challenge

Composition	SFA-rich meal
Percentage of total energy from	
Protein	5.0
Carbohydrate	25.0
Fat	69.5
Percentage of energy from fatty acids	
SFA	46.9
MUFA	15.7
PUFA	6.9

Abbreviations: HF=high fat, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid, SFA=saturated fatty acid

Table 7.3 Participant characteristics at the pre-intervention visit

	Control (n=16)	Pecan ADD (n=15)	Pecan SUB (n=16)
Age (y)	49 ± 14	50 ± 11	46 ± 11
Height (cm)	168.1 ± 11.7	166.9 ± 7.6	166.4 ± 10.6
Weight (kg)	91.9 ± 27.7	84.6 ± 14.0	92.6 ± 20.2
Body Mass Index (kg/m^2)	31.9 ± 6.8	30.3 ± 4.2	33.4 ± 6.7
Waist Circumference (cm)	100.4 ± 16.8	96.6 ± 9.1	101.9 ± 14.8
Hip Circumference (cm)	114.4 ±15.2	112.2 ± 9.3	116.5 ±12.8
Systolic Blood Pressure (mmHg)	123 ± 13	127 ± 17	128 ± 18
Diastolic Blood Pressure (mmHg)	80 ± 10	85 ± 15	83 ± 11
Malondialdehyde (µM)	15.4 ± 5.5	14.4 ± 4.8	13.3 ± 5.8
TAC (µM Trolox Equivalents)	1978 ± 287	1946 ± 315	2058 ± 523
γ-Tocopherol (µg/mL)	1.8 ± 0.8	1.4 ± 0.5	1.8 ± 0.7
α-Tocopherol (µg/mL)	15.6 ± 3.6	15.7 ± 2.6	15.9 ± 4.6
δ-Tocopherol (µg/mL)	nd	nd	nd

All values are mean ± standard deviation (n=47) A one-way analysis of variance of demographic data revealed no differences between groups at baseline. Abbreviations: nd=not detected; Pecan ADD=consumed pecans as part of a free-living diet; Pecan SUB=substituted pecans for isocaloric foods from their habitual diet; TAC=Total Antioxidant Capacity.

Table 7.4 Fasting biochemical markers

	Control			Pecan ADD			Pecan SUB		
	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
MDA (µM)	15.4 ± 5.5	13.6 ± 6.9	13.5 ± 5.6	14.4 ± 4.8	14.2 ± 5.9	14.8 ± 7.9	13.3 ± 5.8	13.6 ± 5.4	12.9 ± 5.5
TAC (µM TE)	1978 ± 287	2002 ± 445	1911 ± 328	1946 ± 315	1982 ± 445	1747 ± 369	2058 ± 523	2183 ± 597	1878 ± 629[#]
γ-Tocopherol (µg/mL)	1.8 ± 0.8	2.0 ± 0.7	1.8 ± 0.7	1.4 ± 0.5	1.8 ± 0.7**	1.8 ± 0.4**	1.8 ± 0.7	2.3 ± 1.0***	2.1 ± 0.8*
α-Tocopherol (µg/mL)	15.6 ± 3.6	16.7 ± 4.5	16.0 ± 4.5	15.7 ± 2.6	14.9 ± 3.3	14.8 ± 2.5	15.9 ± 4.6	16.4 ± 4.8	15.5 ± 5.3
δ-Tocopherol (µg/mL)	nd	nd	nd	nd	nd	nd	nd	nd	nd

All values are mean ± SD. A two-way analysis of variance was used to test for within group differences, and post-hoc analyses were conducted using a least-squares mean test. Abbreviations: nd=not detected; Pecan ADD=consumed pecans as part of a free-living diet; Pecan SUB=substituted pecans for isocaloric foods from their habitual diet; MDA=Malondialdehyde; TAC=Total Antioxidant Capacity; TE=Trolox Equivalents. * Indicates a significant difference from pre-intervention within a group ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$). [#] Indicates a significant difference from mid-intervention (week 4) only within a group ($p \leq 0.05$).

Supplemental Table 7.5

Nutrient breakdown for pecans (68 grams)

Energy (kcal)	469.9
Carbohydrates (g)	9.4
Total Sugars (g)	2.7
Total Dietary Fiber (g)	6.5
Protein (g)	6.2
Fat (g)	48.9
SFA (g)	4.2
MUFA (g)	27.7
Oleic Acid	27.6
Palmitoleic Acid	0.1
PUFA (g)	14.7
ALA (ω-3)	0.7
Linoleic Acid (ω-6)	14.0

Kcal= kilocalorie; g=gram; SFA=Saturated Fatty Acid; MUFA=Monounsaturated Fatty Acid; PUFA=Polyunsaturated Fatty Acid; ALA=Alpha-Linolenic Acid.

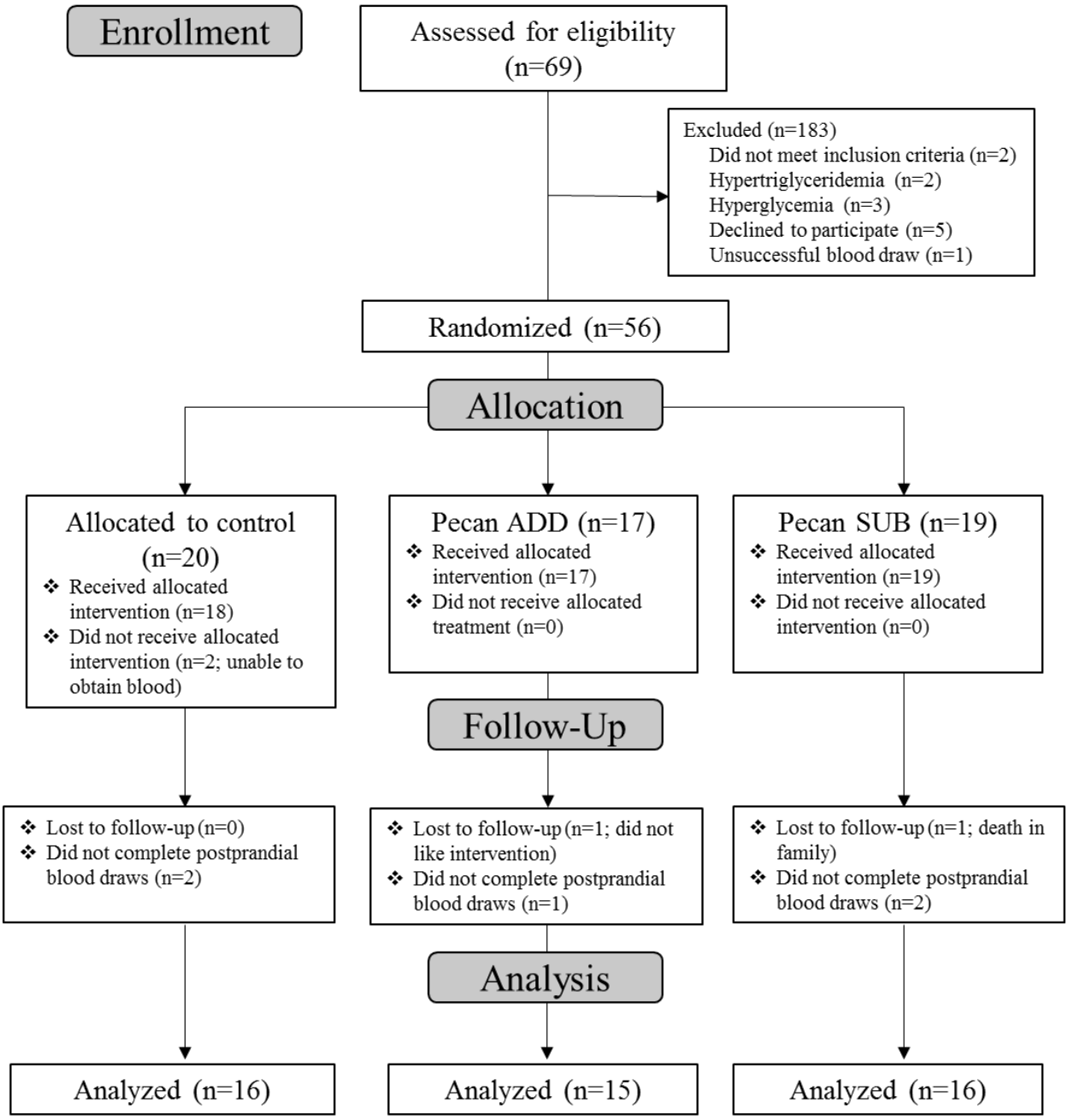

Figure 7.1 Consolidating Standards of Reporting (CONSORT) flow diagram for the selection of participants.

