THE EFFECTS OF DIETARY PECANS AND ALMONDS ON THE PREVENTION OF HIGH FAT DIET – INDUCED METABOLIC DISORDERS

by

CHRISTINE OH

(Under the Direction of Dexi Liu)

ABSTRACT

Tree nuts have been studied for their nutritional value in the food and health industry and are considered an excellent source for vitamins, antioxidants, mono- and poly-unsaturated fats, fibers and proteins. The objective of this thesis study was to study the effects of tree nuts on animals fed a high fat diet in blocking high fat diet-induced obesity and obesity-associated metabolic disorders. Thirty-five C57BL/6 mice were continuously fed regular chow diet as the control or a high fat diet with or without the inclusion of almonds or pecans. Animal growth and food intake were monitored for 12 wk and the body composition was analyzed at the 12-wk feeding period. The impact of different diets on insulin sensitivity, blood – glucose levels, and fat accumulation were assessed after this feeding period. The results obtained suggest that dietary almonds and pecans do not block high fat diet-induced obesity and its associated consequences.

INDEX WORDS: High fat diet – induced obesity, tree nuts, pecans, almonds, insulin resistance, body weight, adipocytes, fatty liver

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DEDICATION

I would like to dedicate this thesis to my parents, who have been supporting me to pursue my dreams through their unconditional love. I also would like to thank my friends, who have always offered their time, support, and encouragements.

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I would like to acknowledge and thank my advisor, Dr. Dexi Liu for his patience and guidance for the past 3 years that I have been in his lab. I would also like to acknowledge my committee members, Dr. Phillip Greenspan and Dr. Gurvinder Rekhi for their criticism and comments that have helped me to learn and enjoy this field.

I would also like to thank my former lab-mates, Dr. Sary Alsanea and Madhura Spandana. Although my time with them in the lab was very brief, I sincerely thank Sary for his help and patience in teaching me lab techniques. I'd also like to thank Madhura, who was involved in this project, for her helpfulness and encouragements.

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CHAPTER 1

INTRODUCTION

Obesity and its Current Status

Obesity is a medical condition of an individual who has an excess amount of body fat. Obesity is normally defined by a body mass index (BMI), which is obtained by dividing an individual's body weight (kg) by the height in square meters. A BMI of 18.5 – 24.9 kg/m^2 is considered normal weight, 25 – 29.9 overweight, 30.0 - 34.9 class I obesity, 35.0 - 39.9 class II obesity, and greater than 40 class III or severe obesity.

According to the 2017 Global Health Observatory from the World Health Organization, the prevalence of overweight and obesity among adults (age >18) in 2016 were 39% and 13%, respectively. 18% of children and adolescents (age 5 – 19) were overweight and 7% were obese (1, 2). According to the 2017 NCHS Data Brief by the Centers for Disease Control and Prevention, 93.3 million American adults (39.6%) and 13.7 million children and adolescents (18.5%) were obese in 2016 (3). Within the last 10 years, obesity was prevalent among approximately 35% American adults, but the prevalence of overweight is expected to rise by 38% and 20% for obesity within the next decade (4). In Georgia, the adult obesity rate was 31.6% in 2017 and was ranked 24th in the US, while the rate of obesity in children (ages 10 – 17) was 18.4% and was ranked the 8th (5). The persistent rising trend of obesity suggests that obesity has become an epidemic issue not only in the developed countries, but also worldwide.

Obesity is the result of an imbalance between energy intake and expenditure, with more energy typically consumed than spent. Over time, the excess energy acquired is converted into body fat and stored in adipocytes, leading to body weight gain and accumulation of white adipose tissue surrounding internal organs in the peritoneal cavity and as subcutaneous fat. Physiological changes associated with fat accumulation are hyperglycemia, insulin resistance, fatty liver, and an increase in blood lipid level (6). Obesity often serves as a starting point for a range of serious medical complications including type-2 diabetes, cardiovascular diseases, steatosis, sleep apnea, atherosclerosis, hypertension, and cancer (6). As a result, obese adults in America spend up to 42% more on healthcare costs for obesity – related diseases and spend more than $147 billion per year for these costs (7).

Excess energy consumption is the direct cause for obesity, but multiple factors are known to influence food consumption. Leptin and leptin receptor genes have been identified as a regulator for satiety and mutations in these gene have been linked to continuous eating and obesity (8). Lifestyle, physical activities and diet are also contributing factors to obesity. Psychology is another factor that influences eating habits and obesity. Some patients eat in response to negative emotions such as boredom, sadness, or anger. While most overweight people have no more psychological disturbances than people at normal weights, about 30% struggle with binge eating or a loss of control of eating (9). Multiple approaches have been explored for treatment of obesity. In 2004, the World Health Organization released a global strategy plan to raise awareness for increasing physical activity and healthy eating habits and to reduce risks for chronic diseases (10). Pharmacological approach has led to development of a few drugs with limited

effectiveness. For example, Orlistat has been developed to inhibit the gastrointestinal absorption of fats for weight loss (11). Administration of orlistat for 36 wk has shown to reduce body weight by 8.3% in overweight patients with non-alcoholic fatty liver or diabetes (12). However, the weight loss can only be achieved when coupled with calorie restriction and physical exercise. Once the treatment or the accompanied behavior intervention discontinues, patients tend to gain back the weight that they lost. Sibutramine is another drug for weight loss for obese patients via suppressing food intake. Unfortunately, this drug was withdrawn from the market in 2010 due to severe side effects including cardiovascular problems and stroke (13). Glucagon-like peptide-1 is a peptide drug recently approved by the FDA for treating obesity (11). The therapeutic effect of this peptide can only be achieved at high doses, presumably due to unfavorable pharmacokinetics. However, in 2011, patients on glucagon-like peptide – based therapy showed increased risk of pancreatitis and pancreatic cancer, which led the FDA to release a warning of pancreatic safety from glucagon-like peptide use (14). Currently, bariatric surgery is the only method that is most widely used for effective and long – lasting weight loss. This procedure is an irreversible open surgery and can only be applied to a small group of patients with severe obesity (15). Moreover, even with such an extremely invasive therapy, 15-20% of patients will regain their body weight and fail to maintain euglycemia (15).

Strategies for Prevention of Obesity

Significant efforts have been made to control obesity epidemics by government administrations such as the Food and Drug Administration and the American Diabetes Association promoting healthier lifestyles with less intake of heavily carbohydrate and

sugar – based foods. In particular, medical nutrition therapy has been implemented to help reduce the risks that come with preexisting medical conditions in an individual The nutrition therapy provides five healthy, dietary patterns that can be personalized to meet the needs of each patient. Of these, the Mediterranean diet has been closely studied after it has shown to induce weight loss in the SUN project and reduce risks of cardiovascular events (i.e., cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke) as seen from the PREDIMED study (16, 17). This diet has also been popular because of its lack of restriction in food choices but encouraging consumption of vegetables, fruits, moderate amounts of red wine, and healthy unsaturated fats such as olive oil. In particular, the diet also promotes the consumption of tree nuts like macadamia nuts, almonds, walnuts, and pecans. Increasing evidence seems to suggest that among many nutritional components in the Mediterranean diet, the tree nuts play a critical role in regulating obesity and obesity-associated metabolic disorders.

Tree nuts are dry fruits with one seed that develops as the ovary wall hardens during maturity (18). The most common edible nuts are almonds, hazelnuts, pecans, walnuts, cashews, macadamias, and Brazil nuts (18). These nuts are highly nutritious, provide 553 – 718 kcal/100 g, and are high in; the calories from fat can range from 71.4% of total fat in cashews to 95% in macadamia nuts (18, 19). All tree nuts have greater proportions of monounsaturated and polyunsaturated fatty acids, though it may be in different amounts, compared to saturated fatty acids (18, 19, 20, 21). These nuts are also good sources of protein (4.41 – 15.5% total protein) and dietary fiber (3.3 – 12.5 g/100 g) (18, 19, 20). In addition, tree nuts have significant amounts of metal ions such as calcium, magnesium, and potassium (18, 19, 20).

