EFFECT OF CONSUMING PISTACHIOS ON THE DIETARY INTAKES AND SERUM CONCENTRATIONS OF GAMMA-TOCOPHEROL AND MAGNESIUM AND ON SERUM LIPID PROFILE IN ADULTS.

A DISSERTATION

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BY

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To the Dean of the Graduate School:

I am submitting herewith a dissertation written by Ladia Hernandez entitled "Effect of Consuming Pistachios on the Dietary Intakes and Serum Concentrations of Gamma-tocopherol and Magnesium and on Serum Lipid Profile in Adults." I have examined this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Nutrition.

John D. Radcliffe, Ph.D., Major Professor

We have read this dissertation and recommend its acceptance:

Department Chair

Accepted:

Dean of the Graduate School

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ABSTRACT

LADIA HERNANDEZ

EFFECT OF CONSUMING PISTACHIOS ON THE DIETARY INTAKES AND SERUM CONCENTRATIONS OF GAMMA-TOCOPHEROL AND MAGNESIUM AND ON SERUM LIPID PROFILE IN ADULTS

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Pistachios are one of the best dietary sources of gamma-tocopherol (γ-tocopherol), a form of vitamin E that may help to protect against diseases such as cardiovascular disease (CVD) and cancer, e.g., lung, prostate, and colon. This study investigated the effect of the incorporation of pistachios (at a level of 68 g per day) on the intakes and serum concentrations of γ-tocopherol, magnesium, and serum lipids, using a six-week randomized, controlled, clinical trial design, having a two-week pre-intervention period and a four-week intervention period. Participants (n = 38) were healthy men and women, randomized to either the control group or the intervention group, which received pistachios. Intakes were calculated using the Nutrition Data System for Research Version 2007. Weekly compliance for pistachio consumption ranged between 84-94%. Dietary γ-tocopherol intake for the intervention group was significantly higher at weeks 3 and 4 ($p < 0.001$) and at weeks 5 and 6 compared to the control group ($p < 0.001$). Additionally, γ-tocopherol intake was significantly higher in the intervention group at weeks 3 and 4 compared to weeks 1 and 2 ($p < 0.001$) and at weeks 5 and 6 compared to weeks 1 and 2 ($p < 0.001$). On the pistachio diet, post-intervention (week 6) serum

concentration of γ-tocopherol and the γ-tocopherol/total cholesterol ratio were significantly higher compared to corresponding pre-intervention values ($p = 0.007$ and $p < 0.001$, respectively). No significant differences between the intervention group and the control group were found for dietary magnesium intake, serum magnesium concentrations, or any indices of serum lipid profile. However, dietary magnesium intake was significantly higher in the intervention group at weeks 3 and 4 compared to weeks 1 and 2 ($p = 0.001$). Serum total cholesterol concentration was significantly lower in the intervention group post-intervention compared to pre-intervention ($p = 0.003$); the same was found for non high density lipoprotein-cholesterol concentration ($p = 0.003$). Thus, consumption of pistachios can lead to an improvement in γ-tocopherol, magnesium, and total cholesterol status. Pistachios could be incorporated into dietary strategies designed to reduce the risk of CVD and certain cancers.

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CHAPTER I

INTRODUCTION

Epidemiological studies suggest that the frequent consumption of nuts is associated with a decreased risk of developing coronary heart disease (CHD). These studies include the Iowa Women's Health Study and the Nurses' Health Study (Ellsworth, Kushi, & Folsom, 2001; Hu et al., 1998), both of which were prospective in nature. In the former study, the frequency of consumption of nuts by 34,111 post menopausal women (during 12 years of follow-up) was compared with mortality from CHD, and an inverse relationship between CHD mortality and frequency of nut consumption was demonstrated. Participants who frequently consumed nuts (two or more servings [28.35 g]) per week had a 20% reduction in the risk of developing CHD compared to those eating less than one serving per week with the relative risk (RR) and 95% confidence intervals (CI) being 0.81 (0.60 – 1.11). In the latter study, the frequency of consumption of nuts by 86,016 women (during 14 years of follow-up) was also associated with a decreased risk of developing CHD. The magnitude of the reduction was similar for both fatal and nonfatal CHD. Women who were classified as having a high frequency consumption of nuts (one serving or more per week) had a 39% reduction in the risk of developing CHD compared to women classified as having rare consumption (less than once a month), with the RR and 95% CI being 0.61 (0.35 – 1.05). The association persisted in subgroups stratified by levels of smoking, body mass, and

exercise. The mechanism by which the consumption of nuts reduces the risk of CHD is not known. One possible mechanism is that consumption of nuts brings about favorable changes in lipid profiles, including decreased serum concentrations of low-density lipoprotein-cholesterol (LDL-C).

Mukuddem-Petersen, Oosthuizen, and Jerling (2005) reviewed 415 published studies having data on the effect of the consumption of nuts on blood lipid profiles. Of these, 24 were determined to have been human intervention trials having designs that permitted the independent effect of nuts on serum lipids to be evaluated. Nuts used in these studies included almonds, pecans, walnuts, and pistachios. Reported decreases in serum cholesterol concentration ranged from 2-16%, whereas reported decreases in LDL-C concentration varied from 2-19%. Some of these decreases were not significant.

The cholesterol-lowering effect of nuts is partially attributable to their high content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). However, other components of nuts (for example, sterols) may also exert a cholesterol-lowering effect (Kris-Etherton et al., 1999). Nuts are good dietary sources of some amino acids (such as arginine) and many micronutrients and non-nutrients. It has been hypothesized that some of these compounds (vitamin E, magnesium, copper, arginine, and phytosterols) may be cardioprotective. However, the mechanisms of these putative cardioprotective effects have yet to be elucidated. The effect of incorporating nuts into the diet on the intake of some of these compounds has been reported. For example, Jambazian, Haddad, Rajaram, Tanzman, and Sabaté (2005) reported that the incorporation of almonds (10% of energy) into the diet increased the intake and serum

levels of vitamin E (as α-tocopherol) and resulted in a decrease in the serum concentrations of total cholesterol and LDL-C, while Alper and Mattes (2003) reported that the incorporation of peanuts into the diet (500 ± 136 kcal per day) resulted in increased intakes and serum levels of magnesium. The latter authors also reported increased intakes of vitamin E, as alpha-tocopherol (α-tocopherol), as the result of the consumption of peanuts, but the effect on the serum concentration of this compound was not reported. Neither the intake nor the serum concentration of gamma-tocopherol (γ-tocopherol), which occurs in peanuts at approximately the same concentration as α-tocopherol, was measured.

Pistachios are one of the more commonly consumed nuts in the United States (U.S.). They are one of the best dietary sources of γ-tocopherol, the leading dietary source of vitamin E in the U.S. (Jiang, Christen, Shigenaga, & Ames, 2001), as well as the carotenoid lutein. (For the purposes of this paper vitamin E is taken to mean a generic descriptor for all tocol and trienol derivatives qualitatively exhibiting the biological activity of α-tocopherol.) In addition, pistachios are a dietary source of copper (19% of the Daily Value [DV] per a 28.35 g serving) and magnesium (9% of the DV per a 28.35 g serving), both of which may be cardioprotective. The two most abundant fatty acids are linoleic acid (an n-6 polyunsaturated fatty acid) and oleic acid (an n-9 monounsaturated fatty acid). There is limited information on the effect of pistachios on serum lipid profile, with only five studies being reported (Edwards, Kwaw, Matud, & Kurtz, 1999; Kocyigit, Koylu, & Keles, 2006; Sheridan, Cooper, Erario, & Cheifetz,

2007; Gebauer et al., 2008; Sari et al., 2009). Of the studies reported, four out of the five used a randomized control design. However, Edwards et al., 1999 used a crossover design with no washout period, and only 10 subjects were enrolled. Additionally, there were no data reported on the intake and serum concentrations of magnesium in any of the five studies and only one study (Gebauer et al., 2008) reported dietary γ-tocopherol intake, but no data on the serum concentrations of γ-tocopherol. Thus, there is a need for appropriately designed trials (such as randomized control trials) to determine the effect of consuming pistachios on serum lipid concentrations and on the intake and serum concentrations of putative cardioprotective nutrients, such as γ-tocopherol and magnesium.

Statement of Purpose

The purpose of this study was to determine the effect of the incorporation of pistachios (at a level of 68 g per day) on the intakes and serum concentrations of γ-tocopherol and magnesium, as well as a serum lipid profile (total cholesterol, LDL-C, high density lipoprotein-cholesterol [HDL-C], and triglycerides) using a six-week randomized, controlled, clinical trial design, having a two-week pre intervention period and a four-week intervention period.

Hypotheses

The following null hypotheses were examined in this study:

1. The intake of γ-tocopherol by the intervention group (receiving 68 g of pistachios per day) during the intervention period will not be significantly different from that

of the control group.

2. The serum concentration of γ-tocopherol for the intervention group will not be significantly different from that of the control group after the four-week intervention period.
3. The intake of magnesium by the intervention group during the intervention period will not be significantly different from that of the control group.
4. The serum concentration of magnesium for the intervention group will not be significantly different from that of the control group after the four-week intervention period.
5. The lipid profile of the intervention group will not be significantly different from that of the control group after the four-week intervention period.

Delimitations

This study had the following delimitations:

1. Participants were recruited from the Texas Medical Center (TMC) area or through individuals who worked at facilities in the TMC.
2. Recruitment of the study participants was limited to those who could attend a series of three visits to Texas Woman's University – Houston Center.
3. Study participants had to be able to speak, read, and understand English.
4. Study participants were not to be taking lipid-lowering medications or a single vitamin.
5. Study participants were adults, age $\geq$ 18 years.

Limitations

This study had the following limitations:

1. Those who participated may represent a more motivated set of individuals interested in the effect of pistachio intake on diet and serum levels.
2. Study participants may not have accurately recorded their diets.
3. The values reported in the Nutrition Data System for Research (NDS-R) software database may not accurately reflect the nutrient profile of foods consumed by the study's participants.
4. Only a single daily dose of pistachios was provided to study participants.
5. The amount of pistachios given to study participants may be greater than would be eaten in a non-experimental setting.

Significance of the Study

In summary, there is a need for appropriately designed trials (such as randomized controlled trials) to determine the effect of consuming pistachios on serum lipids and on the intake and serum concentrations of putative cardioprotective nutrients, such as γ-tocopherol and magnesium. Moreover, this study may provide a foundation for dietary recommendations for clinicians counseling patients on the incorporation of nuts, specifically, pistachios into a healthy diet.

CHAPTER II

REVIEW OF THE LITERATURE

Cardiovascular Disease

An estimated 80,000,000 (approximately 1 in 3) American adults have one or more types of cardiovascular disease (CVD) thus, making CVD the leading cause of morbidity and mortality (Writing Group Members et al., 2009). The American Heart Association (AHA) has set forth guidelines that recognize diet as part of an overall healthy lifestyle (Lichtenstein et al., 2006). The AHA's strategy for CVD risk reduction in the general population acknowledges improving diet and lifestyle as a critical component of their goals and recommendations. Their goals include, but are not limited to, consuming an overall healthy diet and aiming for the recommended levels of LDL-C, HDL-C, and triglycerides for CVD risk reduction.

Several studies suggest that nuts may play a role in reducing total cholesterol and LDL-C concentrations. Nuts contain several components such as, tocopherols and magnesium, which hold CVD risk-reducing properties (Alper & Matts, 2003). According to Alper and Matts, low serum magnesium concentrations can increase risk of CVD due, in part, to diminished lipoprotein lipase and lecithincholesterol acyltransferase activity, which results in hyperlipidemia. These authors also note that magnesium infusion inhibits platelet aggregation. Since dietary intake of magnesium has declined through reduced consumption of magnesium-rich foods and losses during processing

(Alper & Matts, 2003), nut consumption would be a beneficial component of a healthy diet. Thus, there is a need to determine the impact of consuming nuts on the intake and serum levels of magnesium. The intake and serum concentration of magnesium could be obtained in feeding studies that are carried out with the primary objective of determining the effects of nut consumption on serum lipids.

Dietary Tocopherols

According to Devaraj and Jialal (2005) γ-tocopherol is the most prevalent tocopherol in plant seeds and plant seed products and is the leading source of tocopherol intake in the United States diet. These authors also note that plasma and tissue γ-tocopherol are decreased by α-tocopherol supplementation. Q. Jiang et al., (2001) indicated that several studies suggest that γ-tocopherol may be important to human health and that it possesses unique features that distinguish it from α-tocopherol.