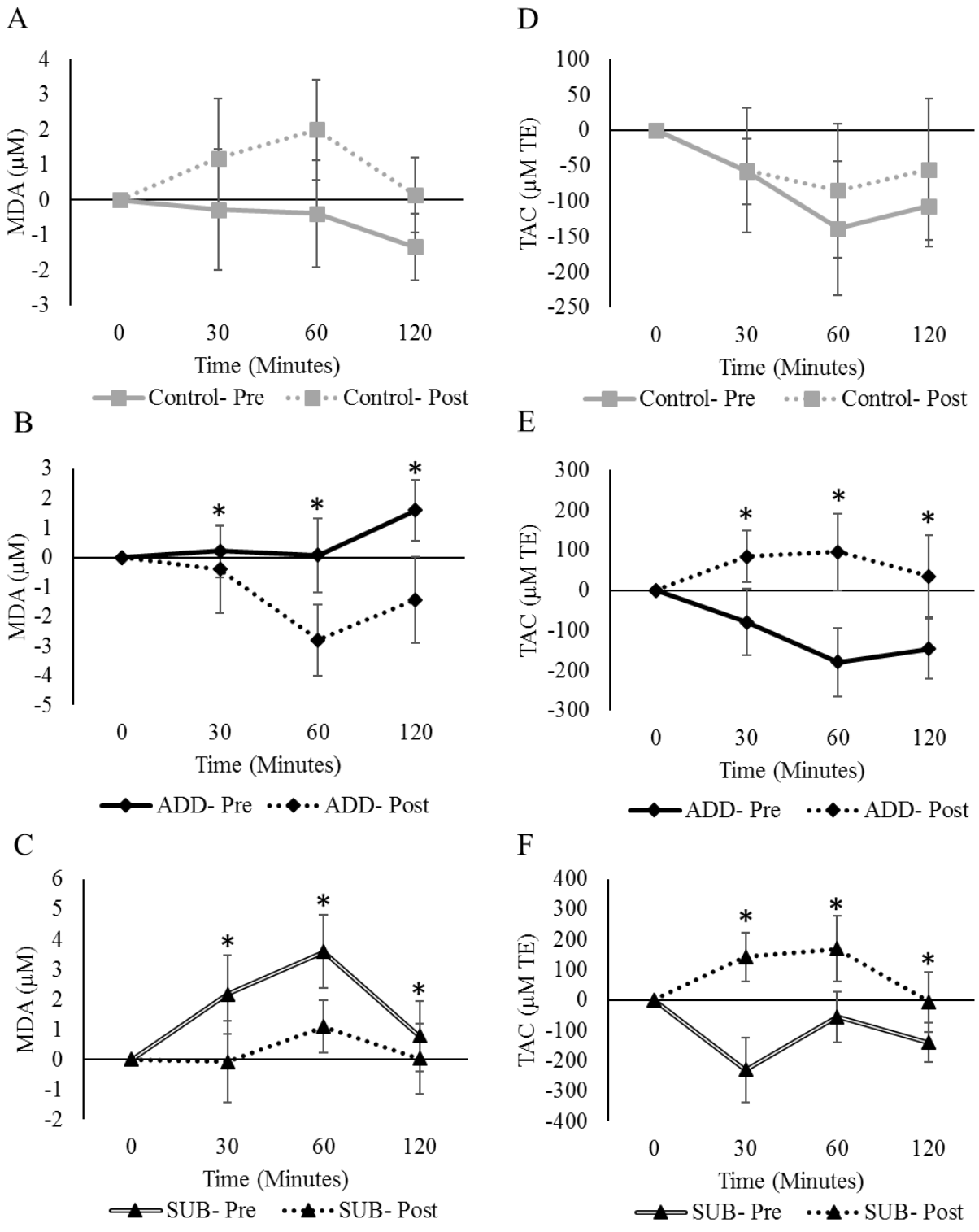
A
MDA (µM)
4
3
2
1
0
-1
-2
-3
0
30
60
120
Time (Minutes)
Control- Pre
Control- Post
D
TAC (µM TE)
100
50
0
-50
-100
-150
-200
-250
0
30
60
120
Time (Minutes)
Control- Pre
Control- Post
B
MDA (µM)
3
2
1
0
-1
-2
-3
-4
-5
*
*
*
0
30
60
120
Time (Minutes)
ADD- Pre
ADD- Post
E
TAC (µM TE)
300
200
100
0
-100
-200
-300
*
*
*
0
30
60
120
Time (Minutes)
ADD- Pre
ADD- Post
C
MDA (µM)
6
5
4
3
2
1
0
-1
-2
*
*
*
0
30
60
120
Time (Minutes)
SUB- Pre
SUB- Post
F
TAC (µM TE)
400
300
200
100
0
-100
-200
-300
-400
*
*
*
0
30
60
120
Time (Minutes)
SUB- Pre
SUB- Post

Figure 7.2 Time course for change in malondialdehyde (MDA), a measure of lipid peroxidation, and total antioxidant capacity (TAC) in the control, ADD, and SUB groups at pre- and post-intervention (Figures 7.2A-C and D-F, respectively). Repeated measures analysis of variance (ANOVA) was used to determine main and interaction effects of time, treatment, and visit. Post-hoc analyses were conducted using a least-squares mean test. There was a significant suppression of MDA at the post- vs. pre-intervention visits in ADD and SUB ($p<0.05$). Furthermore, TAC was elevated at the post- vs. pre-intervention visit in ADD ($p<0.001$) and SUB ($p<0.01$).
* Asterisks denote a statistically significant difference within a group. All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; MDA=malondialdehyde; SUB=substituted pecans for isocaloric foods from their habitual diet; TAC=total antioxidant capacity; TE=Trolox Equivalent.

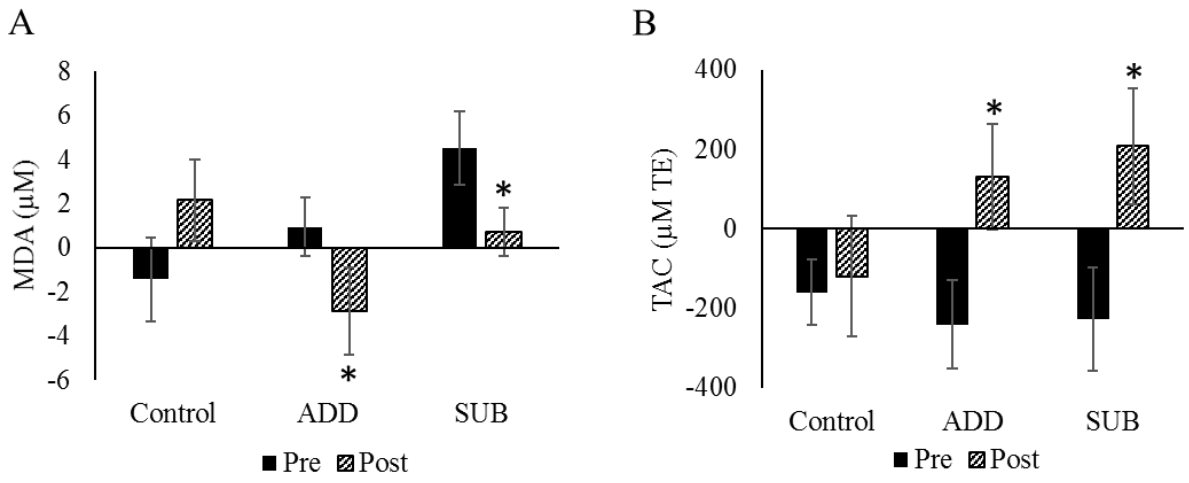

Figure 7.3 Incremental Area Under the Curve (iAUC) for malondialdehyde (MDA), a measure of lipid peroxidation, and total antioxidant capacity (TAC) in the control, ADD, and SUB groups at pre- and post-intervention (Figure 7.3A and 7.3B, respectively). A two-way analysis of variance (ANOVA) was used, and post-hoc analyses were conducted using a least-squares mean test. MDA was significantly suppressed at the post- vs. pre-intervention visits in ADD and SUB ($p<0.05$). Furthermore, TAC was significantly elevated at the post- vs. pre-intervention visit in ADD and SUB ($p<0.01$). * Asterisks denote a statistically significant difference within a group (* for $p<0.05$ and ** for $p<0.01$). All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; MDA=malondialdehyde; SUB=substituted pecans for isocaloric foods from their habitual diet; TAC=total antioxidant capacity; TE=Trolox Equivalents.