Tree nut consumption has been highly encouraged by health organizations (i.e., FDA, AHA, ADA) to reduce the risks for coronary heart disease and diabetes. Several studies have shown that increased nut consumption has maintained body weight despite nuts' high fat content. In a study by Alper and Mattes (2002), healthy and normal weight adults consumed about 2113 kJ/day of peanuts for 8 wk without any dietary or lifestyle advice (22). The subjects gained about 1 kg after the experiment, which was about 28% of what was predicted (3.6 kg) (22). A similar study was done by Hollis and Mattes (2007), which had healthy and overweight adult females consume about 1440 kJ/day of almonds in a cross over trial for 10 wk (23). After a washout period of 3 wk, the participants were followed their usual diets for another 10 wk. By the end of the 23 – wk study, the average weight change decreased by 0.1 kg (23). In addition, three large scale clinical studies were conducted examining the effect of tree nuts (16). The SUN (Seguimiento Universidad de Navarra) project involved a prospective cohort of 8,865 adult men and women in Spain that started in December 1999 and has collected data using a food frequency questionnaire that measured frequency of nut consumption (50-g serving) and weight gain (16). After 28 months follow up, all participants gained weight but those who consumed the most (>2 serving/wk) gained the least amount. The odds of gaining weight (> 5 kg) for participants who consumed nuts at least twice a week was 30% less than those who rarely ate nuts (16). The PREDIMED (PREvencion Dieta MEDiterranea) study was a large, randomized clinical trial that tested if the Mediterranean Diet given with tree nuts or olive oil or a low – fat control diet will reduce the risks of cardiovascular disease in 7,447 Spanish adult men and women (16). After a 1 year follow – up, participants who ate the Mediterranean diet enriched with nuts or olive oil had reduced risks of developing cardiovascular diseases

(16). In addition, the nut – enriched diet decreased obesity prevalence by 4.9% compared to the 2.7% increase in the control low-fat diet (16). In the Nurses' Health Study – 2, 51,188 nurses were followed for 8 years to study the relationship between nut consumption and changes in BMI (16, 24). A reduced average weight gain by 1.1 kg in American women nurses was found among those who consumed >2 serving of nuts/wk (16, 24).

There have been several studies that test the effects of tree nuts on the prevention of type-2 diabetes mellitus, which is signified by decreased blood glucose clearance from insulin resistance and pancreatic beta cell dysfunction. A 16 year follow – up from the Nurses' Health Study-1, which tested the effect of nut consumption on the risks for type-2 diabetes on lean women and women with diabetes, showed that the relative risk for developing diabetes decreased by 27% for diabetic women who consumed nuts 5 times a week compared to those who rarely ate nuts (18, 25). The relative risk for lean women who ate nuts 5 times a week was further reduced to 45%. In another study by Wien et al. (2003), 65 overweight men and women who were diagnosed with diabetes or a metabolic syndrome were given almond-based diet (84 g/day) or carbohydrate-based diet for 24 wk (26). The subjects were evaluated for insulin resistance via HOMA-IR among other factors indicative of weight change and dyslipidemia. HOMA-IR decreased for both almond-based and carbohydrate-based diets, but beta cell functions significantly improved for the almond-based diet (26). These studies suggest that the consumption of nuts may be able to improve insulin sensitivity.

Different results have been reported on the effects of dietary nuts on insulin sensitivity. Lovejoy et al. (2002) reported no improvement on insulin sensitivity in 20 healthy adults with almond consumption (100 g/day) for 4 wk. Insulin sensitivity in women

worsened by 19% and improved by 13% in men, but these results were not statistically significant (27, 28). In a study by Tapsell et al. (2004), 58 type-2 diabetic men and women were given a low-fat control diet, a modified low-fat diet, and a walnut-based (30 g/day) low-fat diet (27). Measurements of body weight, BMI, HbA_{1c} (glycated hemoglobin), total body fat, total cholesterol, and lipid levels were made at beginning, 3 and 6 months later (29). The results show that the addition of 30 g of walnuts to the low fat diet improved lipid and cholesterol levels, but no significant changes in glycated HbA_{1c} (29). The study concluded that nut consumption greater than 4 wk and nuts taking 10% of the total energy in diets is required for positive effects on insulin sensitivity and glycemic control (29). However, the Iowa Women's Health Study (2003) with a 11-year follow up indicated that nut consumption provided little protective effects against the risk of type-2 diabetes in postmenopausal and non-diabetic women (18, 30). The data was readjusted for post-menopause, but the rate did not significantly change. The Physician's Health Study (2010) tested the effect of nut consumption in men against the risk for diabetes, but there were no reductions after a 19-year follow up (18, 31). The inconsistency of the results from different studies suggest that nut consumption may or may not completely impact insulin sensitivity in diabetic and non-diabetic patients, warranting more studies for better understanding of the activity of dietary nuts in regulating metabolic homeostasis and obesity development.

Study Objectives and Design

Despite the inconsistency in beneficial effects of dietary nuts on improving insulin sensitivity reported by different studies involving a large number of people with various physiological conditions, increasing evidence seem to suggest that dietary tree nuts could produce health benefits in in controlling weight gain and glucose homeostasis. The central

hypothesis for this thesis study is that dietary tree nuts will reduce or block high fat-induced obesity and obesity-associated insulin resistance, hyperglycemia and fatty liver development. The objective of this study was to test this hypothesis using inbred animal model with minimal genetic heterogeneity and well controlled environment.

Georgia pecan and almonds were used in the study. The tree nuts were formulated as part of high fat diet with fixed amount of calorie from nuts. Three different high fat diets containing pecan, almonds or without were color coded and therefore the study was run in a double blinded fashion. The high fat diets provided 18% cal from proteins, 32% cal from carbohydrates, and 50% cal from fat. The almond and pecan replaced approximately 10% of the total energy in the high fat diets. Animals were fed continuously with different diets, and the effects of dietary nuts included in high diet on animal growth, food intake, and energy intake were monitored for 12 wk. Body composition was examined at the end of the 12-wk feeding period. Additional tests to measure blood glucose levels using glucose and insulin tolerance tests were taken beyond this feeding period. The mice were then sacrificed to collect the liver and adipose tissues to assess the effects of HFD – inclusion of pecans and almonds on changes in liver and adipose tissue weight and adipocyte diameter. The goals of these experimental assessment were to collect direct evidence to prove or disapprove whether dietary nuts are effective in blocking high fat diet induced obesity and/or improving metabolic homeostasis.

CHAPTER 2

MATERIALS AND METHODS

Materials

The pecans were obtained through contracts at the Pecan Commission of Georgia (Tifton, GA). The almonds were from the Almond Board of California (Modesto, CA) and the California Walnut Commission (Folsom, CA). All high fat diets were custom-made and provided by Research Diets, Inc (New Brunswick, NJ). The diet composition is listed in Table 1. Humulin® was obtained from Eli Lilly (Indianapolis, IN, Catalog# 0002-8215-01). The glucose was purchased from Fisher Diagnostics (Middletown, VA, Catalog# 070800). The 10% neutral buffered formalin (NBF) was purchased by VWR (Radnor, PA, Catalog# 6970). The optimal cutting temperature (OCT) compound was purchased from Sakura Finetek (Torrance, CA, Catalog# 2964). The 100% ethanol was purchased from Decon Labs (King of Prussia, PA; Catalog# 191414). The Oil Red O isopropanol solution was purchased from Electron Microscopy Sciences (Hatfield, PA, Catalog# 26079-05). The Protocol® Eosin Y was obtained from Fisher Scientific Company (Kalamazoo, MI, Catalog# 245-658) and the Mayer's Hematoxylin was obtained from Dako (Santa Clara, CA, Catalog# S3309). Xylene (Catalog# 148642) and Permount® mounting medium were purchased from Fisher Scientific (Fair Lawn, NJ, Catalog# 110165).