Although γ-tocopherol is a slightly less powerful antioxidant than α-tocopherol because it lacks one of the electron-donating methyl groups on the chromanol ring (see Figure 1), the unsubstituted C-5 position of γ-tocopherol appears to make it better able to trap lipophilic electrophiles, such as reactive nitrogen species (Devaraj & Jialal, 2005). Most evidence for γ-tocopherol having antioxidant properties has been established with studies done in vitro.

Recommended Dietary Allowance: Vitamin E

The Recommended Dietary Allowance (RDA) for vitamin E, previously established in 1989, was increased from 10 and 8 mg of α-tocopherol equivalents per day

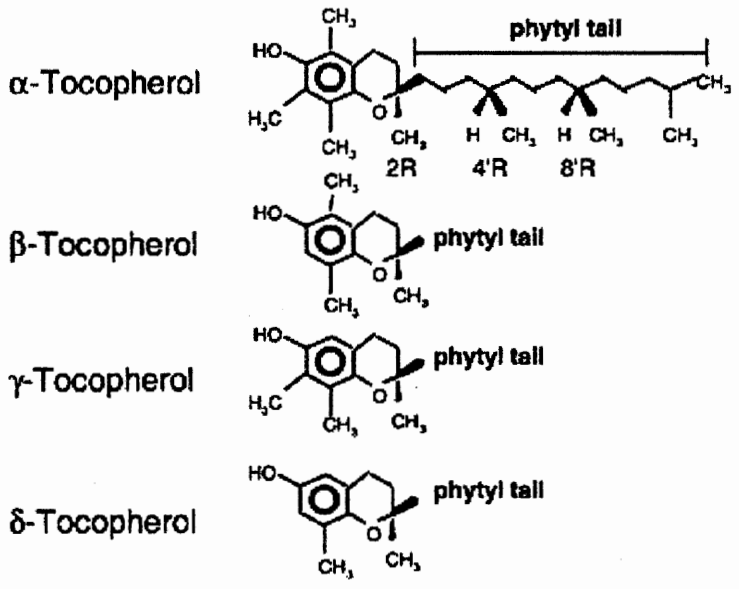

Figure 1. Tocopherol Structures.

From the DRI Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids A Report of the Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition, Washington, D.C.: National Academy Press. Copyright 2000 by the National Academy of Sciences.

for adult men and women, respectively, to 15 mg α-tocopherol per day for both men and women, which would be 18 mg α-tocopherol equivalents, in 2000 (Gao, Wilde, Lichtenstein, Bermudez, & Tucker, 2006). For the 1989 RDA, γ-tocopherol was used to calculate vitamin E intake, but this tocopherol was not used to calculate the new RDA or

other Dietary Reference Intakes, that is, the Estimated Average Requirement (EAR) and the Upper Level.

Gao et al. (2006) noted that only 8% of men and 2.4% of women met the EAR for vitamin E (12 mg per day) from foods alone, in the 1994-96 Continuing Survey of Food Intakes by Individuals, and a similarly low compliance with the EAR was observed in the National Health and Nutrition Examination Survey (NHANES) 1999-2000. The latter authors emphasize the need for more understanding of the way in which foods could be used to meet the new vitamin E recommendations.

Gao et al. (2006) assessed whether the new RDA for vitamin E can be met with the latest national dietary intake data. These authors employed linear programming to formulate diets with maximal intake of α-tocopherol, while also meeting other specified nutritional conditions, such as the RDA or Adequate Intake for a set of nutrients, sodium and fat recommendations, and energy limits in four sex and age strata of the NHANES 2001-2002 participants. They compared the α-tocopherol concentrations of foods with the current RDA and identified the most important foods for meeting the RDA for vitamin E in the U.S. diet. It was reported that increased consumption of nuts and seeds (up to 2.5 oz servings/d) was associated with higher vitamin E intake, whereas a low intake of nuts and seeds was related to an inadequate vitamin E intake. These authors stated that meeting the current RDA could be done with only dietary choices that vary considerably from the current practices of most Americans. They suggested dramatic dietary changes, including not only greater intakes of fruits and vegetables but also of nuts and seeds, were necessary to meet the RDA.

Effects of Nuts on Lipid Profiles

Nuts are recommended as constituents of diets designed to meet the AHA's requirements. It has been of interest to researchers to study the impact of nut consumption on serum lipid levels in feeding studies with adults. Mukuddem-Petersen et al. (2005) stated that there is substantial evidence that nuts may have favorable effects on CVD through a variety of mechanisms. These authors noted that the most extensively studied mechanism is the lipid-lowering effect of nuts. They reviewed 415 published studies having data on the effect of the consumption of nuts on blood lipid profiles. Of these, 24 were determined to have been human intervention trials designed to evaluate the independent effect of nuts on serum lipids. The different types of nuts used in these studies included almonds, pecans, walnuts, and pistachios. The latter nuts are a source of both magnesium and γ-tocopherol. Even though there are many studies examining the association of nut consumption on blood lipid profiles, only a few investigated the effect of pistachios on serum lipids. There is need to have information on the effect of consuming pistachios on the intake and serum concentrations of putative cardioprotective nutrients, such as γ-tocopherol and magnesium.

General Nut and Seed Consumption

According to R. Jiang et al. (2006) and Edwards et al. (1999), nuts and seeds are rich in unsaturated fatty acids, antioxidant vitamins such as vitamin E, dietary fiber, and plant protein. Additionally, frequent nut consumption has been associated with a reduced risk of CHD and type 2 diabetes in prospective studies. R. Jiang et al. noted that clinical

trials suggest that nut and seed consumption may decrease the levels of inflammatory factors. These authors examined the association of nut and seed consumption with levels of inflammatory biomarkers, that is, C-reactive protein, interleukin-6, and fibrinogen in the Multi-Ethnic Study of Atherosclerosis. They hypothesized that frequent consumption of nuts and seeds would be associated with lower serum levels of these inflammatory markers. Their study was comprised of 6,080 U.S. participants aged 45-84 years. A self-administered food frequency questionnaire provided information on a list of food items (e.g., nuts, seeds, peanuts and peanut butter) consumed during the previous year and the average size of items consumed. The consumption of nuts and seeds was categorized as never/rare, less than once/week, one to four times/week, and five or more times/week. Levels of C-reactive protein, interleukin-6, and fibrinogen were measured in baseline serum samples. Frequent nut and seed consumption was inversely associated with levels of inflammatory markers C-reactive protein, interleukin-6, and fibrinogen. The authors reported that associations of nut and seed consumption with these biomarkers were not modified by body mass index (BMI), waist:hip ratio, or race/ethnicity.

Epidemiological Studies

Epidemiological studies suggest that the frequent consumption of nuts is associated with a decreased risk of developing CHD. These studies include the Iowa Women's Health Study and the Nurses' Health Study (Ellsworth et al., 2001; Hu et al., 1998), both of which were prospective in nature. In the former study, the frequency of consumption of nuts by 34,111 post menopausal women (during 12 years of follow-up) was compared with mortality from CHD, and an inverse relationship between CHD

mortality and frequency of nut consumption was demonstrated. Participants who frequently consumed nuts (two or more servings [with one serving being 28.35 g]) per week had a 20% reduction in the risk of developing CHD compared to ones eating less than one serving per week with the RR and 95% CI being 0.81 (0.60 – 1.11). In the latter study, the frequency of consumption of nuts by 86,016 women (during 14 years of follow-up) was also associated with decreased risk of developing CHD. The magnitude of the reduction was similar for both fatal and nonfatal CHD to women who were classified as having a high frequency consumption of nuts (one serving or more per week) had a 39% reduction in the risk of developing CHD compared to women classified as having rare consumption (less than once a month), with the RR and 95% CI being 0.61 (0.35 – 1.05). The increase association persisted in subgroups stratified by levels of smoking, body mass, and exercise. The mechanisms by which nuts exert their protective cardioprotective effects are not fully understood; however, one possible mechanism is that consumption of nuts brings about favorable changes in lipid profiles. The cholesterol-lowering effect of nuts is partially attributable to their high content of MUFA and PUFA. However, other components of nuts (for example, sterols) may also exert a cholesterol-lowering effect (Kris-Etherton et al., 1999).

Impact of Selected Nuts on Intake and Serum Levels of Nutrients and Serum Lipids

Almonds

Jaceldo-Siegl, Sabate, Rajaram, and Fraser (2004) studied the effect of a daily supplement of almonds on the overall habitual diets of healthy participants and reported the impact of almond supplementation on nutrient profile and nutrient displacement of

almond-supplemented habitual diets. Participants were entered into the study at staggered three-month intervals and randomly allocated to each of the four entry periods and followed for 12 months. The first six months was the control diet period and the second six months the intervention period. During the control diet period, participants followed their habitual self-selected diet. The intervention diet period required participants to consume a daily allowance of almonds equivalent to 15% of each participant's mean energy intake during the habitual diet period. Random-order telephone 24-hour diet recalls were obtained unannounced and collected at 3-week intervals. Two food diaries were collected during each diet period. Mean values of the seven diet recalls from each diet period were calculated and a paired *t* test was computed to compare results from the habitual and intervention diet periods. The results indicated that daily supplementation of almonds can induce favorable nutrient modifications to an individual's habitual diet. Significant increases were seen in the intake of MUFA, PUFA, plant protein, fiber, magnesium, copper, and α-tocopherol.

Jenkins et al. (2002) noted that nuts are generally not recommended as snacks for hyperlipidemic participants because of their high fat content. However, the authors stated that almond consumption fits well with current AHA guidelines to replace saturated fats with unsaturated fats and with the National Cholesterol Education Program guidelines to liberalize total fat intake. However, the effective dose is still unknown. Therefore, these authors assessed the effect of whole unblanched almonds at 2 doses on the blood lipids of hyperlipidemic participants when provided as supplements to their self-selected therapeutic diets.

In a randomized crossover study, the dose-response effects of whole almonds, taken as snacks, were compared with low-saturated fat (>5% energy) whole-wheat muffins (control) in therapeutic diets; 27 hyperlipidemic men and women consumed 3 isoenergetic (mean 423 kcal/d) supplements each for one month. This study expressed the results as mean ± standard error of the mean (SEM). The supplements provided 22.2 % of energy and consisted of full-dose almonds (73±3 g/d), half-dose almonds (37±2 g/d) plus half-dose muffins (75±3 g/d), and full-dose muffins (147±6 g/d). The full-dose almonds produced the greatest reduction in levels of blood lipids. Significant reductions from baseline were seen on both half- and full-dose almonds for LDL-C mg/dL (171.6±1.7%, P=0.001, and 9.4±1.9%, P<0.001, respectively) and the LDL-C:HDL-C ratio (7.8±2.2%, P=0.001, and 12.0±2.1%, P<0.001, respectively). A significant reduction was also found for full-dose almonds alone for lipoprotein(a) (7.8±3.5%, P=0.034) and oxidized LDL-C concentrations (14.0±3.8%, P<0.001). The authors concluded that almonds could be substituted for whole-wheat flour muffins in diets designed to lower serum lipids. These authors did not report the effect on the intake and serum levels of magnesium and tocopherols.

Spiller, Jenkins, Bosello, et al. (1998) compared the lipid-altering effects of an almond-based diet with an olive oil-based diet against a cheese and butter-based control diet. The study consisted of 45 free-living hyperlipidemic men (n = 12) and women (n = 33) with a mean plasma total cholesterol of 251 ± 30 mg/dL. One of three diets was followed by each of the participants; that is, almond-based, olive oil-based, or dairy-based, for four-week periods. The researchers matched the total fat content in each of the

three diet regimens 48 g, 47.5 g, and 55.3 g for the almond-based diet, olive oil-based diet, and the dairy-based diet control diet, respectively. They also provided sources of fat that comprised the major portion of fat intake. Significant differences were seen at four weeks between the three groups for plasma total cholesterol ($p<0.001$) and plasma LDL-C concentrations ($p<0.001$). Within groups, the almond-based diet group had a significant reduction in plasma concentrations of total cholesterol ($p<0.05$) and LDL-C ($p<0.001$), as well as the total cholesterol:HDL-C ratio ($p<0.001$). There were no significant changes in plasma concentrations of HDL-C in the almond-diet group. Values for total cholesterol and HDL-C concentrations for participants receiving the control diet were significantly increased from baseline (both $P<0.05$), while consumption of the olive oil-based diet resulted in no significant changes over the study period. Furthermore, weight did not change significantly for any of the diet groups. These authors did not report the effect on either the intake or serum levels of either magnesium or tocopherols. These results suggest that the more favorable lipid-altering effects induced by the almond group may have been due to the interactive or additive effects of the numerous bioactive constituents found in almonds.