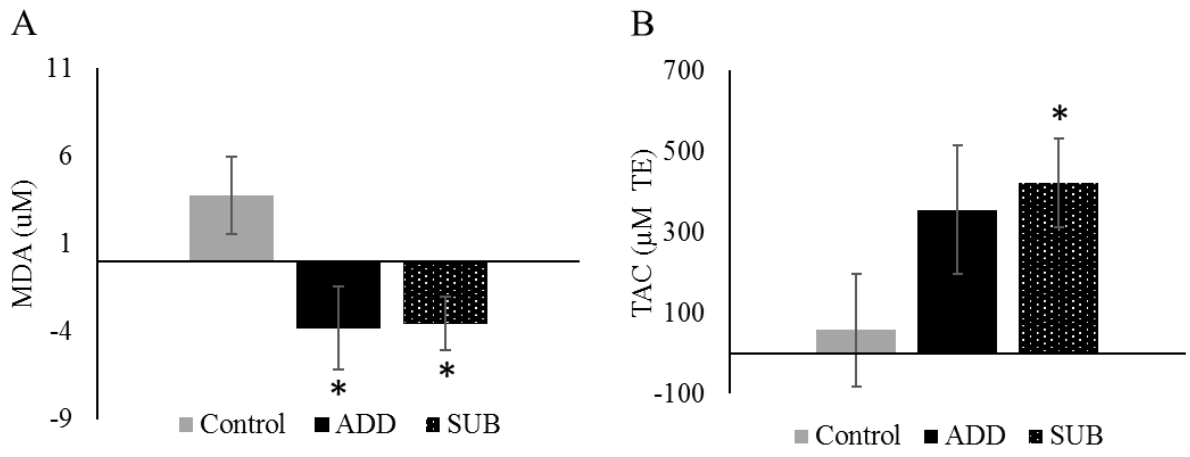

Figure 7.4 The change in incremental Area Under the Curve (iAUC) for malondialdehyde (MDA), a measure of lipid peroxidation, and total antioxidant capacity (TAC) from pre- to post-intervention in control, ADD, and SUB (Figure 7.4A and 4B, respectively). A one-way analysis of variance (ANOVA) was used, and post-hoc analyses were conducted using a least-squares mean test. The change in the iAUC for MDA was significantly more suppressed in ADD and SUB vs control ($p<0.05$). Furthermore, the change in iAUC for TAC was significantly more elevated in SUB vs control ($p<0.05$). * Asterisks denote a statistically significant difference versus the control group, and # denotes a trend for a difference versus the control group ($p=0.06$). All values are presented as mean ± SEM. Abbreviations: ADD=consumed pecans as part of a free-living diet; MDA=malondialdehyde; SUB=substituted pecans for isocaloric foods from their habitual diet; TAC=total antioxidant capacity; TE=Trolox Equivalents.

CHAPTER 8

CHANGES IN BODY WEIGHT IN RESPONSE TO PECAN-ENRICHED DIETS WITH AND WITHOUT SUBSTITUTION INSTRUCTIONS: A RANDOMIZED, CONTROLLED TRIAL[6]

[6]Guarneiri, L.L., C.M. Paton, and J.A. Cooper. *Journal of Nutrition Science*, 2022. 11:16. p. 1-9. Reprinted here with permission of the publisher.

Abstract

Substantial evidence suggests that regular tree nut consumption does not lead to changes in body weight (BW). However, these studies used a variety of dietary substitution instructions which may confound the interpretation of prior BW outcomes. The purpose of this study was to examine the impact of daily pecan consumption, with or without isocaloric substitution instructions, on BW and composition. This was an 8-week randomized, controlled trial with three treatments: a nut-free control group (n=32) and two pecan groups. ADD (n=30) consumed pecans (68g/d) as part of a free-living diet, and SUB (n=31) substituted the pecans (68g/d) for isocaloric foods from their habitual diet. BW and total body fat percentage (BF) were measured, and theoretical change in these outcomes if pecans were consumed without compensation were determined. BW increased in all groups across the intervention, and there was a trend ($p=0.09$) for an increase in ADD (1.1±0.2kg) and SUB (0.9±0.3kg) compared to control (0.3±0.2kg). In addition, there was increased BF in SUB (1.0±0.3%; $p=0.005$) but not ADD (0.1±0.2%) or control (-0.2±0.3%) There was a large difference in the actual vs theoretical change in BW regardless of pecan treatment (actual: 1.1±0.2 and 0.9±0.3 vs theoretical: 3.3±0.0kg and 3.2±0.0kg in ADD and SUB, respectively; $p<0.001$). Furthermore, there was a difference in actual vs theoretical change in BF in ADD (0.1±0.2 vs 1.2±0.1%; $p=0.002$) but not SUB or control. In conclusion, daily pecan consumption for 8 weeks did not result in significant weight gain, regardless of dietary substitution instructions.

Introduction

More than forty percent of U.S. adults have obesity, which is associated with elevated risk for chronic diseases and preventable death [1]. Since weight-loss interventions often result in weight regain [2], promoting obesity prevention is an important approach for combatting the obesity epidemic. One method for achieving energy balance and maintaining weight is to consume nutrient-dense foods that are rich in fiber, protein, and unsaturated fatty acids [3-6]. Tree nuts are rich sources of these nutrients, and there is substantial evidence that suggests regular tree nut consumption, even in large quantities, does not cause weight gain [7, 8].

Despite the promising evidence for tree nuts with respect to body weight (BW) regulation, there are methodological differences between intervention studies to consider. Some tree nut studies provide no dietary instructions [9, 10], while others provide instructions to substitute energy-equivalent foods or specific macronutrients in their typical diet for the nuts provided [11, 12]. Furthermore, other studies have provided all meals in an outpatient feeding setting designed to keep participants in energy balance [13, 14]. We recently conducted a systematic review and meta-analysis to examine the impact of the absence of dietary substitution vs some type of dietary substitution instructions on BW and concluded that neither condition resulted in changes in BW [7].

Contrary to the result of our meta-analysis, two intervention studies involving walnuts or peanuts that directly compared changes in BW and body fat percentage (BF) in participants who received substitution instructions vs no instructions reported that substitution instructions impact these outcomes [15, 16]. Njike et al. [15] reported that a walnut diet without dietary advice increased BF, while the walnut diet with dietary advice improved waist circumference. Likewise, Alper et al. [16] reported that participants gained BW when consuming peanuts without dietary

guidance, but there was no change in BW when peanuts were substituted for other fats in the diet. Since the conclusions from our meta-analysis and these two studies conflict, further research needs to be conducted to directly compare the impact of substitution instructions on weight outcomes during trials involving nuts. The purpose of this study was to examine the impact of daily pecan consumption, with or without isocaloric substitution instructions, for an 8-week period on BW and BF (primary outcomes) in sedentary adults. We hypothesized that the pecan group with no substitution instructions (ADD) would have an increase in BW and BF, and that the increase would be greater than SUB (pecan group with isocaloric substitution instructions) and control (no nuts) groups. We did, however, expect this increase to be less than theoretical calculations. Finally, we expected no differences in weight changes between control and SUB.

Methods

Study Design

This was a randomized, parallel controlled trial (clinicaltrials.gov: NCT04376632) involving an 8-week intervention conducted at the University of Georgia (UGA). The participants were unaware that there were two pecan groups that received different dietary instructions. Data collection occurred August 2018-May 2021, when the goal of at least 26 subjects/group was obtained. The protocol included a screening visit and 3 testing visits (baseline, 4 weeks, and 8 weeks). Subjects were randomly assigned to one of three groups: a "no nut" control group or one of two pecan groups (ADD or SUB). Subjects in ADD and SUB each consumed 68g/day of pecans for 8 weeks; however, dietary instructions for pecan incorporation differed by group. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by

the Institutional Review Board for human subjects at UGA. Written consent was obtained from all subjects.