Table 1. Nutrient content and diet composition of experimental high fat diets.

	HFD	HFD-Almonds	HFD-Pecans
Nutrients			
Carbohydrate (% cal)	32.0	32.0	32.0
Protein (% cal)	18.0	18.0	18.0
Total Fat (% cal)	50.0	50.0	50.0
Cal per gram	4.76	4.79	4.76
Composition (g)			
Almonds	-	82.8	-
Pecans	-	-	83.2
Lard	194	155.3	136.6
Soybean Oil	25	25	25
Casein	200	179.9	192.1
L-Cystine	3	3	3
Corn Starch	51.55	36.2	42.4
Maltodextrin 10	100	100	100
Sucrose	157	153.05	154.65
Cellulose	50	39.7	42
Vitamin and Mineral Mix	12	12	12
Red Dye #40, FD&C	0.05	-	-
Blue Dye #1, FD&C	-	0.05	-
Yellow Dye #5, FD&C	-	-	0.05

Methods

Animals and Animal Treatments

Thirty-five C57BL/6 male mice at 8 wk of age were purchased from Charles River Laboratory (Wilmington, MA). All the mice were divided randomly into 7 groups and placed in mouse cages (5 mice/cage) (Table 2). Two cages of animal (n = 10) were fed either a regular HFD or HFD containing almonds or pecans respectively. One cage of animals served as a control and was fed a regular chow (n = 5). Animals were housed under

a standard condition with a 12 h light-dark cycle. Animal weight was measured using an electronic scale twice per week (Monday and Thursday) for 12 wk. At the end of experiment, the body composition of each animal was determined using an EchoMRI system. All treatments on the mice were approved by the Institutional Animal Care and Use Committee at the University of Georgia, Athens, Georgia.

Determination of Food and Energy Intake

Food intake or consumption was determined by subtracting the total amount of diet left from the amount originally placed for each cage of animals. Food measurement was taken twice a week and the left – over from previous feeding period was discarded and replaced with fresh food. Food intake was calculated by dividing the total amount food consumed by the number of animals in each cage and the number of days involved. The energy intake was determined by multiplying the calculated food intake and the calories from each diet.

Glucose Tolerance Tests

Ten days after the 12-wk feeding period (day 94), one cage of 5 animals from each diet group were fasted for 6 h and intraperitoneally injected with glucose solution (1.5 g/kg). Immediately after and at 30, 60, and 120 min post injections, a small tip at the end of the mouse tail was snipped with surgical scissors to collect a few drops of blood onto blood test strips. The blood strips were inserted into the glucometer to show the blood glucose levels. The same test was repeated on these animals on day 104.

Insulin Tolerance Tests

On day 101 of the experiment, the same groups of mice from those used for the glucose test were fasted for 4 h. Intraperitoneal injection of insulin solution (0.75 U/kg) was performed. Soon after and at 30, 60, and 120 min post injection, a small cut at the tip of the mice's tails was made to collect a few drops of blood onto a blood test strip for determination of glucose level. Animals were returned to their diets after the test.

H&E Staining

On day 105 of the experiment, 5 mice from each diet group were sacrificed to collect blood samples, the liver, epididymal white adipose tissue (eWAT), inguinal white adipose tissue (iWAT), perirenal white adipose tissue (pWAT), and brown adipose tissue (BAT). Each collected tissue and liver sample were weighed before a small piece was cut, which was placed in 45 mL centrifuge tubes and fixed with 10% formalin at room temperature overnight. Formalin fixed liver and adipose tissues were cut, placed in tissue cassettes, and washed with water 3 times. The tissue samples were then gone through the process of dehydration first in 75% ethanol for 30 mins, 85% ethanol for 30 mins, 95% ethanol for 30 mins, and finally 100% ethanol for 60 min. Dehydration in 100% ethanol was repeated twice and tissue samples were placed in xylene for 120 min. The dehydrated tissue samples were placed in plastic tissue molds and filled with paraffin. Using forceps, the tissues were carefully placed at the center of the paraffin – filled molds to ensure better tissue sections. A cassette lid was placed on top of the molds to hold the tissues in place while they were kept at 60^{o}C overnight. The molds were placed on a cold-plate at -5°C for 3 h to solidify. The paraffin blocks were mounted onto the Leica RM2235 rotary microtome (Buffalo Grove, Il) and sectioned at a thickness of 6 µm. The thinly sectioned ribbons of the tissues were placed in a warm water bath at 40 – 45°C to dissolve the paraffin, mounted

onto glass slides, and left to dry for 3 h at room temperature. The glass slides were dipped three times for 3 min each in 100% xylene, 100% ethanol, 95% ethanol, and rinsed with distilled water. The glass slides were then placed in Hematoxylin for 8 min followed by 5 min rinse with tap water. The sections were dipped 4 times very quickly into acid alcohol (1% HCl in 70% ethanol). The sections were rinsed under tap water again for 5 min. After which, the slides were dunked 6 times slowly in ammonia water (0.1% NH_3 in 1 L of water). The tissues were rinsed with tap water again for 5 min and stained with eosin Y for 1 min followed by rinsing using tap water until the water without color. Tissue sections were finally treated with 95% ethanol for 2 min,100% ethanol for 2 min and 100% xylene for 2 min. The slides were left for airdry at room temperature. Small drops of Permount mounting medium was placed onto the area with tissue sections and covered with a cover slip. The glass slides were left to dry in a fume hood overnight and examined under a light microscope (Nikon Eclipse Ti). Photographs were taken at 10x magnification using the NIS imaging system.

Oil Red O Staining

After the mouse dissection, the liver samples were freshly mounted onto tissue cassettes with OCT compound, frozen with liquid nitrogen, and stored in a -80°C freezer until staining. The frozen liver samples were mounted onto the Leica CM1850 Cryostat (Buffalo Grove, IL) and frozen sectioned at a thickness of 8 µm. The sections were mounted onto labeled glass slides and left to dry at room temperature for overnight. After which, the Oil Red O working solution was prepared using 24 mL of Oil Red O stock solution and 16 mL of distilled water. The solution was filtered into a designated container before use. The 60% isopropanol was prepared by combining 60 mL of 100% isopropanol

and 40 mL of distilled water. The dried glass slides were dipped into 10% NBF for 30 min. Next, the liver sample were rinsed under tap water for 10 min and placed in 60% isopropanol for 5 min. Then, the glass slides were vigorously dunked into Oil Red O working solution multiple times for 15 min and placed in 60% isopropanol for 5 min. The tissues were quickly dipped five times into hematoxylin, rinsed with distilled water until no more color was running, and placed in the fume hood at room temperature to dry for overnight. Because there was no glycerin jelly at hand to use as a mounting medium, the glass slides were directly analyzed. The stained liver samples were examined under the light microscope and photographed at different magnifications using the NIS imaging system.

Statistical Analysis

Statistical analysis was performed by ANOVA and Tukey's range test with significance set at $P<0.05$. Each data point represents the mean ± standard deviation.

CHAPTER 3

RESULTS

Effects of Dietary Pecans and Almonds on Body Weight and Composition

By the end of the 12 – wk period, the mice were photographed for body comparisons between mice from each diet. In Figure 1a, all mice given high fat diet with or without nuts looked significantly larger than mice given regular chow. The average weight gain by the end of 12 wk for the chow – fed mice was 32.68 ± 2.41 g (Figure 1b). Mice given HFD gained an average weight of 47.6 ± 2.87 g, 47.06 ± 4.95 g for mice on HFD-almonds, and 46.15 ± 5.87 g for mice on HFD-pecans. There is a 14.50 g difference between the chow – fed mice and HFD – fed mice. However, the difference between each HFD mice was about 1 g.