Spiller, Jenkins, Gragen, et al. (1992) studied the effect of almonds as part of a low saturated fat, low cholesterol, high-fiber diet in 26 adult men and women. Participants consumed their typical food pattern during a two-week baseline diet. During the nine-week intervention phase of the study (almond diet period), baseline diets were modified for all participants by limiting the intake of meat, fatty fish, high-fat milk products, eggs, and saturated fat. The foundation of the diet consisted of grains, beans,

vegetables, fruits and low-fat milk products. During the almond diet period, raw almonds (100 g/day) supplied 34 g/day of MUFA, 12 g/day of PUFA, and 6 g/day of saturated fatty acid (SAFA). There was a rapid and sustained reduction in LDL-C, but no changes in HDL-C were reported. Total plasma cholesterol concentration decreased from 235±5.0 mg/dL mean ± SEM at baseline to 215±5.0 mg/dL at three weeks; and 214±5.0 mg/dL at nine weeks (P<0.001). These authors did not report the effect of consuming almonds on either the intake or serum levels of either magnesium or tocopherols

Walnuts

Several studies have reported that walnuts reduce serum cholesterol concentration. Zambon et al. (2000) designed a dietary intervention study in free-living adult men and women with polygenic hypercholesterolemia to compare the effects of a walnut-rich diet with that of a cholesterol-lowering Mediterranean diet on serum lipid levels, lipoprotein levels, and LDL resistance to oxidation. A randomized, crossover feeding trial was conducted on 55 men and women with a mean age of 56 years; 49 individuals completed the trial. The participants were given a cholesterol-lowering Mediterranean diet and a diet of similar energy and fat content, in which walnuts replaced approximately 35% of energy obtained from MUFA. Participants followed each diet for a period of six weeks. Compared with the Mediterranean diet, the walnut diet produced mean changes of -4.1% in total cholesterol concentration, -5.9% in LDL-C concentration, and -6.2% in lipoprotein(a) concentration. Furthermore, the mean differences in the changes in serum lipid concentrations were -10.8 mg/dL (P<0.001) for total cholesterol concentration, -11.2 mg/dL (P<0.001) for LDL-C concentration, and -0.021 g/L

(P=0.042) for lipoprotein(a) concentration. Lipid changes were similar in men and women, except for serum concentrations of lipoprotein(a), which decreased only in men. Also, LDL particles were enriched with PUFA when walnuts were given, but their resistance to oxidation was preserved. The authors concluded that substituting walnuts for part of the MUFA in a cholesterol-lowering Mediterranean diet further reduced total and LDL-C concentration in men and women with hypercholesterolemia.

Munoz et al. (2001), assessed whether LDL compositional changes produced by a walnut diet, particularly LDL enrichment with PUFA from walnuts, might increase LDL uptake by the liver cells, thereby reducing the number of circulating LDL particles and contributing to a cholesterol-lowering effect. The study was a randomized, crossover feeding trial involving 10 men with polygenic hypercholesterolemia. Participants were given a control, Mediterranean-type cholesterol lowering diet and a diet of similar composition, in which walnuts replaced ~35% of energy from unsaturated fat for a period of six weeks each. The walnut diet reduced serum concentrations total cholesterol and LDL-C by 4.2% (P=0.176) and 6.0% (P=0.087), respectively, compared to the control diet. No significant changes were observed in the serum concentrations of HDL-C, cholesterol, triglycerides, and apolipoprotein A-I levels or in the relative proportion of protein, phospholipids, triglycerides, and cholesteryl esters in LDL particles; LDL obtained during the walnut diet period showed a 50% increase in association rated to the LDL receptor in human hepatoma HepG2 cells in comparison with the LDL obtained during the control diet. Additionally, LDL uptake by HepG2 cells was correlated with the alpha-linolenic acid content of the triglyceride plus cholesteryl ester fractions of LDL

particles (r^2=0.42, P<0.05). The authors noted that changes in the quantity and quality of LDL lipid fatty acids after a walnut-enriched diet facilitate receptor-mediated LDL clearance and may contribute to the cholesterol-lowering effect of walnut consumption. The authors did not report the effect of walnut consumption on either the intake or serum levels of either magnesium or tocopherols.

Ros et al. (2004) tested the hypothesis that walnut intake would improve endothelial function in participants with hypercholesterolemia. These authors conducted a randomized crossover design with 21 hypercholesterolemic men and women. Participants were randomized in a crossover design between two diet sequences (control, Mediterranean-type diet and an isoenergetic diet enriched with walnuts) for four-week periods. Because walnuts are a rich source of γ-tocopherol, serum levels were measured as a biological marker of adherence to the walnut diet, serum levels of α-tocopherol were also measured. The walnut diet significantly (P=0.043) improved endothelium-independent vasodilation (EDV) from (mean ± standard deviation [SD]) 3.6+3.3% to 5.9±3.3%, a relative increase of 64% when compared with control diet. The level of vascular cell adhesion molecule-1 was significantly lower (P=0.045) during the walnut diet than during the Mediterranean diet. No significant differences were found in soluble intercellular adhesion molecule-1 levels between the diets. There was an inverse correlation between changes in EDV and changes of cholesterol:HDL-C ratios. The walnut diet significantly reduced total cholesterol (-4.4±7.45%) and LDL cholesterol (-6.4±10.0%) (P<0.05 for both) compared to the Mediterranean diet. However, a parallel nonsignificant reduction in LDL-C:HDL-C ratio was observed with the walnut diet and

the differences of effect on the lipid profile between the two diets did not change when adjusted for baseline values and gender. Additionally, the authors suggested that walnut ingestion was 100% due to the increase in serum γ-tocopherol level during the walnut diet compared with the control diet (mean±SD), 3.46±1.28 versus 1.89±1.05 μmol/L, (P<0.001).

Peanuts

According to Alper and Matts (2003), consumption of peanuts in the U.S. is greater than all other nuts combined. These authors reported that the incorporation of peanuts into the diet (500 ± 136 kcal/day) resulted in increased intakes and serum levels of magnesium. The latter authors also reported increased intakes of α-tocopherol as the result of the consumption of peanuts, but the effect on the serum concentration of this compound was not reported. Neither the intake nor the serum concentration of γ-tocopherol, which occurs in peanuts at approximately the same concentration as α-tocopherol, was measured. There was no effect of the consumption of peanuts on the serum concentrations of total cholesterol, LDL-C, or HDL-C.

Pecans

Several studies have investigated the effect of a pecan-rich diet on lipid profiles. Morgan and Clayshulte (2000) conducted an eight-week randomized, controlled study in adults with normal lipid levels and compared serum lipid profiles and dietary intakes using a pecan treatment vs. control group design. Participants were randomly assigned to either the treatment group (who consumed 68 g pecans daily) or the control group

(avoided nut consumption). The control group was instructed to consume a self-selected diet during the course of the eight-week study, with the exception of no consumption of nuts. The pecan treatment group also consumed self-selected diets and avoided nut consumption with the exception of the 68 g pecans provided daily for the study (pecans contributed 459 kcal and 44 g fat daily). Blood samples were obtained at baseline, four weeks, and eight weeks. Values for serum total cholesterol concentrations were significantly different between the treatment and control group at eight weeks ($P<0.05$), partially due to the increase in serum cholesterol concentration levels in the control group. Serum total cholesterol concentration levels (mg/dL) for baseline, four weeks, and eight weeks (mean$\pm$SD) were 169$\pm$23.0 mg/dL, 161$\pm$26.1, and 163$\pm$32.4 for the treatment group and 181$\pm$10.1, 176$\pm$17.2, and 194$\pm$21.1 for the control group; LDL-C concentrations (mg/dL) from baseline in the treatment group(101.8$\pm$19.1) were significantly lower at 4 weeks (91.7$\pm$19.1) and at the eighth week (95.9$\pm$23.1), $P<0.05$ for both. Additionally, LDL-C concentrations were significantly lower in the treatment group vs the control group at four weeks and eight weeks, $P<0.05$. The authors reported the effect of pecan consumption on the intake of both magnesium and vitamin E (as α-tocopherol equivalents) but not on the serum levels of magnesium or either the intakes or serum levels of tocopherols.

Rajaram, Burke, Connell, Myint, and Sabate (2001) investigated the effect of a pecan-enriched diet as an alternative to the National Cholesterol Education Program Step 1 diet in modifying serum lipids and lipoproteins in men and woman with normal to moderately high serum cholesterol. These authors conducted a single-blind, randomized,

controlled, crossover feeding study. Following a two- week run-in phase, twenty-three participants were assigned to follow one of two diets for four weeks (a pecan-enriched diet and a Step 1 diet), the groups then reversed their diet intervention and continued their participation in the study for another four weeks. A washout period was not included between the two diet periods based on previous studies with walnuts that had shown no carry-over effects. The pecan-enriched diet was accomplished by proportionately reducing all food times in a Step I diet by one fifth for a 20% isoenergetic replacement to accommodate the consumption of pecans. Pecans were consumed under supervision and overall dietary compliance was estimated to be >95%. Fasting blood samples were drawn at the end of the run-in phase and the end of the two diet periods. Lipid profiles were improved by both diets. The pecan-enriched diet, when compared to the Step I diet, decreased both serum total cholesterol mg/dL (mean±SD) (183.3±27.3 vs. 186.4±29.3) and LDL-C (106.5±19.9 vs. 119.0±21.8) concentrations for a 6.7 and 10.4% decrease in serum total cholesterol and LDL-C, respectively. HDL-C concentrations mg/dL were increased by the pecan-enriched diet (47.2±9.8) when compared to the Step I diet (44.5±9.0) for a 5.6% increase in HDL-C. Additionally, the pecan-enriched diet lowered the LDL-C:HDL-C ratio by 0.44 when compared to the Step I diet and significantly lowered the plasma concentrations of triacylglycerol, apo B, and lipoprotein(a) concentrations by 11.1%, 11.6%, and 15.1%, respectively, and increased apo A1 concentrations significantly by 2.2%. The authors did not report the effect of pecan consumption on either the intake or serum levels of either magnesium or tocopherols.

Haddad, Jambazian, Karunia, Tanzman, and Sabate (2006), who had demonstrated in a previous study that a pecan-enriched diet improved serum lipids in human participants, conducted a study to evaluate the effect of consuming a pecan rich diet on plasma γ-tocopherol and α-tocopherol concentrations, as well as on measures of antioxidant capacity and lipid peroxidation in healthy persons. A randomized, controlled, crossover feeding study was conducted on 24 participants. The participants were assigned to two diets, each for a four-week period (the control diet and a pecan-enriched [20% of energy] diet). Cholesterol-adjusted plasma γ-tocopherol increased by 10.1% ($P<0.001$), whereas cholesterol-adjusted α-tocopherol decreased by 4.6% ($P<0.001$), and malondialdehyde (MDA) concentrations, measured as thiobarbituric acid reactive substances, decreased by 7.4% ($P<0.05$) on the pecan diet. No significant changes were observed for ferric-reducing ability of plasma or Trolox equivalent antioxidant capacity values. The authors concluded that data from this study can possibly provide some evidence for potential protective effects of pecan consumption in healthy individuals.

Pecans are a rich source of γ-tocopherol, yet a poor source of α-tocopherol, containing 24.4 and 1.4 mg per 100 g of nut, respectively (Haddad et al., 2006). Although, several studies have noted the favorable effects of pecan intake on serum lipid profiles, research is limited on the investigation of the role of pecan consumption and their contribution to antioxidant protection. Haddad et al. conducted a randomized, controlled, crossover feeding study to evaluate the effect of consuming a pecan-rich diet on plasma α- and γ-tocopherol concentrations and on measures of antioxidant capacity

and lipid peroxidation in healthy individuals. Consumption of the pecan-rich diet resulted in cholesterol-adjusted plasma γ-tocopherol value being increased by 10.1% ($P<0.001$) and the cholesterol-adjusted α-tocopherol value being decreased by 4.6% ($P<0.001$). No significant difference between the control diet and the pecan-rich diet were observed for ferric-reducing ability of plasma or antioxidant capacity values; however, a significant decrease was observed in MDA concentrations ($P<0.05$). These findings support pecans as a rich source of γ-tocopherol in the diet despite not influencing fasting total antioxidant capacity.