Participants

One hundred twenty four sedentary men and women between the ages of 30-75y with a body mass index (BMI) $\geq$18.5kg/m^2 were assessed for eligibility. Exclusion criteria included habitual nut consumption (>2 servings/week), nut allergies, special diets (i.e. ketogenic diet, intermittent fasting), excessive alcohol use (>3 drinks/d for men or >2 drinks/d for women), tobacco or nicotine use, exercise >3h/week, weight change >5% of body weight in the past 3 months, history of medical events or medication use affecting digestion, absorption, or metabolism, gastrointestinal surgery, and chronic or metabolic diseases. Individuals taking lipid-lowering medications, fish oil supplements, steroid/hormone therapy, or medications for diabetes mellitus or Attention Deficit Hyperactivity Disorder were also excluded. Finally, individuals with the following biomarkers were excluded: fasting glucose >126mg/dL, fasting triglycerides (TG) >350mg/dL, and blood pressure (BP) >180/120mmHg. Eligibility based on these biomarkers were determined from fasting measurements at the screening visit.

Protocol

Screening Visit

Individuals arrived at the Human Nutrition Lab (HNL) following an 8-12h overnight fast and 24h without exercise or alcohol. A lipid panel, glucose, and anthropometrics were assessed to confirm eligibility. If individuals qualified for the study, subjects were randomized to one of the three treatment groups by a researcher that was not involved in data collection or analysis. An allocation ratio of 1:1:1, a permuted block design (balanced for age, sex, and BMI), and a random number generator was used to randomize participants.

Pre-Intervention Visit (Baseline Visit)

Participants completed a two-day food diary containing one weekend day and one week day [17] between the screening visit and the pre-diet intervention visit (baseline). The night before the baseline visit, participants consumed a lead-in dinner meal and snack (provided by research personnel) that contained 50% of total energy from carbohydrate, 15% protein, and 35% fat. For the baseline visit, participants arrived at the HNL following an 8-12h overnight fast and 24h without exercise or alcohol. Participants changed into a hospital gown and removed footwear for the BW measurement, which was recorded to the nearest 0.001kg using a calibrated electronic scale. Next, height was measured to the nearest millimeter using a stadiometer. Waist circumference (WC) and hip circumference (HC) were measured in triplicate to the nearest mm, and BP was measured in triplicate with 30 seconds between each measurement. For WC, HC, and BP, the average of the 3 measurements was used.

Next, body composition was measured by Dual-Energy X-ray Absorptiometry (DXA) (Discovery A; Hologic Inc., Waltham, MA). Weight, waist-hip circumference, and body composition were primary outcomes while BP was a secondary outcome. Finally, physical activity, stress, and preference for fat were evaluated via validated questionnaires (International Physical Activity Questionnaire (IPAQ) Short Form, Perceived Stress Scale (PSS), and fat preference questionnaire, respectively) [18-20]. To quantify the taste and frequency scores for the fat preference questionnaire, the percent of food sets in which high-fat foods were reported to "taste better" and be "eaten more often" were calculated [20]. In addition, the frequency score was subtracted from the taste score to quantify a difference score.

8-Week Dietary Intervention

The day after the baseline visit, all participants began the 8-week intervention. Written diet instructions were provided to all participants. Subjects were instructed to avoid all forms of nuts that were not part of the study and to consume ≤2 servings (64g) of nut butter/week. Participants in ADD were provided with 68g (~0.5 cup or 2.25 ounces) portions of pecans to consume each day as part of their free-living diet with no additional dietary instructions. Participants in SUB were instructed to substitute the 470kcal provided by the 68g of pecans for foods habitually consumed in their free-living diet. Trained research personnel guided the participants on how to make appropriate energy substitutions based on their previously completed food diaries. For example, if the participant habitually consumed snacks throughout the day, the research personnel highlighted the energy content of the snacks and asked the participant if it was feasible to replace the habitual snacks with the provided pecans. The guidance provided was individualized based on each participant's dietary intake. **Table 8.1** shows the complete nutrition information for the 68g portion of pecans. In addition, they were instructed to eat the pecans in their raw form (no roasting, cooking, or baking) but could add them to other foods. Finally, all subjects were instructed to avoid consuming >42g alcohol/d (men) or >28g alcohol/d (women) and were asked not to make any other changes to their diet or activity levels. Participants were unaware of the diet instructions that were provided to other groups to prevent unintentional or intentional changes in behavior.

Weekly Responsibilities

Participants in ADD and SUB also completed a daily nut compliance log that detailed the time of day for pecan consumption. Nut compliance logs were submitted to research staff once per week. Poor compliance was categorized as consumption of <75% of pecans throughout the 8-week intervention. All participants completed a food diary once per week alternating between

week days and weekend days. Daily nutrient intakes based on food diaries were assessed using The Food Processor SQL software (version 10.12.0). The nutrients from the two baseline food diaries, and then the food diaries from weeks 1-8, were averaged before analysis. Physical activity was assessed at baseline and during weeks 2, 4, 6, and 8. Physical activity was averaged for the weeks during the intervention before analysis.

Mid and Post-Intervention Visits (Weeks 4 and 8)

After 4 and 8 weeks of the diet intervention, participants returned to the HNL under the same conditions as baseline. At both visits, participants completed the exact same procedures and measurements that took place at baseline, except the fat preference questionnaire were not evaluated at week 4. The PSS from weeks 4 and 8 were averaged together before analysis.

Statistical Analyses

SAS version 9.2 statistical package (SAS Institute Inc, Cary, NC, USA) and R version 3.6.2 (The R Foundation, Vienna, Austria) were used for statistical analyses. All hypotheses and analytic plans were pre-specified. All values were reported as mean±SEM unless otherwise noted. Statistical significance was set at $p \leq 0.05$. A samples size of 78 (26 per group) was estimated to detect a significant difference in BW between the two pecan groups using G*power 3.19.7 assuming at least 80% power and an α of 0.05. This calculation was based on the mean difference of 0.8kg between the peanut interventions with and without substitution instructions in the study by Alper et al. [16]. The theoretical change in BW and BF in ADD and SUB if pecans were consumed without compensation were calculated for each participant using the National Institute of Health (NIH) Body Weight Planner, which accounts for the physiological energy adaptations during periods of weight change [21]. The NIH Body Weight Planner was also used to calculate the estimated energy intake (EI) during the study based on each participant's weight

change, age, sex, and height. A measure of under- or over-reporting on food diaries (energy report score-ERS) was calculated by subtracting the estimated EI during the study based on changes in BW from the average EI reported on food diaries during the intervention. The ERS for each group was compared using a one-way analysis of variance (ANOVA).

An unpaired t-test was used to assess differences in nut compliance between the two pecan groups. For anthropometrics, dietary intake, and questionnaires, a repeated measures linear mixed model for treatment (ADD, SUB, and control) and visit (baseline, 4-weeks, and 8-weeks) was used to test for differences. In addition, a two-way ANOVA was used to test for differences for the actual vs theoretical change in BW and BF. Post hoc analyses were done using Tukey's test. Finally, exploratory multiple regression analyses were conducted to determine predictors of the change in BW and BF in the two pecan groups. Factors included in the model were treatment (0=ADD, 1=SUB), baseline age, sex, BW, BF, physical activity (total MET-minutes/week), low-density lipoprotein (LDL) (from the screening visit), fat preference difference score, sugar intake, and the ERS. To determine predictors, two-way stepwise multiple regression and best subsets multiple regression approaches were employed. The two-way stepwise multiple regression analysis selected the model that minimized the Akaike Information Criterion (AIC) [22]. Similarly, the best subsets methods selected models that minimized the Bayesian Information Criterion (BIC) and Mallows Cp [22-24]. Multiple regression analysis was used to model the change in BW and BF with the predictors obtained from the model selection methods.

Results

Subjects

One hundred six participants were randomly assigned to an intervention (ADD: n=36, SUB: n=35, control: n=35); however, twelve participants did not start or complete the

intervention and were not included in final analyses **(Figure 8.1).** Three of these twelve participants were excluded after follow-up due to non-compliance (n=1 did not meet >75% pecan compliance; n=2 were non-compliant with study procedures). Therefore, ninety-three participants completed the intervention (n=20 women and 10=men for ADD; 21=women and 10=men for SUB; 23=women and 9=men for control) and were included in the per protocol analyses of primary and secondary outcomes. The average age of participants in ADD, SUB, and control was 47±2, 44±2, and 47±2y. Participants in both ADD and SUB consumed 95% of the pecans provided, and compliance was not different between groups. No participant reported poor compliance, and there was no report of intake of nuts in the control group according to food diaries.