In terms of body composition, mice on HFD-pecans had a lean mass of 29.01 ± 2.94 g and fat mass of 15.38 ± 7.83 g. In comparison, mice on HFD-almonds had 27.48 ± 1.74 g of lean mass and 22.86 ± 1.32 g of fat mass. Mice on HFD resulted in similar body compositions with 27.65 ± 3.12 g of lean mass and 22.66 ± 0.43 g of fat mass. Finally, mice on regular chow had the smallest body mass with 26.19 ± 3.29 g of lean mass and 7.25 ± 1.94 g of fat mass (Figure 2). Of the high – fat diets, the HFD-pecan diet had the lowest fat mass, which is 7.48 g and 7.28 g lower than the fat masses of the HFD-almond diet and the HFD only, respectively.

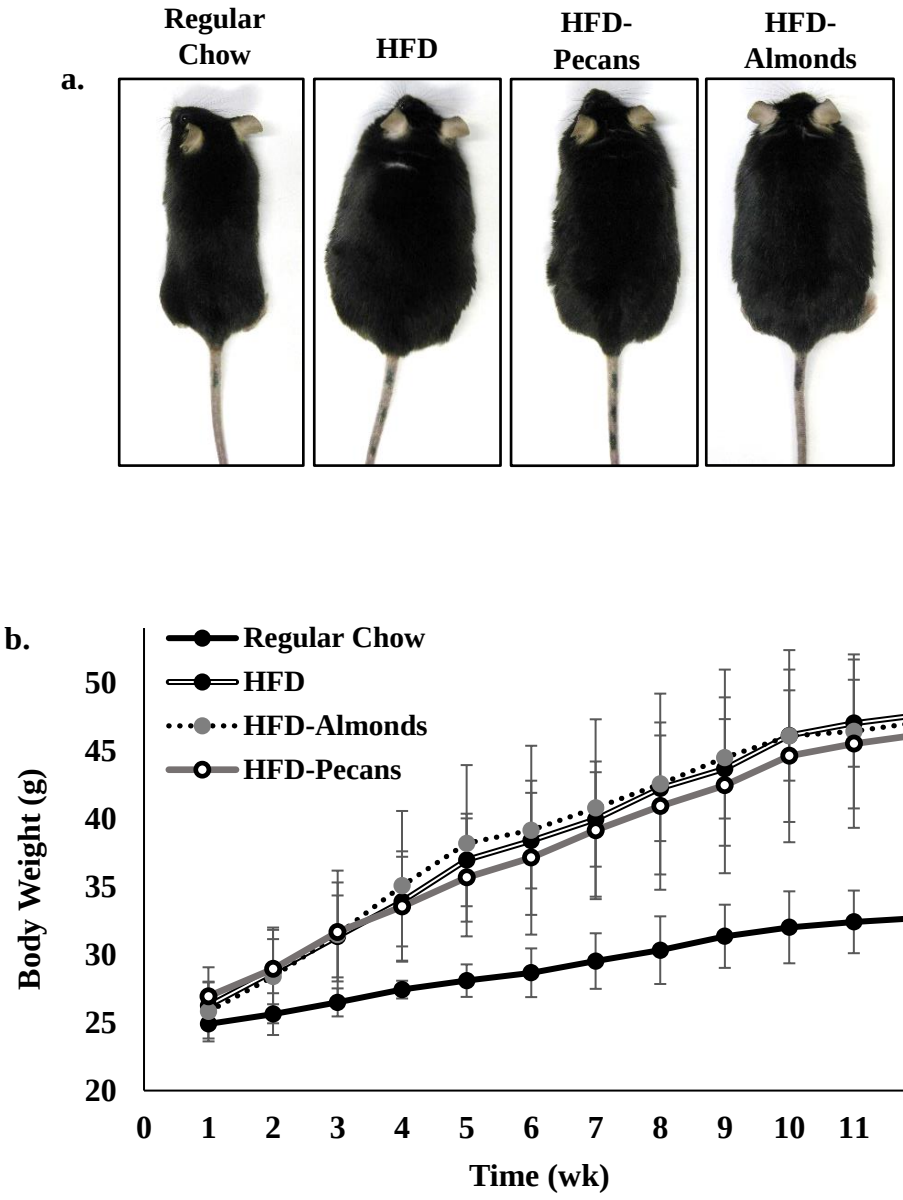


Figure 1. Effects of dietary pecans and almonds on high fat diet – induced weight gain at the end of 12-wk feeding period. Mice were continuously fed regular chow (n=5), HFD (n=10), HFD-almonds (n=10), and HFD-pecans (n=10) for 12 wk. Body weight gain was measured twice a week during the feeding period. (a) Images taken to compare physical changes in mice from regular chow, HFD, HFD-Almonds, and HFD-Pecans. (b) Average body weight gain by 12 wk. All values represent mean ± standard deviation.

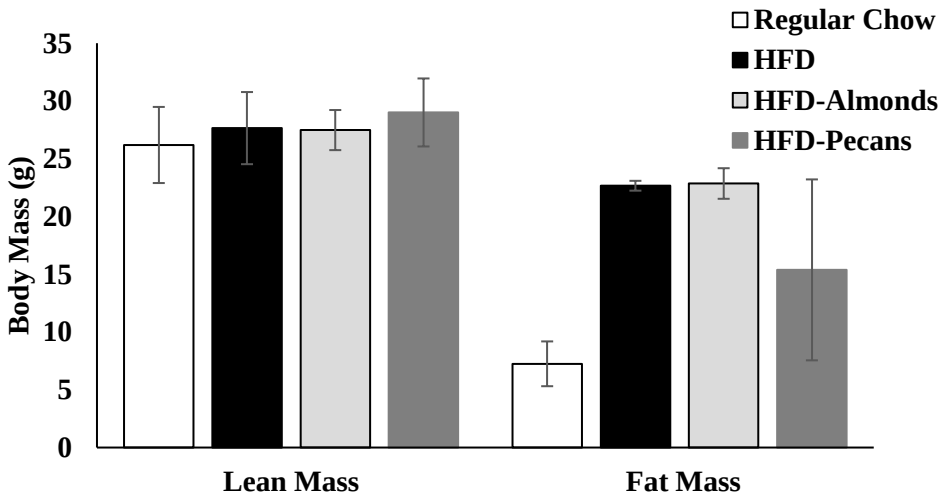


Figure 2. Effects of dietary pecans and almonds on lean and fat mass at the end of 12-wk feeding period. Body mass of mice from all diets (n=5) were measured using EchoMRI. All values represent mean ± standard deviation.

Effects of Dietary Pecans and Almonds on Food Intake and Energy Intake

Although two groups of mice were used to measure food intake for each diet, only one group was measured for the HFD – almond diet because there was some variability in the food intake. Small bits of food were seen on the floor of the cage, which affected the food intake measurements. The inaccurate data has also led to the omitting of some data points in Figures 3a – b. The average food intake per mouse per day were only measured for the HFD diets (Figure 3a). In Figure 3b, the accumulative energy intake per mouse per day presents a similar caloric intake between each mouse of the three high – fat diets; i.e., 26.28 ± 0.023 kcal/mouse/day for mice given HFD with almonds, 24.14 ± 0.12 kcal/mouse/day for mice on HFD with pecans, and 22.8 ± 0.32 kcal/mouse/day for mice on HFD.