Pistachios

Pistachios are one of the more commonly consumed nuts in the U.S. They are one of the best dietary sources of γ-tocopherol, the leading dietary source of tocopherols in the U.S. (Q. Jiang et al., 2001). There is limited information on the effect of pistachios on serum lipid profile, with only five studies being reported (Edwards et al., 1999; Kocyigit et al., 2006; Sheridan et al., 2007; Gebauer et al., 2008; Sari et al., 2009). Although the Kocyigit et al. used a randomized control design, the others used a randomized crossover design (see Table 1). Specifically, Edwards et al. (1999) studied the substitution of pistachio nuts for the high fat snacks (e.g., buttered popcorn, candy bars, potato chips, and the like) to determine if the pistachio nuts would improve the lipid profiles of humans with primary, moderate hypercholesterolemia. The authors utilized a controlled, randomized crossover design whereas participants served as their own controls. Half of the participants were first randomized to a pistachio group (substituting roasted, unsalted

Table 1

Summary of Pistachio Intervention Studies

Study	Design	Participants	Treatment
Edwards et al. (1999)	Controlled, randomized crossover design	Men (n=4) and women (n=6) adults with moderate hypercholesterolemia; median age: 46 y (range: 28 – 64 y)	Three weeks of dietary modification with 20% caloric intake from pistachios
Kocyigit et al. (2006)	Randomized intervention design	Healthy men (n=24) and women (n = 20); mean age: 32.8 y	Three weeks of dietary modification with 20% caloric intake from pistachios
Sheridan et al. (2007)	Randomized crossover trial	Men (n=11) and women (n=4) adults with moderate hypercholesterolemia; mean age: 60 y (range: 36-75 y)	Four weeks of dietary modification with 15% caloric intake from pistachios (about 2-3 ounces per day)
Gebauer et al. (2008)	Controlled, randomized crossover design	Men (n=10) and women (n=18) adults with moderate hypercholesterolemia; mean age: 48 y (range: 35-61 y)	Three isoenergetic diets: (1) lower-fat control diet with no pistachios (control diet), and (2) 1 serving/d of a pistachio diet (10% caloric intake from pistachios) (1 PD) (3) 2 servings /d of pistachio diet (20% caloric intake from pistachios) (2 PD)
Sari et al. (2009)	Controlled environment, crossover design	Healthy men (n=33) mean age: 22 y (range: 21-24 y)	Four weeks of a Mediterranean diet intervention followed by four weeks of Mediterranean diet with addition of 20% caloric intake from pistachios

Note: PD = pistachio diet

pistachio nuts for 20% of their daily caloric intake) and the other half maintained their regular diets for a three-week period. The participants were then crossed over to the other diet group. After a three-week period, there was a decrease in plasma total cholesterol concentration ($P<0.04$), and increase in HDL-C concentration ($P<0.09$), a decrease in total cholesterol/HDL-C ratio ($P<0.01$), and a decrease in the LDL-C/HDL-C ratio ($P<0.02$) (see Table 2). Consumption of the pistachio diet significantly decreased the intake of SAFA and increased the intake of MUFA and PUFA. Table 3 summarizes the results of the median change resulting from the consumption of the pistachio containing diet versus the regular diet. The authors concluded that the substitution of pistachio nuts for other high fat snacks for a consecutive three-week period can significantly improve lipid profiles in individuals with moderate hypercholesterolemia. The authors did not report the effect of pistachio consumption on either the intakes or serum concentration of tocopherols or magnesium.

In a study conducted in Turkey by Kocyigit et al. (2006), the effect of pistachio nut consumption on improving plasma lipid profile and decreasing oxidative status was demonstrated in healthy participants. The four-week study consisted of a one-week pre-experimental period and a three-week experimental period. During the pre-experimental phase, all participants were asked to consume their regular diet not including nuts, nut butters or nut oils. After the pre-experimental phase, participants were randomized to either the regular diet or the intervention (pistachio diet) phase. The group assigned to the regular diet continued to follow normal dietary patterns, but excluding nut and nut products, while the intervention diet incorporated pistachio nuts contributing ~20% of the

Table 2

Serum Lipid Concentrations for Participants Consuming Their Regular Diet vs. a Diet Containing Pistachios

Variable (units)	Initial	Final	Median change	p-value
Total cholesterol (mg/dL)	243	239	-9	<0.04
HDL-C (mg/dL)	50	56	+4	<0.09
LDL-C (mg/dL)	180	158	-11	N.S
Triglycerides (mg/dL)	113	108	-6	N.S.
Total cholesterol/HDL-C	4.8	4.5	-0.7	<0.01
LDL-C/HDL-C	3.2	3.1	-0.3	<0.02

Note: HDL-C = high density lipoprotein-cholesterol; LDL-C = low density lipoprotein-cholesterol; N.S. = non significant p-value. From Edwards K., Kwaw I., Matud J., & Kurtz I. (1999). Effect of pistachio nuts on serum lipid levels in patients with moderate hypercholesterolemia. *Journal of the American College of Nutrition*, 18, 229-232.

Table 3

Intakes of Energy and Macronutrients for Participants Consuming a Regular Diet or a Diet Containing Pistachios

Dietary variable	Regular diet	Pistachio diet	Median change	p-value
Total energy (kcal/day)	1900	1905	5	N.S
Protein (% energy)	17	16	1	N.S.
CHO (% energy)	47	44	3	N.S.
Fat (% energy)	37	39	2	N.S.
SAFA (g/day)	23	16	7	<0.01
MUFA (g/day)	23	32	9	<0.01
PUFA (g/day)	9.6	14	4.4	<0.01
Fiber (g/day)	12	20	8	<0.01

Note: CHO = carbohydrate; SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; N.S. = non significant p-value. From Edwards K., Kwaw I., Matud J., & Kurtz I. (1999). Effect of pistachio nuts on serum lipid levels in patients with moderate hypercholesterolemia. *Journal of the American College of Nutrition*, 18, 229-232.

total daily energy intake (65 to 75 g) into the diet. Fasting blood samples were obtained at the end of the pre-experimental phase and at the end of the intervention phase. Plasma total cholesterol, MDA (a marker of lipid peroxidation), total cholesterol/HDL-C, and LDL-C/HDL-C ratios were significantly decreased while HDL-C and total antioxidant potential /MDA ratios were significantly increased in the pistachio diet intervention group after three weeks. Intakes or serum concentration of α-tocopherol, γ-tocopherol, and magnesium were not reported by these authors.

Sheridan et al. (2007) also utilized a randomized crossover design in an outpatient setting with participants serving as their own controls. Fifteen participants, including both men and women, with moderate hypercholesterolemia completed the study. At baseline, normal diets were consumed for a five-day period. Eight participants were randomized to continue their regular diet for four weeks followed by the pistachio diet (15% of energy intake or about 2-3 ounces per day) for four weeks. Subsequently, the other seven participants followed these diets in reverse order. Consumption of the pistachio diet compared to the regular diet resulted in a decrease in SAFA (as % of energy) intake (mean difference, -2.7%: 95% CI, -5.4% to -0.10%; p = 0.04) as well as, an increase in the intake PUFA (mean difference, 6.5%: 95% CI, 4.2% to 8.9%; $P<0.001$) and total dietary fiber (mean difference, 15 g/day: 95% CI, 8.4 g/day to 22 g/day; $P=0.003$). There were no effects of diet on the dietary intakes of total energy or percent total energy from protein, carbohydrate, total fat or monounsaturated fat per day. Additionally, there were no effects of the pistachio diet compared to the regular diet on the plasma concentrations of total cholesterol, triglycerides, LDL-C, very low density

lipoprotein-cholesterol (VLDL-C), apolipoprotein A-1 or apolipoprotein B-100. However, consumption of the pistachio diet resulted in decreases in the ratios for TC/HDL-C , LDL-C/HDL-C, and B-100/A-1 compared to baseline intake. Additionally, there was an increase was in plasma HDL-C concentrations for the pistachio diet . Lastly, no changes in BMI and blood pressure were observed in the participants. No statistically significant differences were reported for body weight and BMI between treatments.

Gebauer et al. (2008) conducted a randomized crossover, controlled-feeding study with twenty-eight participants with serum LDL-C $\geq$ 2.86 mmol/L (11.5 mg/dL). Three isoenergetic diets previously described in Table 1 were each consumed for four weeks. When compared to the control diet, the 1 pistachio diet (PD) and 2 PD had significantly lower values for total cholesterol, LDL-C, and nonHDL-C (the sum of LDL-C and VLDL-C). The 2 PD diet had significantly lower triglycerides, total cholesterol/HDL-C, LDL-C/HDL-C when compared to the control diet. In addition, ratios of total cholesterol/HDL-C, LDL-C/HDL-C, and non-HDL/HDL-C were significantly lower in the 2 PD compared to the 1 PD. Although HDL-C concentrations were significantly higher in women only after the 2 PD than after the control, no significant differences were found in HDL-C when the control diet was compared to the pistachio diets. As well, there were no significant differences in the changes in BMI from baseline compared to the three diets.

In a most recent study in humans, Sari et al. (2009) demonstrated that a pistachio diet improved blood glucose level, endothelial function, and some indices of

inflammation and oxidative status. The authors enrolled participants living in a controlled environment free of any acute or chronic medical disorders. A Mediterranean-type diet was administered for four weeks followed by the Mediterranean diet plus pistachios constituting 20% of daily caloric intake. When participants consumed the diet without pistachios, higher concentration values were reported for blood glucose ($P<0.001$), LDL-C ($P<0.001$), total cholesterol ($P<0.001$), and triglycerides ($P=0.008$). There was no effect of diet on HDL-C concentration. Consumption of both diets was associated with decreased total cholesterol/HDL-C and LDL-C/HDL-C ratios. EDV was significantly improved on the pistachio diet. Additionally, consumption of the pistachio diet significantly decreased the serum concentrations of interleukin-6, lipid hydroperoxide, MDA, and total oxidant status and increased superoxide dismutase ($P<0.001$ for all). No significant differences were found for the serum concentrations of C-reactive protein and tumor necrosis factor-α. Lastly, no significant differences were found for body weight and blood pressure during the study.

In a study in rats, researchers investigated the effect of pistachio intake on lipid oxidation and serum antioxidant levels (Aksoy et al., 2007). All rats were fed a standard basic diet for ten days before the study. The rats were then randomized into one of three groups, the control group (basic diet); the basic diet plus pistachios, which provided 2.5 g/day (20% of caloric intake); the basic diet plus pistachios, which provided 5 g/day (40% of caloric intake). Fasting blood samples were collected after ten weeks of feeding; serum HDL-C concentrations were significantly increased in both of the groups receiving pistachios; however, no difference was found between the two pistachio groups for this

parameter. Total cholesterol/HDL-C ratio was significantly decreased for both pistachio diet groups when compared to the control group with no difference shown between the two pistachio diet groups. Relative to the control group, the serum activity of paraoxonase and arylesterase (two enzymes that reduce LDL-C oxidation) was increased by 35% and 60%, respectively, for the group receiving 20% of energy as pistachios. When pistachio intake was increased to 40% of the daily caloric intake, antioxidant activity was blunted and differences were not statistically significant between the two pistachio diet groups.

All six pistachio intervention studies (four human and one rat) did not report the effect of pistachio consumption on either the intake or serum levels of tocopherols. Moreover, all six pistachio intervention studies failed to report intake or serum levels of magnesium. Thus, there is a need for more appropriately designed trials (such as randomized control trials) to determine the effect of consuming pistachios on serum lipid and on the intake and serum concentrations of putative cardioprotective nutrients, such as γ-tocopherol and magnesium.

CHAPTER III

METHODOLOGY

Study Design

The design of the study, the length of the intervention phase, and the amount of nuts are consistent with previous studies (Mukudden-Petersen et al., 2005). See appendix A for a flow diagram of the study design.

Participants

The participants, all of whom were healthy adults age 18 years or older, were randomly assigned (by the toss of a coin) to either the control group or the intervention group. The number of participants who agreed to participate in this research was 38. Of the participants enrolled, 18 were randomized to the intervention group (10 women and 8 men) and 20 randomized to the control group (10 women and 10 men).

Protection of Human Participants

Permission to conduct this study was obtained from Texas Woman's University Institutional Review Board for the Houston Center. All participants were informed that participation was voluntary, and there would be no penalty for refusing to take part or discontinuing participation at any time.

Data Collection Procedure

Participants were recruited via fliers. Participants were given a verbal description of the study, time to read the informed consent, ask questions, decide on participation,

and sign the consent form. Inclusion criteria were that all participants must be healthy adults at least 18 years of age and willing to consume 68 g of pistachios per day. Neither race nor ethnicity was used as an exclusion criterion. Exclusion criteria included that female participants must not be pregnant nor should they plan to become pregnant during the six-week study period. Participants could not have an allergy to pistachios, any condition that would prevent them from undergoing an overnight fast of 8-12 hours, or be currently taking single vitamin supplements or medications that would affect serum lipid concentrations, including hormone replacement therapy.