Anthropometrics and Blood Pressure

Anthropometrics and blood pressure at baseline, 4 weeks, and 8 weeks are presented in **Table 8.2.** There were no differences between groups at baseline for any outcome. For BW, there was a significant effect of visit (time) ($p<0.001$), no effect of treatment ($p=0.39$), and a trend for a visit by treatment interaction ($p=0.09$). The post hoc analyses revealed that there was an increase in BW from baseline to 4 weeks ($p=0.01$), baseline to 8 weeks ($p<0.001$), and 4 weeks to 8 weeks ($p<0.001$) regardless of treatment. The trend for the interaction effect, however, was driven by BW changes from baseline to 8 weeks in ADD (77.6±3.0 to 78.7±3.1kg; p=<0.001) and SUB (84.7±3.5 to 85.6±3.5kg; $p<0.001$), but not the control group (80.1±4.4 to 80.4±4.4kg; $p=0.85$). For BF, there was a significant visit by treatment interaction ($p=0.005$) but no main effect of treatment ($p=0.35$) or visit ($p=0.13$). The interaction was for an increase in BF within SUB from baseline to 8 weeks ($p=0.001$) with no change in either ADD or control. Similar to BW data, for BMI, there was a main effect of visit ($p<0.001$) but no treatment effect

(p=0.18) or interactions (p=0.14). The effect of visit was an increase in BMI from baseline to 4 weeks (p=0.006), baseline to 8 weeks (p<0.001), and 4 weeks to 8 weeks (p=0.008) regardless of treatment. For SBP, there was also a visit effect (p=0.04) but no treatment effect (p=0.70) or interactions (p=0.63). The main effect of visit was an increase in SBP from baseline to 8 weeks only (p=0.04) regardless of treatment. Finally, there were no main or interaction effects for WC, HC, WHR, or DBP (ns).

Theoretical Weight Change

The actual and theoretical changes in BW and BF from baseline to 8 weeks in the ADD and SUB groups are presented in **Figure 8.2**. There was a significant effect of the type (actual vs theoretical) for BW (p<0.001) but no effect of treatment (p=0.53) or a type by treatment interaction (p<0.001). The significant main effect was for a difference between actual vs theoretical changes in BW in both pecan groups (actual: 1.1±0.2 and 0.9±0.3 vs theoretical: 3.3±0.0kg and 3.2±0.0kg in ADD and SUB, respectively; p<0.001) **(Figure 8.2A)**. Furthermore, there was a main effect of treatment (p=0.01) and type (p<0.001) and a treatment by type interaction (p=0.002) for BF. Post hoc analyses indicate that the actual change in BF was less than theoretically expected for ADD (0.1±0.2 vs 1.2±0.1%; p<0.001) but not SUB (1.0±0.3 vs 1.1±0.1%; p=0.97) **(Figure 8.2B)**. Finally, the change in actual BF was smaller in ADD vs SUB (0.1±0.2 vs 1.0±0.3; p<0.001).

Regression Analysis

Exploratory two-way stepwise multiple regression and best subsets multiple regression approaches were utilized to determine predictors of the change in BW and BF in the two pecan groups. For BW, the two-way stepwise model and Mallow's Cp indicated the best model to be the one in which the ERS and the difference score from the fat preference questionnaire were

included. BIC indicated the best model to be the one in which only the ERS was included. We chose the model that included the energy report and the difference scores since these were selected by the majority of the model selection methods. When the multiple regression model was analyzed, the ERS (β=-0.001; $p<0.001$) and the difference score (β=0.02; $p=0.12$) explained 28% of the variability in the change in BW within the two pecan groups (Full Model: intercept=0.30; $p<0.001$). For BF, all three model selection methods determined the best model to be one which included treatment and baseline sugar intake. When the multiple regression model was analyzed, treatment (β=0.82; $p=0.02$) and baseline sugar intake (β=0.01; $p=0.01$) explained 22% of the variability in the change in BF (Full Model: intercept=-0.63; $p<0.001$).

Dietary Intake

Average dietary intake at baseline and throughout the intervention is presented in **Table 8.3.** There were no differences in dietary intake between groups at baseline. For total energy intake (kcals/d) there was a significant main effect of time ($p=0.005$) but no effect for treatment ($p=0.17$) or a time by treatment interaction ($p=0.20$). The main effect of time indicates that energy intake increased from baseline regardless of treatment. For the percentage of energy from carbohydrate, fat, and protein, there was a significant main effect of time ($p<0.01$ for all), treatment ($p<0.01$ for all), and a time by treatment interaction ($p<0.001$ for all). Post hoc analyses revealed that was an increase in the percentage of energy from fat ($p<0.001$ for both) and a decrease in the percentage of energy from carbohydrate ($p<0.001$ for both) and protein ($p=0.01$ and $p=0.05$, respectively) within ADD and SUB, but not control, from baseline to throughout the intervention.

For dietary fiber and sugar, there was a main effect of time ($p<0.001$ and $p=0.04$) and a time by treatment interaction ($p<0.001$ for both) but no effect of treatment ($p=0.45$). The

interaction was for an increase in fiber within ADD ($p<0.001$) and SUB ($p=0.04$) with no change in control, and a decrease in sugar within SUB only ($p=0.03$) from baseline to intervention. There were significant main effects for time ($p<0.001$ for both), treatment ($p<0.01$ for both), and a time by treatment interaction ($p<0.001$) for MUFA and PUFA intake (g/d). The interaction was for an increase in MUFA ($p<0.001$ for both) and PUFA ($p<0.01$ for both) within ADD and SUB but not control. For omega-6 fatty acids, there was a main effect of time ($p=0.001$) but no treatment ($p=0.86$) or time by treatment interaction ($p=0.90$), indicating that intake of omega-6 fatty acids increased from baseline regardless of group. There were no main or interaction effects for saturated fat, trans-fat, cholesterol, or omega-3 fatty acids (ns).

Questionnaires

Average questionnaire responses for stress, physical activity, and fat preferences at baseline and throughout the intervention are presented in **Table 8.3.** There were no differences between groups at baseline for any questionnaire outcome. For perceived stress, there was a main effect of visit ($p=0.04$) but no treatment effect ($p=0.15$) or interaction ($p=0.47$). The visit effect indicates a reduction in perceived stress from baseline regardless of treatment. For all self-reported measures of physical activity and fat preference, there were no significant main or interaction effects. Finally, the ERS, a measure of under- or over-reporting on food diaries, was not different between groups ($p=0.27$).

Discussion

There was an increase in BW regardless of treatment, which was predominately driven by the two pecan groups that were trending for an increase in BW compared to control. Although not statistically significant, the average weight gain of 0.9-1.1kg in the pecan groups is clinically meaningful since the average annual weight gain for adults is 0.5-1 kg/y [25]. It is likely that the

slight increase in BW (0.3±0.2kg) and self-reported EI (75±117kcal/d) in the control group inhibited our ability to observe differences between groups for these outcomes. The estimated theoretical increase in BW in the two pecan groups was considerably higher than actual changes in BW by approximately 2.2kg, indicating a fairly large degree of compensation from the added energy content of the pecans and/or other nutrients in the nuts such as fiber, total fat, MUFA, or PUFA. It is likely this partial compensation also contributed to the non-significant differences in weight change between pecan vs. control groups. We did, however, observe increases in BF in the group receiving the isocaloric substitution instructions (SUB) only. While significant, that change in BF was less than the theoretical change in ADD (0.1±0.2 vs 1.2±0.1%) but not SUB. There were no changes in total physical activity (MET-minutes/week), suggesting that any anthropometric changes were likely influenced by EI.

Based on our initial hypothesis, it was surprising that significant weight gain did not occur in the ADD group compared to the other two groups. Two previous intervention studies that directly compared the impact of different dietary practices during nut interventions showed that BW or BF increased in groups without dietary instructions, while the dietary instructions protected against these unfavorable changes [15, 16]. It is possible that the divergence in results between those studies and ours were due to differences in methodology. For example, the walnut intervention was 6 months in duration in the study by Njike et al. [15], thus our study duration may not have been long enough to capture differences between our two pecan groups. Furthermore, the crossover study by Alper et al. [16] involved 3 treatment arms that all consumed peanuts with varying degrees of dietary instructions. The lack of a true control group in that study may explain why they were able to capture differences between peanut groups. Although our BW results are not in line with these two previous studies, our findings do

corroborate with the substantial epidemiological and interventional evidence that tree nuts (such as almonds and walnuts) are beneficial for weight management, even without isocaloric substitution instructions [7, 26].