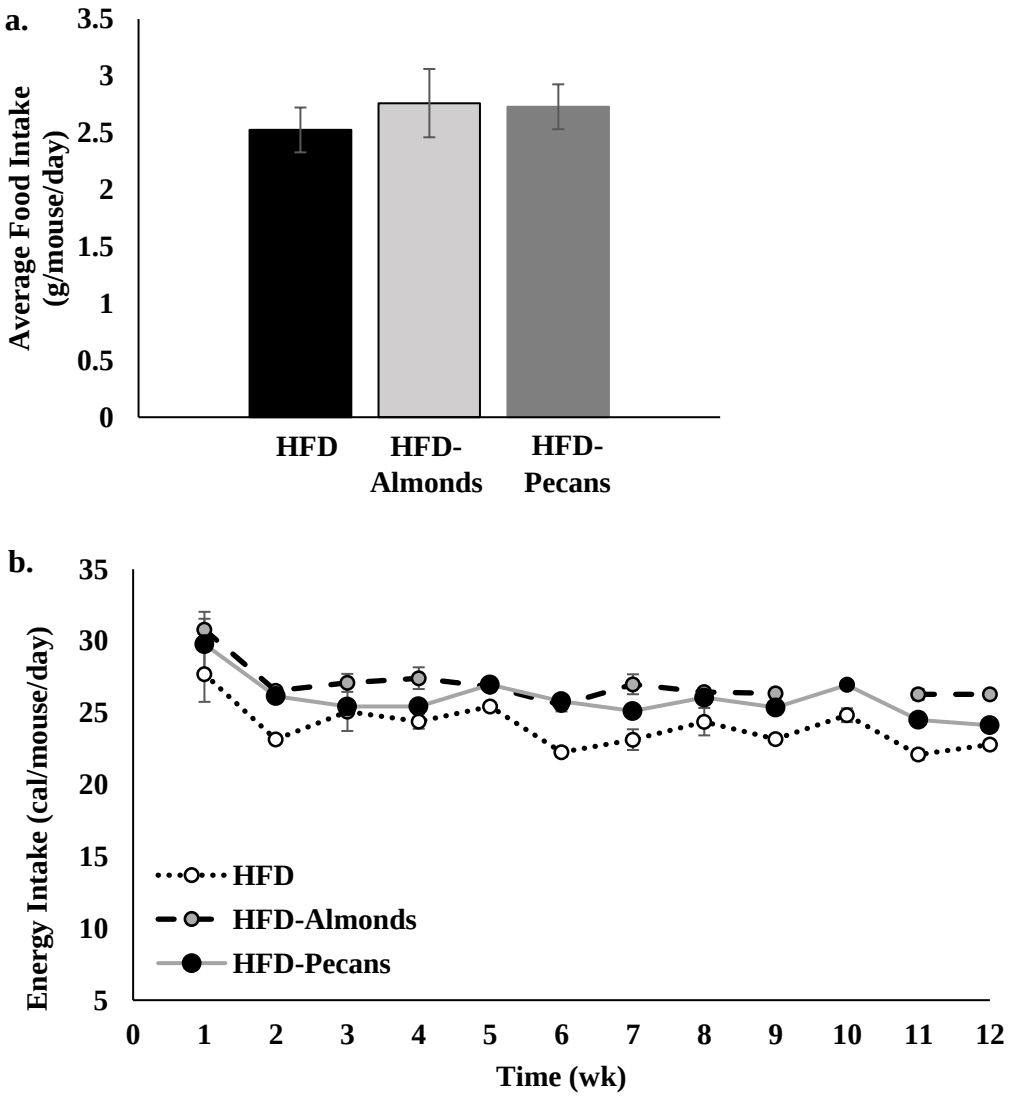


Figure 3. Effects of dietary pecans and almonds on food and energy intake at the end of 12-wk feeding period. Mice given HFD (n=10), HFD-almonds (n=5*), and HFD-pecans (n=10). (a) Average food intake (g/mouse/day). (b) Accumulative energy intake per mouse (cal/mouse/day). All values represent mean ± standard deviation.
*Due to variability in food intake from mice in HFD-almonds, data results from only 5 mice were analyzed.

Effects of Dietary Pecans and Almonds on Lipid Accumulation in the Liver

The physical comparisons of the livers are shown in Figure 4a. The livers belonging to the high fat diet groups appeared to be significantly larger than the liver from the regular chow group. According to Figure 4b, liver weights from mice given HFD, HFD – almonds,

and HFD – pecans were, respectively, about 1.13 g, 1.86 g, and 1.18 g higher than livers from chow – fed mice. Mice given HFD-almond had the highest liver weight with 3.04 ± 0.59 g, followed by mice on HFD-pecan with 2.37 ± 0.59 g, and mice on HFD with a liver weight of 2.32 ± 0.23 g. The H&E staining of the livers indicated greater lipid accumulation in mice given any high fat diet compared to the regular chow group. (Figure 4a). The lipid droplets are also visible in the Oil – Red – O stained livers of the high fat diet – induced mice than seen in that of chow – fed mice (Figure 4a). The additions of pecans or almonds did not lower the lipid accumulation to the extent that is seen with mice given regular chow.

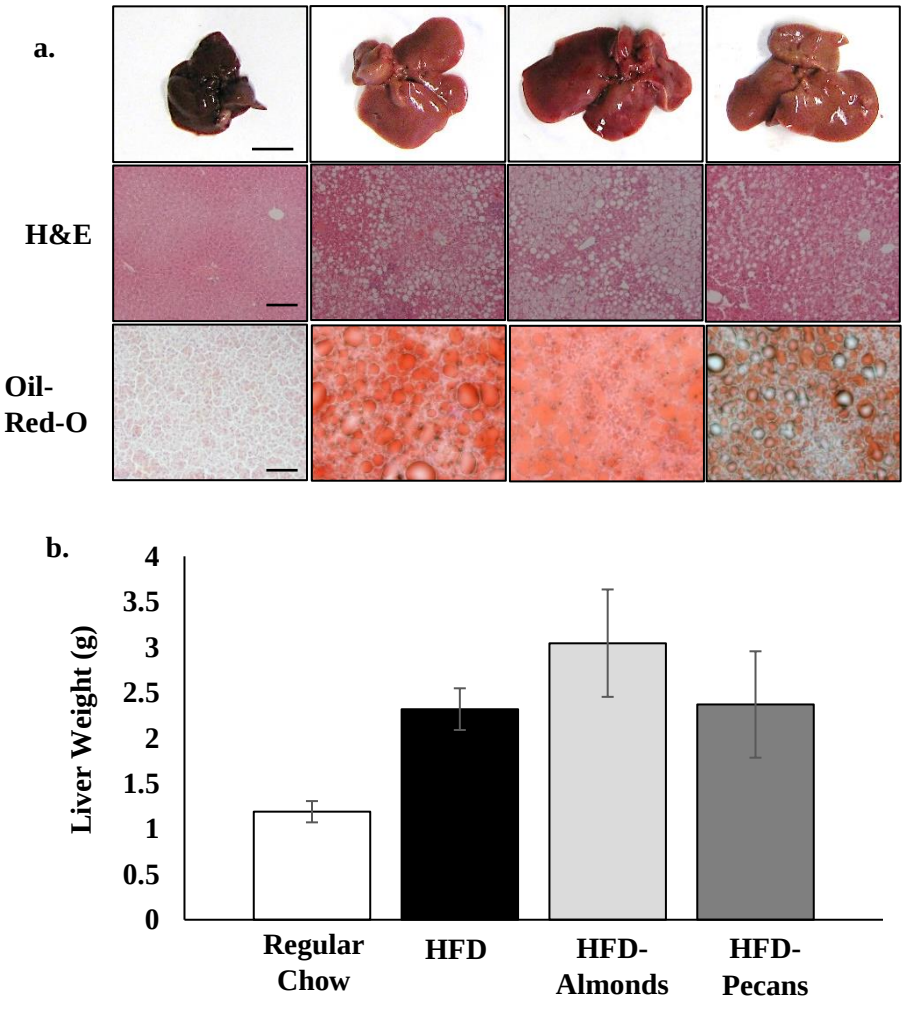


Figure 4. Effects of dietary pecans and almonds on lipid accumulation in the liver after 12 wk. After the 12-wk feeding period, the mice were sacrificed. The consumption of the high – fat diets, particularly the HFD-almonds diet, caused increase in the amount and size of adipocytes in the liver at scale bar of 1 cm. (a) Photo images of liver and H&E and Oil-Red-O staining of liver sections at 10x magnification and scale bar: 100 µm. (b) Average weight (g) of liver, (n=5). All values represent mean ± standard deviation.