Participants made four visits to the Department of Nutrition and Food Sciences at Texas Woman's University, Houston Center. During the first visit, participants reported their age, were randomized to either the control group or the intervention group, and assigned a code number. Height and weight were measured without shoes using an instrument called a Health-O-Meter® (Sunbeam Products, Inc.), which is a combination of a balance and a stadiometer. Instructions were given for a 3-day diet diary collection (three days per week, consisting of two nonconsecutive week days, and one weekend day). The instructions included describing the nature of the food, the amount, and the time eaten; for example, 1 glass of 2% milk @ 8 am. Additionally, participants were given standard measuring spoons and cups to estimate quantities of foods eaten. Participants were instructed to record their normal diet over two 3-day periods. Blank diet diaries were provided to record two 3-day diet diaries for the instructed time period: Each page of the diary was pre-marked with the participant's code number. Participants were also given a schedule of dates and times for their subsequent visits and instructed to

not eat anything 8-12 hours before the second visit to obtain a fasting blood sample under fasting conditions.

At the second visit, 2 weeks after the first visit, weight was measured without shoes and recorded. Returned diet diaries for the previous two weeks were reviewed for completeness (for example, if cream or sugar had been added to coffee) and additions made as necessary; 10 ml of blood was drawn by a trained phlebotomist from the antecubital vein under fasting (8-12 hours) conditions and used to prepare serum by spinning in a clinical centrifuge at 2000 x g at 5°C. Serum samples were stored at -80°C. Participants assigned to the control group were provided blank diet diaries to record their normal diet for two 3-day diet diaries as previously instructed for the next two weeks. Participants in the intervention group were given 28 numbered packets (two per day) containing 34 g per packet of pistachios, obtained from the Arizona Pistachio Company, to be consumed over the course of the next two weeks. Two packets were assigned for each day. Additionally, participants were provided blank diet diaries to record their diet and pistachio intake for two 3-day diet diaries for the next two weeks. They were also asked to return any uneaten pistachio at the next visit.

At the third visit, two weeks after visit 2, weight was measured and recorded. Returned diet diaries for the previous two weeks were reviewed for completeness and additions made as necessary. Participants assigned to the control group were provided blank diet diaries to record their normal diet for two 3-day diet diaries as previously instructed for the next two weeks. Participants in the intervention group returned any uneaten pistachio and were given a new set of 28 numbered packets of pistachios to be

consumed over the course of the next two weeks. Additionally, they were provided blank diet diaries to record their diet and pistachio intake for two 3-day diet diaries for the next two weeks and instructed to return uneaten pistachios.

The final visit occurred two weeks after visit 3. A final weight was measured and record. A final fasting blood sample of 10 ml of blood was obtained by a trained phlebotomist from the antecubital vein under fasting (8-12 hours) conditions and used to prepare serum by spinning in a clinical centrifuge at 2000 x g at 5°C. These serum samples were stored at -80°C. Diet diaries were reviewed for completeness and additions made as necessary. Participants in the intervention group returned uneaten pistachios. No participants reported any illnesses during the course of the study.

Analysis

Nutrient Intakes and Compliance with Consumption of Pistachios

Information collected from the diet diaries (18 days for each subject) was entered into the Nutrition Data System for Research (NDS-R) version 2007. A standardized process for resolving questions about foods not found in the database was established. Data entry rules created by NDS-R and documented in the software manual were implemented to maintain consistency in data entry throughout the study; NDS-R's data entry rules provided guidance when the diet diary was missing information on foods items such as preparation, unknown food type, unknown food amounts, and food equivalent terminology. All diet diaries were reviewed and edited for correctness by a registered dietitian trained in NDS-R data collection.

Intakes of dietary γ-tocopherol and dietary magnesium were calculated using the information collected from the diet diaries in conjunction with NDS-R. The value for γ-tocopherol concentration for pistachios is 22.45 mg/100 grams for both NDS-R and the USDA National Nutrient Database for Standard Reference, Release 22 (USDA SR22). The analytical value provided by Craft Technologies, Inc. was 24.3 mg/100g. Because the analytical value was close to the values in NDS-R, no adjustments were made to the NDS-R value for γ-tocopherol. In addition, the intakes of energy, as well as other relevant nutrients (α-tocopherol, MUFA, PUFA, fat, protein, copper, arginine, and fiber) were obtained for each participant. Dietary data for each subject were aggregated into three two-week blocks, giving one pre-intervention and two post-intervention values for each subject. Compliance was assessed by reviewing diet diaries and reviewing the collected weights of returned pistachios. BMI was computed by dividing weight in kilograms by height in meters^2.

Serum Analyses

Serum γ-tocopherol concentration was determined as described in detail by Natta, Stacewicz-Sapuntzakis, Bhagavan, & Bowen (1988), which involves deproteinization with ethanol, extraction with hexane and quantification by high performance liquid chromatography. (This method also allows for the quantification of α-tocopherol). The concentration of γ-tocopherol and α-tocopherol were determined in serum, as well as serum magnesium concentrations using a standard colorimetric procedure (Stanbio, San Antonio, Texas). The components of the lipid profile (total cholesterol and HDL-C, and

triglycerides) were determined as described by Radcliffe and Czajka-Narins (1998). The concentration of LDL-C was determined using Friedewald's equation: LDL-C= total cholesterol - (HDL-C) - (Triglycerides/5). See appendices B-E for descriptions of the procedures used for determination of serum concentrations of total cholesterol, HDL-C, triglycerides, and magnesium.

Statistical Analyses

Pearson's X^2 test was computed to test differences between the intervention group and the control group by gender. The student's *t*-test was used to test differences in mean age, weight, and height. Data were checked for normal distribution by exploration of the 5% trimmed mean, the Shapiro-Wilk test for normality, shape of the histogram, detrended normal Q-Q plots, and boxplots of the distribution scores. Data that were non-normally distributed were log transformed to normalize distribution for analyses. Untransformed values were reported in the results tables and were expressed as means $\pm$ SD.

A repeated measures analysis of variance (ANOVA) was computed to determine the effects of treatment, time, and treatment X time. If the overall repeated measures ANOVA was significant ($P<0.05$), further all pair-wise comparisons for the post hoc analyses were performed. The within-group comparison for the dietary intake variables was carried out using a Bonferroni adjusted *P* value of 0.0083 (0.05/6). Between-group comparisons for dietary intake variables (control group vs. intervention group) were carried out at a *P* value of 0.0167 (0.05/3). Post hoc comparisons for serum

concentration variables were carried out at a P value of 0.025. All statistical analyses were performed with SPSS for Windows Statistical Software (version 15).

CHAPTER IV

RESULTS

The purpose of this study was to determine the effect of the consumption of pistachios (at a level of 68 g per day) on the intakes and serum concentrations of putative cardioprotective nutrients, that is γ-tocopherol and magnesium, and on serum lipid profiles (total cholesterol, LDL-C, HDL-C, and triglycerides) using a six-week randomized, controlled, clinical trial design.

This study was designed to test the following null hypotheses:

1. The intake of γ-tocopherol by the intervention group (receiving 68 g of pistachios per day) during the intervention period will not be significantly different from that of the control group.

   REJECTED: There was a significant difference between the intervention group and control group during the intervention period for both weeks 3 and 4 and weeks 5 and 6.

2. The serum concentration of γ-tocopherol for the intervention group will not be significantly different from that of the control group after the four-week intervention period.

FAILED TO REJECT: There was no significant difference in the serum concentration of γ-tocopherol between the intervention group and control group during the intervention period.

3. The intake of magnesium by the intervention group during the intervention period will not be significantly different from that of the control group.

   FAILED TO REJECT: There was no significant difference in the intake of magnesium between the intervention group and control group during the intervention period.

4. The serum concentration of magnesium for the intervention group will not be significantly different from that of the control group after the four-week intervention period.

   FAILED TO REJECT: There was no significant difference in the serum concentration of magnesium between the intervention group and control group during the intervention period.

5. The lipid profile of the intervention group will not be significantly different from that of the control group after the four-week intervention period.

   FAILED TO REJECT: There was no significant difference in the lipid profile between the intervention group and control group during the intervention period.

Characteristics

There were no between-group differences for mean height, initial body weight, age, and gender (see Table 4). Mean ages of the 20 control group participants and 18

Table 4

Characteristics by Group Status

Variable	Control group ($N = 20$)		Intervention group ($N = 18$)		p-value
Gender, number (%)					
Men	10	(50.0)	8	(44.4)	
Women	10	(50.0)	10	(55.6)	0.732
Height, mean (SD), m	1.7	(0.1)	1.71	(0.1)	0.668
Initial weight, mean (SD), kg	76.1	(19.6)	81.8	(18.8)	0.378
Age, mean (SD), years	35.8	(9.7)	40.5	(14.6)	0.241

Note: SD = standard deviation. P-values were derived from the χ^2 test for categorical variables and Student's *t* test for continuous variables. All P-values are two-sided.

intervention group were 35.8 and 40.5 years ($p = 0.241$), respectively. There were no significant within-group and between-group differences in BMI (see Table 5). All participants were reported as non-smokers and two participants reported taking a multivitamin. Weekly compliance for pistachio consumption ranged between 84-94% (see Table 6).

Dietary Intake

Dietary intake was examined using both absolute dietary intake values and energy-adjusted values. As indicated in Table 7, there were no significant differences for the main effect of time, the main effect of treatment, or interaction of time and treatment for total energy (kcal/day), percent energy from protein, percent energy from SAFA, or energy-adjusted cholesterol (mg/1000 kcal) intake between the control group and intervention group.

Percent energy from carbohydrate was significantly lower in the intervention group at weeks 5 and 6 compared to the control group ($p = 0.012$). Within the intervention group, percent energy from carbohydrate was significantly lower at week weeks 3 and 4 and weeks 5 and 6, $p < 0.001$ for both. Between groups, percent energy from fat was lower in the controls (32.3%) than the intervention group (38.8%) at weeks 5 and 6 ($p < 0.001$). Percent energy from fat was significant higher for the intervention group at weeks 3 and 4 and weeks 5 and 6 compared to weeks 1 and 2 ($p = <0.001$; $p < 0.001$, respectively). Between groups, percent energy from MUFA were higher in the

Table 5

BMI for Participants by Group Status and Time

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
BMI, mean (SD)					0.662	0.350	0.183
Week 1	26.4[a]	(5.8)	28.0[a]	(5.4)			
Week 3	26.4[a]	(5.8)	28.1[a]	(5.2)			
Week 6	26.3[a]	(5.8)	28.1[a]	(5.2)			

Note: SD = Standard deviation; BMI = Body mass index, weight (kg)/ height (meters)^2. For the within group time points, values having the same superscript are not significantly different.

Table 6

Pistachio Consumption by the Intervention Group

	Week 3		Week 4		Week 5		Week 6	
Variable	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Total, g/day								
All	63.5	(6.2)	60.1	(9.6)	59.0	(9.3)	59.6	(11.4)
Men	63.8	(4.1)	61.1	(8.0)	57.4	(13.1)	57.1	(12.1)
Women	63.4	(7.7)	59.3	(11.0)	60.3	(5.1)	61.7	(10.9)
% Consumed								
All	93.4	(9.1)	88.4	(14.1)	86.7	(13.7)	87.7	(16.8)
Men	93.7	(6.0)	89.9	(11.8)	84.3	(19.3)	83.9	(17.8)
Women	93.2	(11.3)	87.3	(16.2)	88.7	(7.5)	90.7	(16.1)
% Energy from pistachios								
All	19.2	(5.8)	17.1	(5.9)	18.0	(7.8)	17.7	(7.2)
Men	16.5	(4.6)	14.7	(4.6)	12.7*	(5.1)	14.4	(6.1)
Women	21.4	(5.9)	19.0	(6.5)	22.3*	(7.0)	20.2	(7.2)

Note: SD = standard deviation. Asterisks indicate significant differences between men and women within week time period. *$P < 0.0125$.