There are several potential mechanisms for how tree nuts promote weight maintenance, despite their high energy density, which may explain our lack of significant weight gain between pecan groups versus control in the present study. Tree nuts are rich in protein, fiber, and energy, which may prevent further food intake by inducing satiety [27]. We previously showed that a 7d PUFA-rich diet (containing walnuts) improved fasting and postprandial peptide YY, a satiety hormone [28]. Furthermore, previous research involving 8-19 weeks of peanut or pecan consumption resulted in a 5-11% increase in resting metabolic rate[16, 29, 30]. Finally, we know that not all the energy in some nuts is fully metabolized and absorbed[31, 32]. The metabolizable energy (ME) of almonds [31], walnuts [32], cashews [33], and pistachios [34] is 32%, 21%, 16%, and 5% less than predicted by the Atwater factors, respectively. The ME of pecans has not been elucidated, but we might speculate that it is less than expected due to our observed weight maintenance. Altogether, the results of the present study may be due to a combination of increased satiety and energy expenditure and decreased absorption of the energy from pecans.

Although we did not find a significant difference in weight gain between pecan groups vs. control, we did observe a large variation of weight change in the pecan groups (SD of 1.4kg). Therefore, we conducted exploratory regression analyses to investigate predictors of changes of BW and BF during pecan interventions. The exploratory regression analyses indicated that the energy report and difference scores explained 28% of the variability in the change in BW within the two pecan groups. A positive ERS corresponded with over-reporting on food diaries, while a negative score indicated under-reporting on food diaries. The beta coefficient suggests that for

every one kcal decrease in the ERS (under-reporting), the change in weight increased by an additional 0.001kg when the difference score is held constant. Previous research indicates that under-reporting EI is also positively associated with dietary restraint, the tendency to restrict food [35, 36]. Furthermore, the difference score from the fat preference questionnaire is a measure of dietary fat restraint and is also associated with standard measures of dietary restraint [37]. The beta coefficient suggests that for every 1% increase in the difference score, the change in weight increase by an additional 0.02kg when the ERS is held constant. At first glance, it appears contradictory that increased dietary restraint would predict weight gain, but previous research suggests that dietary restraint may increase vulnerability to weight gain, especially in women[38]. Although pecans have been shown to provide a variety of health benefits [39-41], a history of dietary restraint may be an important consideration before recommending daily pecan consumption, especially in high doses.

It was unexpected that BF increased in the SUB and not the ADD group. Although surprising, many other tree nut studies have also observed changes in one, but not both, of these outcomes [16, 42-44]. Although DXA is more accurate and precise than other methods for measuring body composition [45], it is still vulnerable to inaccuracies of approximately one percent [46]. Therefore, it is possible that 1.1% increase in BF within SUB, or the lack of change within ADD, falls within measurement error. The free-living nature of this study is another potential limitation, as extraneous factors such as weather and family circumstances may have influenced lifestyle behaviors that impact BW. However, the design of this study was intentional in effort to increase the generalizability of the results. The self-report nature of the assessments for dietary intake, physical activity, and stress were another limitation as they are vulnerable to under- and over-reporting. Likewise, we utilized 2d food records instead of the standard 3d food

records at baseline to reduce participant burden. Since participants completed weekly food records once per week alternating between week days and weekend days, our reporting of the average intake during the intervention may over-emphasize weekend days since those days occur less frequently throughout a week. Finally, this study was not powered or designed to detect differences between sexes or races, and the short duration of the study limits conclusions for long-term weight management.

In conclusion, daily pecan consumption (68g/d), regardless of isocaloric substitution instruction, did not result in significant weight gain. The slight, non-significant, increase in weight in the control group likely affected our ability to detect a significant change in BW in either pecan group compared to control. Although the non-significant weight gain with pecan consumption may be clinically meaningful, it was much less than the theoretical weight gain, indicating at least partial compensation for the added energy from the pecans. We did observe an increase in BF if one of the two pecan groups, although it is unclear why this occurred in the group performing the dietary substitution instructions. Future research should further investigate the ME of pecans and the impact of dietary restraint on changes in BW and BF during tree nut interventions.

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Table 8.1 Nutrient breakdown for pecans (68 grams)

Energy (kcal)	469.9
Carbohydrates (g)	9.4
Total Sugars (g)	2.7
Total Dietary Fiber (g)	6.5
Protein (g)	6.2
Fat (g)	48.9
SFA (g)	4.2
MUFA (g)	27.7
Oleic Acid	27.6
Paullinic Acid	0.1
PUFA (g)	14.7
ALA (ω-3)	0.7
Linoleic Acid (ω-6)	14.0

Kcal= kilocalorie; g=gram; SFA=Saturated Fatty Acid; MUFA=Monounsaturated Fatty Acid; PUFA=Polyunsaturated Fatty Acid; ALA=Alpha-Linolenic Acid.

Table 8.2 Anthropometrics Across the Intervention

	ADD (n=30)						SUB (n=31)						Control (n=32)					
	Week 0		Week 4		Week 8		Week 0		Week 4		Week 8		Week 0		Week 4		Week 8	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Weight (kg)[a]	77.6	3.0	78.2	3.0	78.7[#]	3.1	84.7	3.5	85.1	3.5	85.6[#]	3.5	80.1	4.4	80.2	4.4	80.4	4.4
BMI (kg/m^2)[a]	27.6	0.9	27.8	0.9	28.0	0.9	30.3	1.2	30.5	1.2	30.6	1.2	28.1	1.2	28.1	1.2	28.2	1.2
WC (cm)	89.0	2.3	88.9	2.0	88.9	2.3	93.7	2.6	94.1	2.8	94.4	2.6	89.0	3.0	89.4	3.2	89.8	3.1
HC (cm)	106.8	1.9	107.6	1.9	107.3	1.8	111.9	2.1	111.9	2.0	113.4	2.2	108.2	2.3	108.4	2.1	108.4	2.3
WHR	0.83	0.01	0.83	0.01	0.83	0.01	0.84	0.02	0.84	0.02	0.83	0.01	0.82	0.02	0.82	0.02	0.83	0.02
Body Fat (%)	30.3	1.4	30.6	1.5	30.4	1.4	32.4	1.2	33.0	1.3	33.5*	1.3	31.1	1.4	30.8	1.3	30.8	1.2
SBP (mmHg)[a]	124	3	123	3	127	3	124	3	127	3	128	3	122	3	123	3	125	3
DBP (mmHg)	80	3	80	3	82	2	79	2	80	2	81	2	78	2	76	3	77	2

There were no differences between groups at baseline. ADD, consumed pecans as part of a free-living diet; SUB, substituted pecans for isocaloric foods from their habitual diet; BMI, body mass index; HC, hip circumference; WC, waist circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure. * Indicates a significant treatment by visit interaction with an increase in body fat % within the SUB group only ($p \leq 0.05$). [#] Indicates a trend for a treatment by visit interaction for greater increases in weight for both pecan groups compared to control ($p < 0.10$). [a] Indicates a significant main effect of visit at $p \leq 0.05$.

Table 8.3 Lifestyle factors at baseline and during the intervention

	ADD (n=30)				SUB (n=31)				Control (n=32)			
	Baseline		Intervention		Baseline		Intervention		Baseline		Intervention	
Dietary Intake	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Distribution of Energy												
Energy (kcal)[a]	2,141	143	2,468	145	2,161	104	2,280	79	1,998	108	2,073	116
Kcal from carbohydrate (%)[a,b]	48	2	37*	1	49	1	38*	1	48	1	46	1
Kcal from protein (%)[a,b]	16	0.4	14*	0.5	14	0.5	13*	0.3	15	0.5	16	0.5
Kcal from fat (%)[a,b]	36	2	48*	1	36	1	47*	1	36	1	37	1
Kcal from alcohol (%)[a]	1	0.3	1	0.4	1	0.5	1.5	0.4	1	0.4	2	0.6
Carbohydrates												
Fiber (g)[a]	14	1	21*	1	14	2	18*	1	16	1	15	1
Sugar (g)[a]	81	6	75	5	105	12	79*	8	78	5	79	8
Fats												
MUFA (g)[a,b]	34	3	62*	4	35	3	55*	2	28	2	33	2
PUFA (g)[a,b]	19	2	33*	2	21	2	30*	1	18	2	20	1
Omega-3 FA (g)	2	0.5	2	0.2	2	0.4	2	0.1	2	0.6	2	0.3
Omega-6 FA (g)[a]	13	2	29	2	15	2	26	1	11	1	26	12
SFA (g)	30	3	31	3	28	2	28	1	30	2	28	2
Trans-FA (g)	0.8	0.1	0.8	0.1	0.8	0.2	0.7	0.1	0.8	0.1	1.0	0.2
Cholesterol (mg)	330	58	316	54	293	34	225	16	236	24	260	21
IPAQ Short Form												
Total PA (MET-min/week)	1,647	248	1,799	278	1,331	296	1,068	185	1,116	164	1,295	181
Vigorous PA (MET-min/week)	725	141	672	122	335	173	313	109	389	89	501	99
Moderate PA (MET-min/week)	336	120	574	127	285	76	290	80	246	53	327	73
Walking (MET-min/week)	556	104	559	114	711	171	464	98	481	82	468	69
Sitting Time (minutes/d)	375	37	374	32	415	37	444	50	399	39	433	32
Activity EE (kcal/d)	289	44	333	55	254	50	208	38	215	43	257	53
Fat Preference Questionnaire												
Taste Score (%)	65	3	63	4	67	3	66	3	70	3	71	3
Frequency Score (%)	42	4	41	4	48	4	47	3	50	3	49	4
Difference Score (%)	22	3	22	3	19	3	19	3	20	2	23	3
Other Variables												
Perceived Stress Scale Score[a]	14	0.6	12	0.9	15	1.3	14	1.1	12	1.1	11	1.2
Energy Report Score	--	--	-230	104	--	--	-432	107	--	--	-427	91