Effects Dietary Pecans and Almonds on White and Brown Adipose Tissues

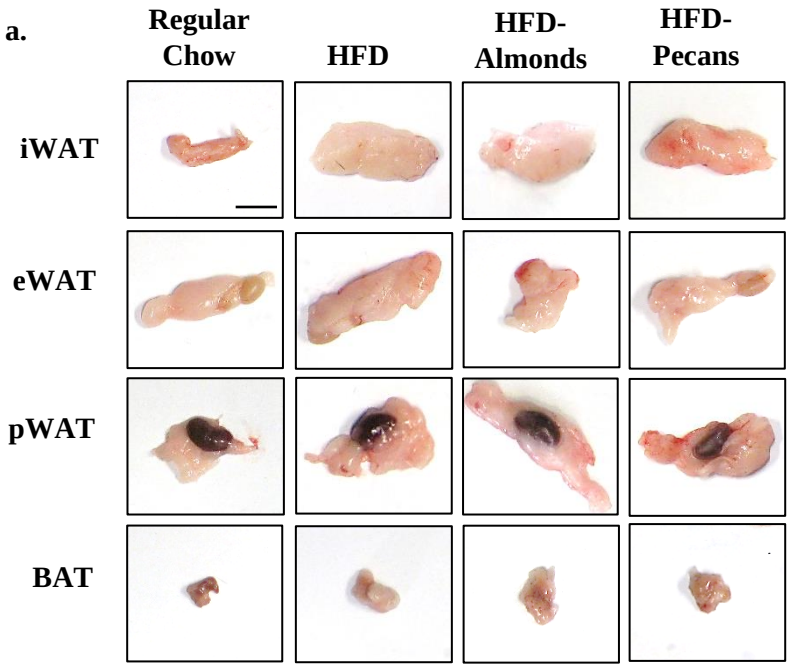
The inguinal white adipose tissues (iWAT) from the high fat diets were significantly larger than the regular chow group (Figure 5a). The adipose tissues are noticeably larger in high fat diet – induced mice than in chow – fed mice. The average weights of iWAT from the HFD, HFD-pecans, and HFD-almonds diets were, respectively, 1.94 ± 0.55 g, 1.93 ± 0.55 g, and 1.56 ± 0.32 g, whereas the chow – fed mice had an average tissue weight of 0.26 ± 0.088 g (Figure 5b). The H&E staining further confirms the larger adipocytes seen in HFD – fed mice than those from the chow – fed mice (Figure 5c). The average tissue diameter for mice given regular chow, HFD, HFD-almonds, and HFD-pecans diets were, respectively, 65.02 ± 12.9 µm, 154.8 ± 28.4 µm, 132.4 ± 30.2 µm, and 158.2 ± 24.1 µm (Figure 5d).

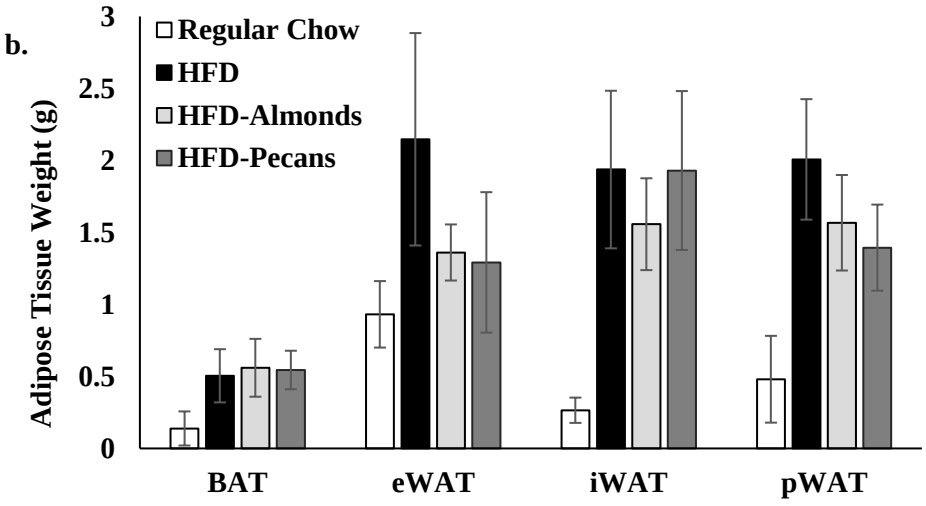
The accumulation of the perirenal white adipose tissue (pWAT) around the kidneys were significantly larger in high fat diet groups than that seen in the regular chow group (Figure 5a). However, the accumulation appeared to be less in the HFD-pecan group, which is also reflected in the tissue weight. The average tissues weights from regular chow, HFD only, HFD-almonds, and HFD-pecans diets were, respectively, 0.48 ± 0.3 g, 2.01 ± 0.42 g, 1.57 ± 0.59 g, and 1.39 ± 0.59 g (Figure 5b). The H&E staining show the adipocytes increased significantly in all mice given high – fat diets compared to mice given regular chow (Figure 5c). The average tissue diameters for mice given regular chow, HFD only,

HFD – almond, and HFD – pecan diets were, respectively, 93.9 ± 21.6 μm, 149.5 ± 30.5 μm, 162.3 ± 46.9 μm, and 155.5 ± 39.2 μm (Figure 5d).

The physical comparisons of the epidydimal white adipose tissues (eWAT) indicated significant fat accumulation in mice given high fat diets (Figure 5a). Although there was some accumulation seen in the regular chow group, this group had the lowest average tissue weight of 0.93 ± 0.23 g (Figure 5b). Mice given HFD had the highest average tissue weight of 2.15 ± 0.74 g (Figure 5b), and tissues from mice on HFD-almonds and HFD-pecan were, respectively, 1.36 ± 0.19 g and 1.29 ± 0.49 g. The H&E staining indicated significant lipid accumulation in HFD – almond diet compared to the other high fat diets (Figure 5c). Although the H&E stained adipocytes in the regular chow diet seemed similar to the other high fat diet groups, the tissue diameter analysis showed that the regular chow – fed mice had the lowest average tissue diameter (Figure 5d). The average tissue diameter was the slightly higher for HFD – almond diets compared to mice given HFD only. The average diameters were 98.97 ± 15.97 μm for chow-fed mice, 119.03 ± 26.1 μm for HFD-fed mice, 135.4 ± 37.2 μm for mice on HFD – almonds, and 137.1 ± 28.3 μm for mice on HFD – pecan (Figure 5d).

The brown adipose tissues from diet – induced obese mice were much larger than that of chow – fed mice, as seen in the physical comparisons and average tissue weights (Figure 5a-b). The average weights from mice given regular chow, HFD only, HFD – almonds, and HFD – pecans were, respectively, 0.14 ± 0.12 g, 0.50 ± 0.18 g, 0.56 ± 0.20 g, and 0.54 ± 0.13 g (Figure 5b). The H&E staining results indicated higher fat deposits in the high fat diets, particularly from mice given HFD-pecans and almonds (Figure 5c).


a.
Regular Chow
HFD
HFD-Almonds
HFD-Pecans
iWAT
eWAT
pWAT
BAT


b.
Regular Chow
HFD
HFD-Almonds
HFD-Pecans
Adipose Tissue Weight (g)
3
2.5
2
1.5
1
0.5
0
BAT
eWAT
iWAT
pWAT