Table 7

Dietary Intakes of Energy and Selected Macronutrients and Micronutrients by Group Status and Time

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
Energy (kcal/day)					0.221	0.201	0.142
Week 1 - 2	1862.4[a]	(675.9)	1918.7[a]	(620.2)			
Week 3 - 4	1824.7[a]	(450.5)	2108.1[a]	(697.0)			
Week 5 - 6	1738.7[a]	(448.8)	2171.4[a]	(878.2)			
% Protein					0.232	0.493	0.401
Week 1 - 2	17.6[a]	(5.0)	17.2[a]	(3.0)			
Week 3 - 4	18.1[a]	(4.5)	16.9[a]	(2.4)			
Week 5 - 6	17.8[a]	(4.8)	16.3[a]	(2.6)			
% Carbohydrate					< 0.001	0.326	0.002
Week 1 - 2	50.8[a]	(8.1)	51.4[a]	(7.6)			
Week 3 - 4	47.2[a]	(8.7)	45.8[b]	(7.0)			
Week 5 - 6	48.9[a]	(7.3)	43.0[b,*]	(6.0)			
% Fat					< 0.001	0.167	< 0.001
Week 1 - 2	31.7[a]	(6.5)	29.4[a]	(6.9)			
Week 3 - 4	34.6[a]	(5.9)	36.9[b]	(5.0)			
Week 5 - 6	32.3[a]	(5.4)	38.8[b,*]	(4.0)			
MUFA (%)					< 0.001	0.025	< 0.001
Week 1 - 2	12.3[a]	(2.9)	11.0[a]	(3.0)			
Week 3 - 4	13.5[a]	(2.7)	15.5[b,*]	(2.1)			
Week 5 - 6	12.4[a]	(2.6)	16.5[b,*]	(1.6)			

Table 7 (Continued, 2)

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
PUFA (%)					< 0.001	< 0.001	< 0.001
Week 1 - 2	6.8[a]	(1.6)	6.5[a]	(1.2)			
Week 3 - 4	7.2[a]	(1.2)	9.2[b,*]	(1.2)			
Week 5 - 6	6.5[a]	(1.8)	9.4[b,*]	(1.7)			
SAFA (%)					0.198	0.171	0.275
Week 1 - 2	10.0[a]	(3.0)	9.4[a]	(2.8)			
Week 3 - 4	11.1[a]	(2.8)	9.4[a]	(2.2)			
Week 5 - 6	10.7[a]	(2.5)	10.0[a]	(1.7)			
Dietary fiber (g/1000 kcal)					0.452	0.507	0.009
Week 1 - 2	10.7[a]	(4.3)	10.2[a]	(3.1)			
Week 3 - 4	10.0[a]	(3.8)	11.5[b]	(3.3)			
Week 5 - 6	9.9[a]	(3.3)	10.6[a,b]	(3.2)			
Cholesterol (mg/1000 kcal)					0.863	0.573	0.181
Week 1 - 2	119.7[a]	(55.6)	122.7[a]	(42.7)			
Week 3 - 4	140.1[a]	(70.7)	115.4[a]	(55.2)			
Week 5 - 6	126.2[a]	(52.2)	117.4[a]	(69.5)			

Note: SD = standard deviation; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SAFA = saturated fatty acids. . For the within group time points, values having the same superscript are not significantly different; significance level set at $P < 0.0083$. Asterisks indicate significant difference from the control at the same time point; significance level set at $P < 0.0167$.

intervention group (15.5%) than the control group (13.5%) at weeks 3 and 4 ($p = 0.011$). Similar results were seen at weeks 5 and 6 between the intervention group (16.5%) and control group (12.4%), $p < 0.001$. Within the intervention group, percent energy for MUFA were significantly higher for weeks 3 and 4 and weeks 5 and 6 compared to weeks 1 and 2 ($p < 0.001$; $p < 0.001$, respectively). Percent energy from PUFA, within in the intervention group, was significantly higher at weeks 3 and 4 (9.2%) and weeks 5 and 6 (9.4%) compared to weeks 1 and 2 (6.5%), $p < 0.001$ for both time periods. Energy-adjusted dietary fiber intake was significantly higher in the intervention group at weeks 3 and 4 compared to weeks 1 and 2, $p = 0.005$.

No significant differences were found for the main effect of time, the main effect of treatment, or interaction of time and treatment for protein (g/day), carbohydrate (g/day), SAFA (g/day), and cholesterol (mg/day) (see Table 8). Fat intake (g/day) was significantly higher for the intervention group at weeks 5 and 6 compared to the control group, $p = 0.001$. Within the intervention group, fat intake was higher for weeks 3 and 4 and weeks 5 and 6 compared to weeks 1 and 2 ($p < 0.001$; $p < 0.001$, respectively) for both absolute values. When compared to the control group, MUFA and PUFA intakes (g/day) were significantly higher in the intervention group at weeks 3 and 4 ($p = 0.010$) and weeks 5 and 6 ($p < 0.001$). Within the intervention group, MUFA and PUFA intakes were significantly higher at weeks 3 and 4 and weeks 5 and 6 compared to weeks 1 and 2 ($p < 0.001$ for both time periods). There were no between group differences for dietary

Table 8

Dietary Intakes of Macronutrients by Group Status and Time

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
Protein (g/day)					0.111	0.392	0.617
Week 1 - 2	78.4[a]	(26.5)	81.9[a]	(26.8)			
Week 3 - 4	80.0[a]	(20.0)	88.7[a]	(27.9)			
Week 5 - 6	74.9[a]	(19.1)	89.0[a]	(38.6)			
Carbohydrate (g/day)					0.127	0.452	0.569
Week 1 - 2	234.5[a]	(88.2)	239.7[a]	(67.5)			
Week 3 - 4	215.4[a]	(66.1)	239.3[a]	(77.3)			
Week 5 - 6	211.9[a]	(60.9)	230.8[a]	(88.0)			
Fat (g/day)					0.002	0.118	0.003
Week 1 - 2	68.0[a]	(31.7)	64.6[a]	(27.9)			
Week 3 - 4	71.1[a]	(22.2)	84.6[b]	(22.0)			
Week 5 - 6	63.4[a]	(23.5)	91.9[b,*]	(31.2)			
MUFA (g/day)					< 0.001	0.050	< 0.001
Week 1 - 2	26.4[a]	(12.8)	24.6[a]	(11.4)			
Week 3 - 4	27.7[a]	(9.5)	35.5[b,*]	(9.0)			
Week 5 - 6	24.4[a]	(10.6)	39.0[b,*]	(13.0)			
PUFA (g/day)					< 0.001	0.003	< 0.001
Week 1 - 2	14.3[a,b]	(6.5)	13.8[a]	(5.0)			
Week 3 - 4	14.8[b]	(4.5)	21.0[b,*]	(5.2)			
Week 5 - 6	12.4[a]	(4.0)	21.9[b,*]	(6.4)			
SAFA (g/day)					0.502	0.896	0.192
Week 1 - 2	21.7[a]	(11.3)	20.8[a]	(9.6)			
Week 3 – 4	22.7[a]	(8.2)	21.7[a]	(7.0)			
Week 5 – 6	21.2[a]	(9.1)	24.2[a]	(10.0)			

Table 8 (Continued, 2)

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
Dietary fiber (g/day)					0.039	0.126	0.001
Week 1 - 2	19.3[a]	(9.0)	18.8[a]	(7.4)			
Week 3 - 4	18.1[a]	(7.9)	23.9[b]	(9.7)			
Week 5 - 6	17.2[a]	(7.9)	22.2[a,b]	(9.8)			
Cholesterol (mg/day)					0.698	0.812	0.617
Week 1 - 2	217.6[a]	(116.3)	253.8[a]	(120.3)			
Week 3 - 4	248.6[a]	(127.8)	249.6[a]	(133.9)			
Week 5 - 6	216.0[a]	(104.1)	271.0[a]	(102.7)			

Note: SD = standard deviation; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SAFA = saturated fatty acids. For the within group time points, values having the same superscript are not significantly different; significance level set at $P < 0.0083$. Asterisks indicate significant difference from the control at the same time point; significance level set at $P < 0.0167$.

fiber (g/day); however, intake was significantly higher in the intervention group at weeks 3 and 4 compared to weeks 1 and 2, $p = <0.001$.

There was a between group difference for dietary intake of γ-tocopherol (mg/day). Intake was significantly higher for the intervention group at weeks 3 and 4 ($p < 0.001$) and weeks 5 and 6 ($p < 0.001$) compared to the control group. Additionally, dietary intakes for γ-tocopherol were significantly higher in the intervention group at weeks 3 and 4 ($p < 0.001$) and weeks 5 and 6 ($p < 0.001$) compared to weeks 1 and 2 (see Table 9). No significant differences were seen for α-tocopherol intakes (mg/day) and total α-tocopherol intakes (mg/day) between and within groups. There were no between group differences for magnesium intake (mg/day). However, within the intervention group, magnesium intake was significantly higher at weeks 3 and 4 compared to weeks 1 and 2 ($p = 0.001$). Similar results were seen for total magnesium (diet + supplement intake) in the intervention group between the same time periods ($p = 0.001$). No significant differences were seen for dietary arginine (g/day) between the intervention group and the control group at any of the time periods. Within the intervention group, arginine intakes were higher at weeks 3 and 4 compared to weeks 1 and 2 ($p = 0.001$). Intakes of dietary copper (mg/day) were significantly higher at weeks 3 and 4 and weeks 5 and 6 for the intervention group compared to the control group, $p < 0.001$ for both. Within the intervention group, dietary copper intake was significantly higher at weeks 3 and 4 and weeks 5 and 6 compared to weeks 1 and 2 ($p < 0.001$ for both). Similar results were seen

Table 9

Dietary Intakes of Selected Micronutrients by Group Status and Time

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
γ-tocopherol (mg/day)					< 0.001	< 0.001	< 0.001
Week 1 - 2	13.8[a]	(6.9)	13.3[a]	(6.6)			
Week 3 - 4	13.8[a]	(5.2)	25.5[b,*]	(5.6)			
Week 5 - 6	12.7[a]	(4.5)	27.2[b,*]	(7.22)			
α-tocopherol (mg/day)					0.131	0.364	0.165
Week 1 - 2	8.6[a]	(4.6)	8.4[a]	(4.1)			
Week 3 - 4	7.9[a]	(3.1)	8.2[a]	(4.2)			
Week 5 - 6	6.4[a]	(3.2)	8.3[a]	(3.9)			
Total α-tocopherol (mg/day)					0.196	0.778	0.208
Week 1 - 2	9.7[a]	(6.1)	9.0[a]	(4.7)			
Week 3 - 4	9.2[a]	(4.9)	8.8[a]	(5.3)			
Week 5 - 6	7.5[a]	(4.2)	8.9[a]	(5.3)			
Magnesium (mg/day)					0.367	0.197	0.002
Week 1 - 2	314.7[a]	(114.2)	308.3[a]	(116.1)			
Week 3 - 4	286.4[a]	(96.0)	360.5[b]	(144.6)			
Week 5 - 6	275.5[a]	(85.5)	355.4[a,b]	(174.1)			
Total magnesium (mg/day)					0.380	0.194	0.002
Week 1 - 2	316.7[a]	(115.0)	312.1[a]	(118.3)			
Week 3 - 4	288.4[a]	(96.5)	364.2[b]	(146.7)			
Week 5 - 6	277.5[a]	(85.5)	360.0[a,b]	(176.7)			

Table 9 (Continued, 2)

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
Arginine (g/day)					0.003	0.108	0.085
Week 1 - 2	4.3^{a}	(1.5)	4.5^{a}	(1.5)			
Week 3 - 4	4.5^{a}	(1.1)	5.4^{b}	(1.7)			
Week 5 - 6	4.1^{a}	(1.1)	$5.5^{a,b}$	(2.6)			
Copper (mg/day)					0.001	0.004	< 0.001
Week 1 - 2	1.35^{a}	(0.7)	1.3^{a}	(0.5)			
Week 3 - 4	1.27^{a}	(0.5)	$2.0^{b,*}$	(0.5)			
Week 5 - 6	1.31^{a}	(0.6)	$2.0^{b,*}$	(0.7)			
Total copper (mg/day)					< 0.001	0.081	0.001
Week 1 - 2	1.6^{a}	(0.9)	1.4^{a}	(0.6)			
Week 3 - 4	1.5^{a}	(0.8)	$2.1^{b,*}$	(0.7)			
Week 5 - 6	1.5^{a}	(1.0)	$2.1^{b,*}$	(0.9)			

Note: SD = standard deviation; γ = gamma; α = alpha; total nutrient values = diet + supplement intake. For the within group time points, values having the same superscript are not significantly different; significance level set at $P < 0.0083$. Asterisks indicate significant difference from the control at the same time point; significance level set at $P < 0.0167$.

for total copper intake in the intervention group between the same time periods ($p < 0.001$ for both time periods).