For fat preference questionnaire, taste and frequency scores were calculated based on the percentage of food sets in which high-fat foods were reported to "taste better" and be eaten more often", respectively. Difference scores were calculated by subtracting the frequency score from the taste score. The energy report score, a measure of under- or over-reporting on food diaries, was calculated by subtracting the estimated energy intake during the study from the average energy intake reporting on food diaries during the intervention. The estimated energy intake during the study was calculated based on changes in body weight using the National Institute of Health Body weight Planner[21]. ADD, consumed pecans as part of a free-living diet; SUB, substituted pecans for isocaloric foods from their habitual diet; FA, fatty acid; g, gram; kcal, kilocalorie; MET, metabolic equivalent; MUFA, monounsaturated fatty acid; PA, physical activity; PUFA, polyunsaturated fatty acid; IPAQ, International Physical Activity Questionnaire; MET, metabolic equivalent.* Indicates a significant time by treatment interaction with greater changes in a group compared to the control ($p<0.05$). [a] Indicates a significant main effect of visit and [b] indicates a significant main effect of treatment at $p<0.05$.

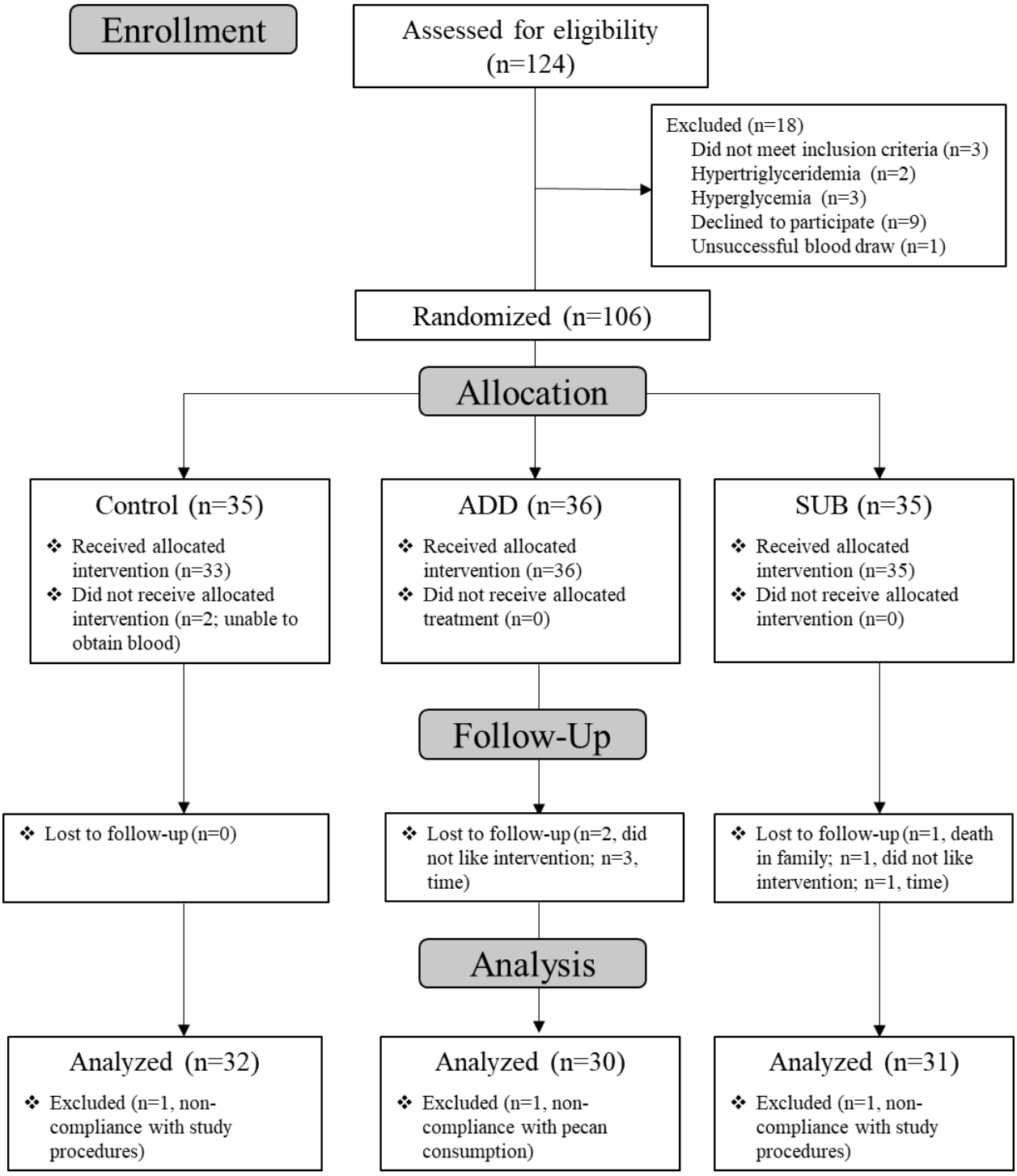

Figure 8.1 Consolidating Standards of Reporting (CONSORT) flow diagram for selection of study participants.

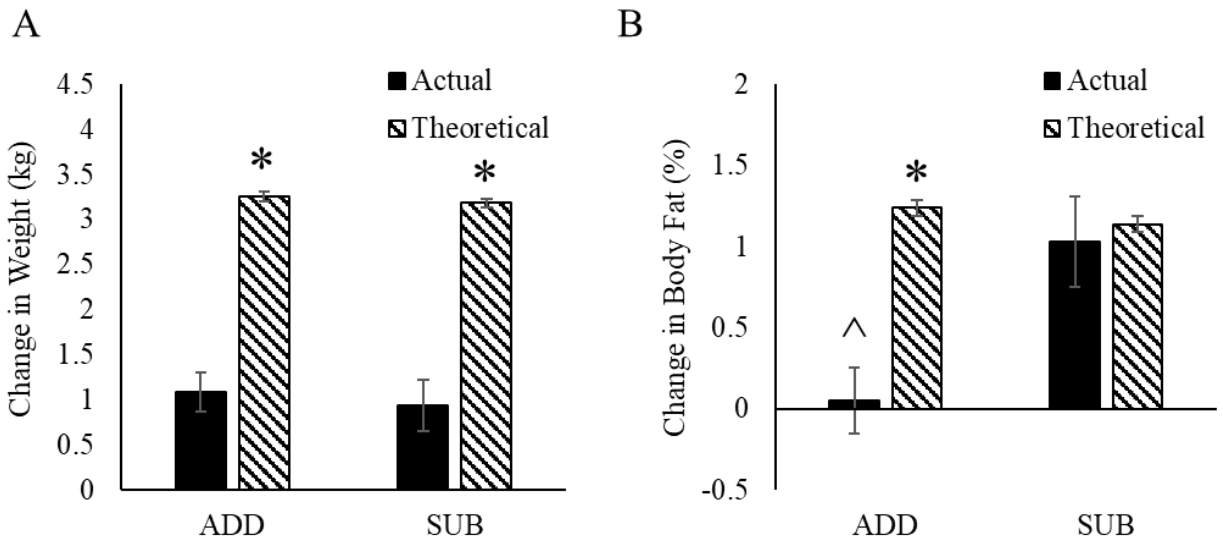

Figure 8.2 Changes in actual and theoretical (A) body weight and (B) total body fat percentage in ADD and SUB from baseline to 8 weeks. A two-way analysis of variance (ANOVA) was used to test for differences. Tukey's test was used for post-hoc analyses. * Indicates a significant difference between the actual and theoretical BW within ADD and SUB ($p \leq 0.05$). * Indicates a significant difference between the actual and theoretical BF within ADD only ($p \leq 0.05$). ^ Indicates a significant difference between groups. Abbreviations: ADD=consumed pecans as part of a free-living diet; SUB=substituted pecans for isocaloric foods from their habitual diet.