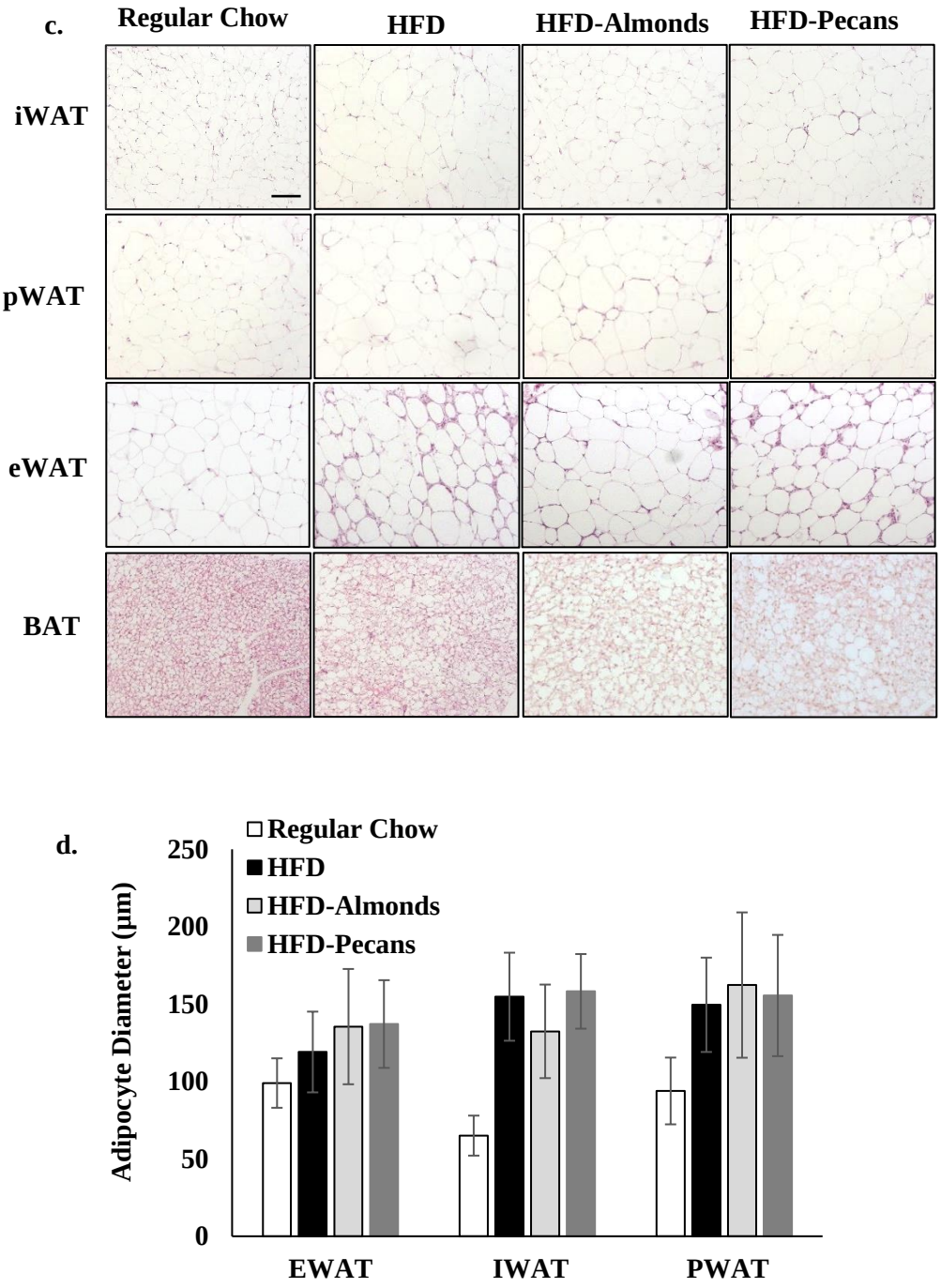


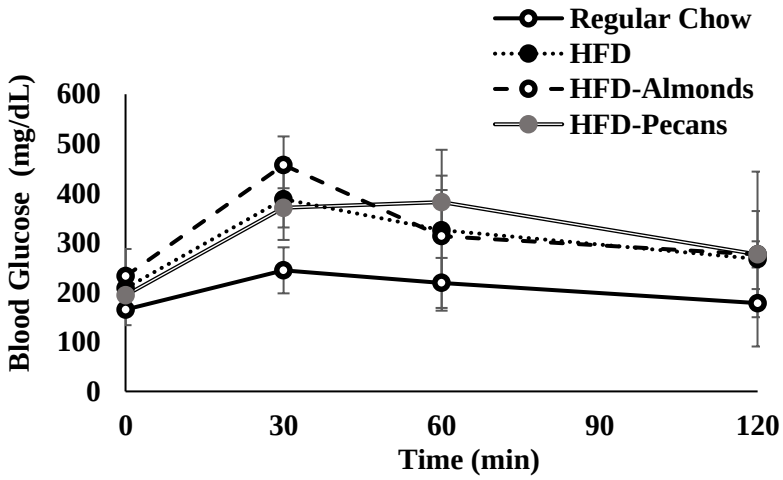
Figure 5. Effects of dietary pecans and almonds on white and brown adipose tissues after 12 wk. After the 12-wk feeding period, the mice were sacrificed. (a) Image comparisons of inguinal, perineal, epididymal white adipose tissues and brown adipose tissue at scale bar of 1 cm. (b) Average tissue weights. (c) H&E and Oil-Red-O staining of white and brown adipocytes at 10x magnification and scale bar of 100 µm. (d) Average fat cell diameter (µm). All values represent mean ± standard deviation.

Effects of Dietary Pecans and Almonds on Insulin Sensitivity and Glucose Tolerance

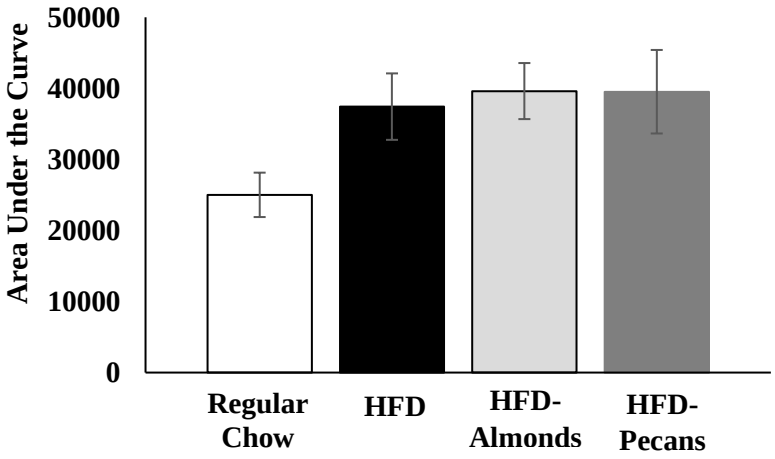
The glucose and insulin tolerance tests revealed that the high fat diets included with nuts did not improve insulin sensitivity in obese mice compared to mice given only HFD. The results for the glucose tolerance test are presented in Figure 6a. Fasting blood – glucose levels (0 min) were elevated for mice given high fat diets and remained so after 120 min, which indicated impaired glucose tolerance (Figure 6a). Mice fed regular chow maintained low blood – glucose levels throughout the test in comparison (Figure 6a). The area under the curve calculations indicate a 33.2%, 36.9%, and 36.7% increase in blood glucose levels from mice given HFD, HFD-almonds, and HFD-pecans, respectively, compared to glucose levels from chow – fed mice (Figure 6b).

After 1 wk, an insulin tolerance test was performed on the same batches of mice from each diet. In Figure 6c, the results of the insulin tolerance test show relatively low glucose clearance after injection of 0.75 U/kg of insulin for all diets, which indicated the high fat diets with almonds or pecans did not improve insulin sensitivity. The blood – glucose levels between mice on regular chow and HFD were not statistically different, but mice on HFD-almonds had a 14.2% increase in blood – glucose level compared to chow-fed mice (Figure 6d). Mice on HFD-pecan increased blood – glucose levels the most by 22.8% compared to control mice, which further confirms that the inclusion of pecans or almonds were not able to have any effect on insulin sensitivity.