When adjusted for energy, similar results were found for intakes of γ-tocopherol (mg/1000 kcal) as for absolute values. Dietary intake of energy-adjusted γ-tocopherol (mg/1000 kcal) was significantly higher for the intervention group at weeks 3 and 4 ($p < 0.001$) and weeks 5 and 6 ($p < 0.001$) compared to the control group. Additionally, dietary intakes for energy-adjusted γ-tocopherol were significantly higher in the intervention group at weeks 3 and 4 ($p < 0.001$) and weeks 5 and 6 ($p < 0.001$) compared to weeks 1 and 2 (see Table 10). No significant differences were seen for energy-adjusted intakes of α-tocopherol (mg/1000 kcal) and total α-tocopherol intakes (mg/1000 kcal) between and within groups. No between group differences were found for energy-adjusted intakes of magnesium (mg/1000 kcal). However, within the control group, energy-adjusted intakes of magnesium (mg/1000 kcal) were significantly lower at weeks 3 and 4 compared to weeks 1 and 2 ($p = 0.006$), the same was found for energy-adjusted total magnesium intake ($p = 0.006$). No significant differences between and within group differences were seen for energy-adjusted intakes of arginine (g/1000 kcal). Within the intervention group, energy-adjusted arginine was weeks 3 and 4 compared to weeks 1 and 2 ($p = 0.004$). Energy-adjusted copper and energy-adjusted total copper intakes (mg/1000 kcal) were higher in the intervention group compared to the control at weeks 3 and 4 and weeks 5 and 6 ($p < 0.001$ for both time periods). Additionally, within the

Table 10

Energy-Adjusted Dietary Intakes of Selected Micronutrients by Group Status and Time

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)[a]	Mean	(SD)[a]	p-value	p-value	p-value
γ-tocopherol (mg/1000 kcal)					< 0.001	< 0.001	< 0.001
Week 1 - 2	7.4[a]	(2.3)	6.8[a]	(2.1)			
Week 3 - 4	7.5[a]	(2.1)	12.5[b,*]	(1.7)			
Week 5 - 6	7.5[a]	(3.0)	13.2[b,*]	(2.8)			
α-tocopherol (mg/1000 kcal)					0.052	0.948	0.302
Week 1 - 2	5.0[a]	(3.3)	5.0[a]	(4.0)			
Week 3 - 4	4.5[a]	(2.3)	4.2[a]	(2.5)			
Week 5 - 6	3.7[a]	(1.6)	3.9[a]	(1.4)			
Total α-tocopherol (mg/1000 kcal)					0.073	0.606	0.207
Week 1 - 2	5.8[a]	(5.1)	5.2[a]	(4.0)			
Week 3 - 4	5.4[a]	(3.7)	4.4[a]	(2.8)			
Week 5 - 6	4.4[a]	(2.6)	4.1[a]	(1.7)			
Magnesium (mg/1000 kcal)					0.580	0.814	0.011
Week 1 - 2	177.4[a]	(67.8)	162.2[a]	(36.4)			
Week 3 - 4	159.7[b]	(46.9)	171.7[a]	(36.2)			
Week 5 - 6	159.4[a,b]	(31.5)	163.6[a]	(31.5)			
Total magnesium (mg/1000 kcal)					0.584	0.786	0.011
Week 1 - 2	179.4[a]	(72.9)	163.6[a]	(35.4)			
Week 3 - 4	161.3[b]	(50.4)	173.2[a]	(36.1)			
Week 5 - 6	160.8[a,b]	(33.4)	165.2[a]	(30.5)			

Table 10 (Continued, 2)

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)[a]	Mean	(SD)[a]	p-value	p-value	p-value
Arginine (g/1000 kcal)					0.004	0.602	0.496
Week 1 - 2	2.4[a]	(0.8)	2.4[a]	(0.5)			
Week 3 - 4	2.5[a]	(0.7)	2.6[b]	(0.4)			
Week 5 - 6	2.4[a]	(0.7)	2.5[a,b]	(0.5)			
Copper (mg/1000 kcal)					< 0.001	0.018	< 0.001
Week 1 - 2	0.7[a]	(0.2)	0.7[a]	(0.2)			
Week 3 - 4	0.7[a]	(0.2)	1.0[b]	(0.2)			
Week 5 - 6	0.8[a]	(0.4)	1.0[b]	(0.2)			
Total Copper (mg/1000 kcal)					< 0.001	0.315	< 0.001
Week 1 - 2	0.9[a]	(0.2)	0.7[a]	(0.2)			
Week 3 - 4	0.9[a]	(0.2)	1.0[b,*]	(0.2)			
Week 5 - 6	0.9[a]	(0.4)	1.0[b,*]	(0.2)			

Note: SD = standard deviation; γ = gamma; α = alpha; total nutrient values = diet + supplement intake. For the within group time points, values having the same superscript are not significantly different; significance level set at $P < 0.0083$. Asterisks indicate significant difference from the control at the same time point; significance level set at $P < 0.0167$.

intervention group, energy-adjusted copper and total copper intakes were higher at weeks 3 and 4 and weeks 5 and 6 compared to weeks 1 and 2 ($p < 0.001$).

Serum Concentrations

Post-intervention, the serum concentration of γ was significantly higher for the intervention group than the control group ($p = 0.007$) and the post-intervention value for the intervention group was lower than for the pre-intervention value. The same was found for the γ-tocopherol/total cholesterol ratio. For serum concentrations, means ± SD are shown in Table 11. No between group differences were found for serum α-tocopherol concentration. In the intervention group, serum α-tocopherol concentration was significantly lower at post-intervention with consumption of pistachios ($p = 0.008$). No between and within group differences were observed for the α-tocopherol/total cholesterol ratio. No between or within group differences were found for serum magnesium.

No significant differences between the intervention group and the control group were found for any of the indices of serum lipid status (see Table 12). Serum total cholesterol was significantly lower in the intervention group at post-intervention compared to pre-intervention ($p = 0.003$), the same was found for nonHDL-C concentration ($p = 0.003$). No within group differences were observed for serum concentrations of triglycerides, HDL-C, LDL-C, VLDL-C, and the ratios of total cholesterol/HDL-C, total cholesterol/nonHDL-C, LDL-C/HDL-C ratio, nonHDL-C C/HDL-C, and triglyceride/HDL-C.

Table 11

Serum Concentrations of Tocopherols and Magnesium by Group Status and Time

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
γ-Tocopherol (μmol/L)					0.064	0.533	0.030
Pre-intervention	4.2[a]	(1.9)	4.2[a]	(1.8)			
Post-intervention	4.1[a]	(1.5)	4.9[b]	(2.1)			
α-Tocopherol (μmol/L)					0.016	0.592	0.123
Pre-intervention	24.9[a]	(5.1)	27.1[a]	(7.1)			
Post-intervention	24.5[a]	(5.2)	25.1[b]	(7.2)			
γ-Tocopherol/total cholesterol (μmol/millimoles)					0.027	0.758	0.151
Pre-intervention	0.9[a]	(0.4)	0.8[a]	(0.3)			
Post-intervention	0.8[a]	(0.3)	1.0[b]	(0.4)			
α-Tocopherol/total cholesterol (μmol/millimoles)					0.087	0.678	0.589
Pre-intervention	5.1[a]	(1.1)	5.2[a]	(1.3)			
Post-intervention	4.9[a]	(1.1)	5.1[a]	(1.2)			
Magnesium (mEqu/L)					0.119	0.271	0.149
Pre-intervention	1.8[a]	(0.1)	1.8[a]	(0.1)			
Post-intervention	1.8[a]	(0.1)	1.8[a]	(0.1)			

Note: SD = standard deviation; γ = gamma; α = alpha. For the within group time points, values having the same superscript are not significantly different; significance level set at $P < 0.025$.

Table 12

Serum Concentrations of Lipids by Group Status and Time

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
Total cholesterol (mg/dL)					0.214	0.811	0.002
Pre-intervention	190.9[a]	(28.6)	201.2[a]	(28.2)			
Post-intervention	196.1[a]	(26.3)	189.8[b]	(24.4)			
Triglycerides (mg/dL)					0.072	0.768	0.316
Pre-intervention	97.5[a]	(44.3)	106.9[a]	(43.3)			
Post-intervention	93.1[a]	(39.8)	91.8[a]	(53.5)			
HDL-C (mg/dL)					0.729	0.594	0.656
Pre-intervention	55.0[a]	(14.9)	52.9[a]	(14.5)			
Post-intervention	55.7[a]	(14.1)	52.8[a]	(14.8)			
LDL-C (mg/dL)					0.602	0.695	0.018
Pre-intervention	116.4[a]	(34.5)	127.0[a]	(25.6)			
Post-intervention	121.8[a]	(31.7)	118.6[a]	(27.3)			
VLDL-C (mg/dL)					0.072	0.768	0.316
Pre-intervention	19.5[a]	(8.9)	21.4[a]	(8.7)			
Post-intervention	18.6[a]	(8.0)	18.4[a]	(10.7)			
nonHDL-C (mg/dL)					0.178	0.659	0.003
Pre-intervention	135.9[a]	(33.8)	148.4[a]	(30.2)			
Post-intervention	140.4[a]	(33.4)	137.0[b]	(31.1)			
Total cholesterol/HDL-C ratio					0.280	0.516	0.100
Pre-intervention	3.7[a]	(1.2)	4.1[a]	(1.3)			
Post-intervention	3.8[a]	(1.1)	3.9[a]	(1.3)			

Table 12 (Continued, 2)

	Control group		Intervention group		Time	Treatment	Time x treatment
Variable	Mean	(SD)	Mean	(SD)	p-value	p-value	p-value
Total cholesterol/nonHDL-C ratio					0.210	0.515	0.087
Pre-intervention	1.4[a]	(0.2)	1.4[a]	(0.1)			
Post-intervention	1.4[a]	(0.2)	1.4[a]	(0.2)			
LDL-C/HDL-C ratio					0.630	0.546	0.147
Pre-intervention	2.3[a]	(1.0)	2.6[a]	(1.0)			
Post-intervention	2.4[a]	(1.0)	2.5[a]	(1.1)			
nonHDL-C/HDL-C ratio					0.280	0.516	0.100
Pre-intervention	2.7[a]	(1.2)	3.1[a]	(1.3)			
Post-intervention	2.8[a]	(1.1)	2.9[a]	(1.3)			
Triglyceride/HDL-C ratio					0.108	0.518	0.588
Pre-intervention	2.0[a]	(1.2)	2.3[a]	(1.5)			
Post-intervention	1.9[a]	(1.1)	2.1[a]	(1.7)			

Note: SD = standard deviation; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; VLDL = very low density lipoprotein cholesterol. The concentration of LDL-C was determined using Friedewald's equation: LDL-C= total cholesterol - (HDL-C) - (Triglycerides/5). For the within group time points, values having the same superscript are not significantly different; significance level set at $P < 0.025$.

CHAPTER V

DISCUSSION

This intervention study demonstrated that a pistachio-enriched diet had favorable effects on dietary intakes of γ-tocopherol and magnesium, as well as for the serum concentration of γ-tocopherol and total cholesterol within the intervention group. Thus, this discussion will focus on these two nutrients and other nutrients of interest mentioned in the introduction, e.g. copper, arginine, as well as serum lipids.

Peanut, walnuts, pecans, and almonds make up a majority of the intervention studies on nut-enriched diets. Nonetheless, it appears that there are only five studies in humans and one study in rats that have examined the effect of a pistachio-enriched diet on dietary intakes and/or plasma and serum concentrations on selected macronutrients, micronutrients, and lipid profiles. (Edwards et al., 1999; Kocyigit et al., 2006; Sheridan et al., 2007; Gebauer et al., 2008; Sari et al., 2009, Aksoy et al., 2007).

γ-Tocopherol and α-Tocopherol

This study is the first feeding study that has demonstrated the effects of a pistachio-enriched diet on the dietary intakes and serum concentrations of γ-tocopherol. Dietary intake of γ-tocopherol was significantly higher at weeks 3 and 4 and weeks 5 and 6 for the intervention group when compared to the control group. Additionally, within the intervention group, intakes were significantly higher at weeks 3 and 4 and weeks 5 and 6 when compared to weeks 1 and 2. These differences remained when adjusted for

energy intake. Despite there being no significant post-intervention difference between the intervention group and the control group, the serum concentration of γ-tocopherol for the intervention group was significantly higher than the pre-intervention concentration. Similarly, when adjusted for the serum concentration of total cholesterol, no significant differences were observed between the intervention group and the control group. The intervention group's post-intervention value for γ-tocopherol/total cholesterol ratio was significantly higher than the pre-intervention value. There were no significant between group differences for α-tocopherol dietary intake or the α-tocopherol/total cholesterol ratio. However, the intervention group had a significantly lower serum concentration of α-tocopherol post-intervention than pre-intervention, but there was no effect when values for serum α-tocopherol were adjusted for cholesterol concentration. Pistachios are not a good source of α-tocopherol; therefore, a favorable effect was not expected for consumption of pistachios. Although, there are no current feeding studies on pistachios to compare with this study's results, two studies have reported the effect of nut consumption on dietary intake and plasma concentration of γ-tocopherol.