CHAPTER 9

SUMMARY AND CONLUSIONS

The main purpose of this dissertation project was to investigate the impact of pecan-enriched diets on cardiovascular disease (CVD) risk (blood lipids, angiopoietin-like proteins (ANGPTL), antioxidant status, and weight management through appetite and metabolism). To best address our research question, we conducted an 8-week randomized, controlled trial with two pecan groups and a nut-free (control) group (n=16). The ADD group (n=15) consumed pecans as part of a free-living diet, and the SUB group (n=16) substituted the pecans for isocaloric foods from their habitual diet. To investigate body weight as a primary outcome, we increased sample size and expanded the inclusion criteria to obtain adequate power (ADD: n=30; SUB: n=31; control: n=32).

The results presented in Chapter 3 demonstrated that daily pecan consumption, regardless of dietary substitution instructions, improved total cholesterol, low-density lipoprotein cholesterol, triglycerides (TG), and apolipoprotein-B. In addition, postprandial TG and glucose improved following a high-saturated fatty acid meal within ADD and SUB, respectively. These findings are clinically meaningful because the magnitude of reduction in LDL cholesterol in the pecan groups could correspond to a 6.5-11.4% reduction in the risk for coronary artery disease [1, 2]. In addition, elevated postprandial TGs and glucose are independent risk factors for CVD, so reductions in either of these following 8 weeks of pecan consumption may reduce CVD risk [3-5]. In Chapter 4, we reported that postprandial ANGPTL3 was suppressed in both ADD and SUB, which could explain the potential mechanism behind the suppressed postprandial TG and

glucose. Finally, in Chapter 7, we demonstrated that daily pecan consumption decreased postprandial lipid peroxidation by 84-133% and increased postprandial total antioxidant capacity by 154-192%. These findings are clinically meaningful because postprandial rises in lipids following a high-fat meal are associated with increased oxidative stress and chronic disease risk. Altogether, the improvements in blood lipids, ANGPL3, and antioxidant status supports the notion that pecan consumption reduces CVD risk.

In Chapter 5, postprandial CCK, PYY, ghrelin, and subjective markers of appetite improved in ADD but not SUB or control. The agreement between the subjective and physiological outcomes highlights the potency of the effect of pecans on appetite since many studies show improvements for either hormones or VAS, but not both. Previous research indicates that improvements in appetite hormones and VAS ratings are associated with decreased energy intake, although we did not observe reductions in energy intake based on self-reported food diaries [6-9]. In addition to some improvements in measures of appetite, in Chapter 6, we showed that daily pecan consumption increased RMR, improved fasting substrate oxidation (lower RER and higher fat oxidation), and increased DIT in at least one of the two pecan groups. Together these findings suggest that daily pecan consumption could improve long-term weight management possibly through small modifications to energy intake and/or energy expenditure, which would subsequently reduce CVD risk.

For the body weight outcome discussed in Chapter 8, we increased the sample size and expanded the inclusion criteria. Contrary to our hypothesis, we observed a non-significant tendency for weight gain in ADD and SUB but not control. The theoretical change in body weight was much higher than the actual changes in body weight, suggesting that participants partially compensated for the added energy content of the pecans in the diet. However, since the

change in weight was not different between ADD and SUB, it appears that the dietary substation instructions did not impact body weight. The findings from this manuscript illustrate the need for future research on the metabolizable energy of pecans, the effect of a lower dose of pecans on health and adiposity, and the impact of different types of dietary instructions, such as fat substitution, during pecan interventions. Since pecans provide many benefits that reduce the risk for developing CVD, it is essential to identify the optimal dose and dietary instructions to support weight maintenance.

Participant recruitment was one of the greatest challenges during the dissertation process. It was difficult to find participants that qualified for the study based on the inclusion criteria, did not take any of the excluded medications, and were willing to attend two 6-hour testing visits. The obstacles for recruitment escalated with the onset of the COVID-19 pandemic in March of 2020. Recruitment halted from March through August 2020 and resumed only after extensive modifications of the institutional review board protocols. The recruitment process could have been improved by establishing partnerships with the local hospitals and doctors' offices at the onset of the study. Furthermore, a larger advertisement budget is warranted when recruiting a difficult population and should be considered for future studies. Finally, since recruitment continued for 3 years, the pecans in the study were from different crops and could have had varying nutrient contents. Pecans are harvested during September and October every year and commonly undergo alternative bearing in which one crop year will produce many nuts of a lower quality nuts while the next year will produce a less plentiful crop with well-developed kernels [10, 11]. In addition, the color and nutrient content of pecans can be affected by temperature, oxygen, light, and post-harvest processing [12]. We kept the pecans fresh by storing them in their original vacuum sealed packages inside the freezer. However, it is possible that the nutrients in

the pecans may have degraded throughout the year. Future studies should measure the fatty acid profile and antioxidants of each crop of pecans periodically throughout the year to control for this limitation.

There are additional aspects of the study that should be noted. First, the cost of the daily dose of pecans (68 g) was approximately $2.88/d, which may not be financially feasible for many households. Determining the lowest effective dose of pecans would help reduce cost barriers. Furthermore, the pecans in the study were Desirable, Stuart, and Sumner cultivars from the South Georgia Pecan Co. This is important because the quality and nutrient content of different cultivars is slightly variable [10, 12]. For example, Desirable pecans produce a high-quality nut with low susceptibility to alternative bearing but high vulnerability to scab fungus. Stuart trees produce a large nut that is more resistant to scab fungus. However, the Stuart trees also take a longer time to mature than other cultivars and are susceptible to developing sooty mold on the leaves. Finally, the Sumner variety produces moderately large nuts that are resistant to scab but susceptible to a pest called black pecan aphid. Prabhakar et al. [12] reported that the total phenol content of Desirable, Stuart, and Sumner cultivars to be 1950, 1472, and 1930 mg ellagic acid (EA)/100 mg, respectively. In contrast, the Schley cultivar contains 830 mg EA/100 mg, and the Choctaw cultivar contains 2300 mg EA/100 mg. Altogether, it's possible that the findings from our study may not be replicated if a different cultivar is used.

Another important consideration is the impact of health research on consumer sales. According to the United States Department of Agriculture, the annual consumption of tree nuts increased from 1.4 lbs. to 3.7 lbs./year between 1970 and 2016 [13]. Almond consumption increased by 1.35 lbs. during these years, while pecan consumption only 0.01 lbs. During these same years, there has been a surge of health research on almonds. To date, 27 trials have investigated the

impact of almond consumption on blood lipids, which does not include other studies that investigated other health outcomes [14]. It is likely that the high volume on health research on almonds contributed to the increases in consumer consumption of almonds. Prior to this dissertation, there were four published studies on the impact of daily pecan consumption on markers of health, thus the published chapters of this dissertation more than doubled the availability of high-quality, peer-reviewed research on pecans. Due to our recent publications, 60 media releases highlighting the health benefits of pecans have been published, which has generated over 18 million media impressions through social media, newspapers, online media sources, and radio newscasts. These media releases occurred in well-known news sources such as Dr. Oz and Yahoo News in five continents. Therefore, we expect to see increased consumer demand for pecans soon, which further illustrates the importance of this research.

Overall, blood lipids, ANGPTL3, antioxidant status, appetite, and energy metabolism improved in at least one of the two pecan groups. The free-living nature of the study and elevated risk of the participants for CVD strengthens the generalizability and clinical significance of these findings. Furthermore, the observed improvements in the postprandial response to the high-saturated fat meal (devoid of pecans) suggests that daily pecan consumption can help protect against the detrimental effects of occasionally eating an unhealthy meal. In conclusion, daily pecan consumption could be a simple and cost-effect method for reducing CVD risk.

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