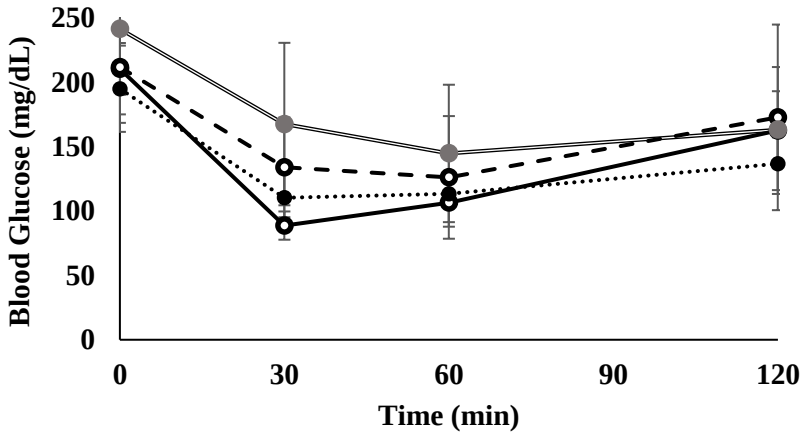
a.


Regular Chow
HFD
HFD-Almonds
HFD-Pecans
Blood Glucose (mg/dL)
600
500
400
300
200
100
0
0
30
60
90
120
Time (min)

b.


Area Under the Curve
50000
40000
30000
20000
10000
0
Regular Chow
HFD
HFD-Almonds
HFD-Pecans

c.


Blood Glucose (mg/dL)
250
200
150
100
50
0
0
30
60
90
120
Time (min)

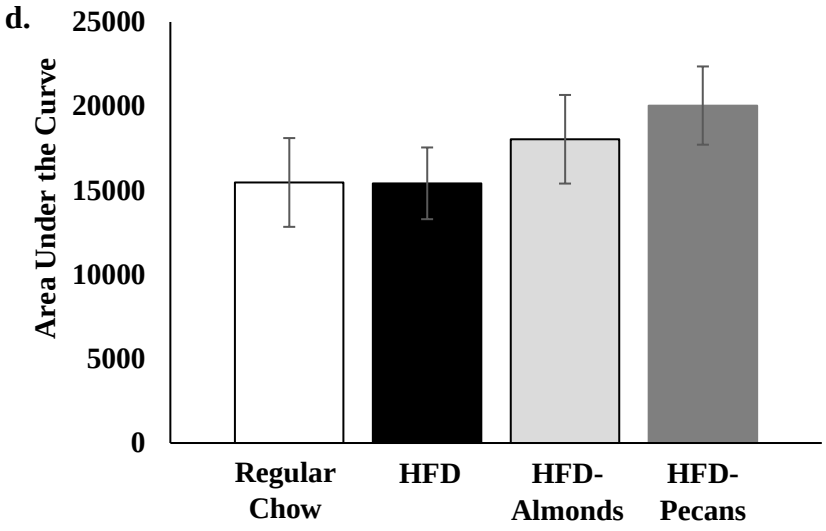


Figure 6. Effects of dietary pecans and almonds on insulin sensitivity and glucose tolerance after 12 wk. (a) Time – dependent blood glucose level after IP injection of glucose (1.5 g/kg). (b) AUC for GTT for 120 mins. (c) Time – dependent blood glucose concentration after IP injection of insulin (0.75 U/kg). (d) AUC for ITT for 120 mins. All values represent mean ± standard deviation.

CHAPTER 4

DISCUSSION

A systematic comparison was carried out in the study to examine the effects of almonds or pecans included in HFD on HFD-induced weight gain, adipogenicity, fat accumulation in the liver, insulin resistance, and glucose homeostasis in C57BL/6 male mice. Significantly higher weight gain was seen in mice fed an HFD comparing to those fed regular chow. This weight gain is also visually seen in the photo images of the mice (Figure 1a), as well as the increased weight of white and brown adipose tissues and adipocyte diameters (Figure 5). However, mice fed HFD with almonds or pecans showed no clear differences in body weight gain by the end of the 12 – wk feeding period. The average food intake was similar for all high fat diets (Figure 3a). The accumulative energy intake of high fat diets with or without almonds and pecans was not statistically significant (Figure 3b). The H&E staining of the adipose tissues revealed no difference in the average adipocyte diameter in mice given HFD with or without nuts (Figure 5c-d). The H&E and Oil-Red-O staining of the liver sections from animals fed HFD with or without tree nuts showed significant fat accumulation in the liver, which is indicative of fatty liver (Figure 4a). The lean masses from all HFD – fed mice and chow – fed mice were similar, but in comparison to the fat mass from mice given regular chow, the fat masses from mice fed HFD and HFD-almonds were 15.41 – 15.61 g higher compared to the 8.13 g increase from mice fed HFD-pecans (Figure 2). The insulin tolerance tests indicated insulin resistance in mice fed with HFD with or without tree nuts and not in mice fed with regular chow (Figure 6c-d). However, the GTT results contradicted this low insulin sensitivity (Figure 6a-b). The

$AUC_{glucose}$ of HFD only from the glucose tolerance test increase significantly compared to chow – fed mice but was lower than that of HFD-pecans and HFD-almonds.

The results of several human clinical trials have suggested the health benefits of dietary nut consumption on high fat diet – induced body weight gain, fat development, and insulin resistance. In a 12-wk crossover trial, 20 type 2 diabetic adult patients, with stable blood – lipid and sugar levels and receiving no insulin treatment, were randomly assigned to an 1800 calorie high – fat or low – fat control diet and a high – fat or low – fat diet supplemented with almonds that replaced 20% of the total caloric intake (approximately 56 g of almonds) (32). After 4 wk, patients given an almond high fat diet decreased body fat by 1.8% compared to those given the control diet. There were also no significant differences in BMI and body weight between the control and almond – supplemented diets. Improvement in glycemic control was also indicated from reduction in fasting insulin (4.1%) and glucose levels (0.8%) and HOMA – IR (9.2%) in patients given the almond diets. However, the results of other studies indicated no clear benefits on body weight and glycemic control from dietary tree nut intake. A study conducted on 34 type 2 diabetic patients who were given almond – supplemented high – fat or low – fat diets assessed the effects of almonds on glycemic control (27). The patients who consumed high fat diets showed increases in glucose and insulin concentrations from the high energy intake, as well as decreased HDL levels by 0.12 mmol/L in men and 0.18 mmol/L in women given almond diets. The difference in results may be due to the different almond composition within the diets, which led to the assumption that the high levels of nuts in the diets used in this study (4.79 cal of almonds and 4.76 cal of pecans) would maintain body weight and glycemic control in mice. However, the high content of fat and energy from the nuts may

have induced greater energy intake in the mice, which would resulted in significant increase in body weight and high blood – insulin and glucose levels.

Studies testing diets with the inclusion of pecans have also demonstrated improved total body fat and sustained insulin and glucose levels. Thirty male Wistar rats were given a control diet, high fat diet, or a high fat diet with either whole pecans, pecan oil, or pecan polyphenols for 9 wk (33). The whole pecan – high fat diet contained about 4.56 cal/g of pecans, which was similar to the diet composition from this study (4.76 cal/g). The whole pecan – diet improved total body weight gain in comparison to the other two high fat diets even though the energy intake for this diet was the highest. Insulin levels from mice given the whole pecan – diet was lower than that of the control, and the HOMA – IR was lower than the results from the remaining high fat diets, which suggested the capability for pecans to prevent insulin resistance. However, the current study showed significant increases in blood – glucose levels in mice given HFD – pecans, which may be attributed to the slightly higher energy composition of the diet used in this current study and the different mice species used.

In conclusion, this study was not able to demonstrate the prevention of high fat diet – induced obesity from the consumption of dietary almonds and pecans in mice. There were no clear differences in body weight, food and energy intake, insulin sensitivity, and glucose tolerance in mice given high fat diet with or without nuts. Although there have been studies that were able to show the health benefits from dietary tree nut consumption, it is possible that the differences between the current study and previous studies are the diet composition and the relative amount of tree nuts included. The use of rats in previous study could be another reason because C57BL/6 mice were used in our study. Regardless, the

results obtained from this thesis project clearly show that inclusion of almonds or pecans into the HFD with the composition listed in Table 1 does not influence the metabolic homeostasis of animals fed an HFD.

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