Haddad et al. (2006) conducted a randomized, controlled, crossover feeding study with pecans. Like pistachios, pecans are a good source of γ-tocopherol and a poor source of α-tocopherol (see Table 13). There was a substantial increase in the mean intake of γ-tocopherol on the pecan diet. Additionally, serum α-tocopherol and the α-tocopherol/total cholesterol ratio were significantly lower on the pecan diet. These authors reported a significantly higher serum γ-tocopherol/total cholesterol ratio on the

Table 13

Selected Macronutrients and Micronutrients of Nuts per 1 ounce serving

	Pistachio	Peanut	Walnut	Pecan	Almonds
Energy, kcal	162.0	166.0	185.0	201.0	169.0
Protein, g	6.1	6.7	4.3	2.7	6.3
Carbohydrate, g	7.8	6.1	3.9	3.8	5.5
Fat, g	13.0	14.1	18.5	21.1	15.0
MUFA, g	6.9	7.0	2.5	12.5	9.5
PUFA, g	3.9	4.5	13.4	5.8	3.6
SAFA, g	5.6	2.0	1.7	1.8	4.0
Fiber, g	2.9	2.3	1.9	2.7	3.3
γ-tocopherol, mg	6.4	Not available	5.9	6.7	0.3
α-tocopherol, mg	0.6	1.9	0.2	0.4	7.4
Magnesium, mg	34.0	50.0	45.0	37.0	81.0
Arginine, g	0.6	0.8	0.6	0.3	0.7
Copper, mg	0.4	0.2	0.5	0.3	0.3

Note: γ = gamma; α = alpha; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SAFA = saturated fatty acids. Values from U.S. Department of Agriculture, Agricultural Research Service. 2009. USDA National Nutrient Database for Standard Reference, Release 22. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl

pecan diet. These results differ from the current pistachio study in that this study did not find a significantly lower α-tocopherol/total cholesterol ratio post-intervention for the control group compared to the intervention group. Nor did this study find a significantly higher serum γ-tocopherol/total cholesterol ratio post-intervention for the intervention group compared to the control group. This current study did find significantly lower values for serum α-tocopherol concentration and significantly higher serum values for γ-tocopherol concentration post-intervention for the intervention group compared to pre-intervention values.

Ros et al. (2004) conducted a randomized, crossover feeding that substituted a walnut-enriched diet for a healthy Mediterranean-type diet. There was a higher concentration of serum γ-tocopherol during the walnut diet period compared with the control diet period. The intake of a walnut enriched diet produced a significantly higher LDL γ-tocopherol concentration and lower α-tocopherol concentration. Dietary intake of γ-tocopherol was not reported by these authors. It is reasonable that pistachios would have a favorable effect on serum concentrations of γ-tocopherol as their concentration of γ-tocopherol is similar to that of walnuts (see Table 13).

Pistachios are a good source of γ-tocopherol, which may play an important role in prevention of certain chronic diseases and human health. Evidence from several studies suggested that γ-tocopherol possesses unique features that distinguish it from α-tocopherol and that are potentially important in the defense against CVD and risk of certain cancers (Jiang, Q. et al., 2001). In addition to its potential role in risk reduction

for certain cancers, it appears that γ-tocopherol is effective in trapping lipophilic electrophiles and inhibiting cyclooxygenase activity (Jiang, Q.). These antioxidant defense features may contribute to the protection against oxidative damage, a major contributor to the development of both CVD and cancer (Jiang, Q.).

Several studies suggest that γ-tocopherol may play a role in the reduction of risk for certain cancer, particularly lung and prostate. In a case-control study on tocopherol intake and lung cancer risk, Mahabir et al. (2008) reported that with increasing quartiles of intake of γ-tocopherol there were 16, 24, and 44% reductions in the risk of lung cancer. Weinstein et al. (2005) examined baseline serum concentrations of α-tocopherol and γ-tocopherol to compare their association with prostate cancer risk. These authors conducted a nested case-control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study of male smokers, randomly selecting 100 incident prostate cancer patients and 200 matched control participants. They reported that men with higher circulating levels of α-tocopherol and γ-tocopherol had a lower risk of developing prostate cancer.

Helzlsouer et al. (2000) conducted a nested case-control within a cohort study to examine the associations of α-tocopherol, γ-tocopherol, and selenium with incident prostate cancer. Median concentrations of α-tocopherol and γ-tocopherol were lower for prostate cancer patients than among the control participants, with the difference being significant for γ-tocopherol. Additionally, the risk of prostate cancer was lower among men with higher serum concentrations of α-tocopherol, γ-tocopherol, and selenium. The

strongest significant association was found for γ-tocopherol with men in the highest fifth of the distribution compared to the lowest fifth with γ-tocopherol having a fivefold reduction in the risk of developing prostate cancer. Furthermore, protective associations with prostate cancer risk for serum concentrations of α-tocopherol and selenium were observed when serum concentrations of γ-tocopherol were above the control-based median value. Thus, according to the authors, consideration should be given to supplementation of combined α-tocopherol and γ-tocopherol for the prevention of prostate cancer.

Mixed tocopherols may possibly play a beneficial role in protecting against prostate cancer, as well as improving endothelial function. Liu, Wallmon, Olsson-Mortlock, Wallin, and Saldeen (2003) conducted a controlled clinical study to evaluate the impact of mixed tocopherol supplementation (100 mg/day of γ-tocopherol and lower doses of several other tocopherols) on platelet function ex vivo. There was a reduction in ADP-induced platelet aggregation with mixed tocopherol supplementation, an effect associated with increased nitric oxide production by the stimulated platelets. In a previous study with γ-tocopherol supplementation, by Li, Saldeen, Romeo, and Mehta (1999), it was reported that administration of γ-tocopherol in rats (100 mg/kg diet) led to an increase in the ex vivo production of nitric oxide by arterial tissues. These findings suggest that γ-tocopherol may play a role in promoting nitric oxide synthase activity, providing possible protection against oxidative damage.

In one study on walnuts, Spaccarotella et al. (2008) assessed the effect of walnuts on markers of prostate and vascular health in men at risk for prostate cancer. These authors conducted an eight-week crossover intervention study to examine the effect of walnut supplementation on the serum concentrations of α-tocopherol, γ-tocopherol, and prostate specific antigen (PSA). There was a significant decrease in the α-tocopherol:γ-tocopherol ratio with an increase in serum γ-tocopherol concentration and a trend towards an increase in the ratio of free PSA:total PSA with consumption of walnuts. They suggested that walnut consumption by men may be beneficial and improve serum concentration of γ-tocopherol and α-tocopherol:γ-tocopherol ratio, two biomarkers that are important in the maintenance of prostate and vascular health. The data suggest that a diet rich in γ-tocopherol may possibly play role in the prevention of certain chronic diseases, that is CVD and cancers such as lung and prostate.

Magnesium

As with γ-tocopherol, previous feeding studies with pistachios did not report the intake and serum concentrations of magnesium. In this study, no significant differences in the dietary intake and serum concentrations of magnesium appeared between the intervention and the control groups. However, a significantly higher intake of magnesium was observed in the intervention group at weeks 3 and 4 compared to weeks 1 and 2. When adjusted for energy intake, a significantly lower intake in the control group was seen at weeks 3 and 4 compared to weeks 1 and 2. Alper and Mattes (2003) investigated the effect of peanut consumption on dietary intake and serum concentration

of magnesium using a cross-over intervention study comparing three diets, that is 500 kcal as peanuts were provided daily during an eight-week free feeding (FF) diet, added during a three-week addition (ADD) diet or replaced an equal amount of fat during an eight-week substitution (SUB) diet. Dietary magnesium intake was significantly higher from baseline for all three diets. The authors reported that dietary magnesium intakes were 49% higher in the FF diet, 67% higher in the ADD diet, and 57% higher in the SUB diet compared to baseline. Final serum magnesium concentrations were significantly higher in the FF diet compared to baseline; there were no significant changes for both the ADD diet and the SUB diet. Although consumption of both the ADD diet and the SUB diet increased dietary magnesium intakes, there was no affect on serum magnesium concentration. The magnesium concentration of peanuts (50 mg/100 g) is higher than that of pistachios (34 mg/100 g). This may partially explain the different effect on dietary magnesium and serum magnesium found by Alper and Mattes as opposed to the present study.

Due to processing of foods and reduced consumption of traditionally magnesium-rich foods, dietary magnesium intake has declined adults. (Alper & Mattes, 2003). According to What We Eat in America, NHANES 2005-2006, usual intakes of magnesium for adult men and women range between 45-58% and 55-65% below the EAR, respectively. Several studies have shown that low serum concentrations of magnesium can increase risk of CVD due to diminished lipoprotein lipase and lecithincholesterol acyltransferase activity which results in hyperlipidemia (Alper & Mattes, 2003). King, Mainous, Geesey, and Woolson (2005) investigated the association

of dietary magnesium intake with C-reactive protein (CRP), a marker of vascular inflammation associated with increased risk of CVD. These authors examined the association between dietary magnesium consumption and serum CRP using data from the 1999-2000 National Health and Nutrition Examination Survey. They reported that among adults, 68% consumed less than the RDA of magnesium and that those who consumed <RDA were 1.48-1.75 times more likely to have elevated CRP than adults who consumed $\geq$ RDA. Also, adults over age 40 with a BMI > 25 and who consumed < 50% of the RDA were 2.24 times more likely to have elevated CRP than adults who consumed $\geq$ RDA.

Several cohort studies have shown an inverse association between magnesium intake and colorectal cancer in women. Van den Brandt, Smits, Goldbohm, and Weijenberg (2007) investigated the association of magnesium intake and colorectal cancer in men and women, including in overweight participants. These authors examined 2328 colorectal cancer cases and 4125 healthy members of the Netherlands Cohort Study and found non significant inverse associations with risk of colorectal and colon cancer in men and women. Yet, when they stratified by BMI, a significant inverse association was observed for only those with BMI $\geq$ 25. They suggested that magnesium intake is possibly inversely associated with colorectal cancer risk through improved insulin sensitivity, as overweight is related to decreased insulin sensitivity. Thus, several studies suggest that regular consumption of foods rich in magnesium, such as nuts, may play an important role in CVD and colon cancer risk reduction.

Lipid profile

Previous feeding studies investigating the effect of pistachios on lipid profiles have demonstrated that pistachio supplementation has favorable effects on lipid profiles. Four of five studies reported significantly lower concentrations of total cholesterol with consumption of a pistachio-enriched diet (Edwards et al., 1999; Kocyigit et al., 2006; Sheridan et al., 2007; Gebauer et al., 2008; Sari et al., 2009). In this study, no differences were observed between the intervention and the control groups for total cholesterol concentration; however, a significantly lower serum concentration of total cholesterol post-intervention compared to pre-intervention concentrations was noted for the intervention group. Gebauer et al. (2008) using a randomized crossover controlled-feeding study reported significantly lower nonHDL-C concentrations for the two pistachio enriched-diets compared to the control diet. Similarly, this study observed a significantly lower concentration of nonHDL-C as a result of feeding pistachios.

In the present study, no within or between group differences were observed for serum concentrations of triglycerides, HDL-C, LDL-C, VLDL-C, total cholesterol/HDL-C ratio, total cholesterol/nonHDL-C ratio, LDL-C/HDL-C ratio, nonHDL-C/HDL-C ratio. Three of the five previous studies reported significantly lower concentrations of triglycerides with a pistachio-enriched diet (Kocyigit et al., 2006; Gebauer et al., 2008; Sari et al., 2009). Three of the five previous studies (Edwards et al., 1999; Kocyigit et al., 2006; Sheridan et al., 2007) reported significantly higher concentrations of HDL-C with a pistachio-enriched diet. Four of the five studies reported significantly lower LDL-C concentrations with consumption of pistachios (Kocyigit et al., 2006; Sheridan et al.,

2007; Gebauer et al., 2008; Sari et al., 2009). Both the present study and that by Sheridan et al. reported no significant changes in VLDL-C. All five previous studies reported significantly lower total cholesterol/HDL-C and LDL-C/HDL-C ratios.

Although, this study did not find a favorable effect of a pistachio-enriched diet on all lipid parameters, a favorable effect was found for the serum concentration of total cholesterol. Edwards et al. (1999) investigated a pistachio-enriched diet in individuals with moderate hypercholesterolemia ($\geq$ 210 mg/dL) and found a significant decrease in total cholesterol concentration. Likewise, the present study also demonstrated a statistically significant lowering effect in the intervention group with a mean serum concentration of total cholesterol 201 mg/dL pre-intervention compared to 190 mg/dl post-intervention. However, there was no effect on serum LDL-C, which is predictive of a reduction in CVD risk. These results suggest that a pistachio-enriched diet may be beneficial in reducing the risk of CVD.

Exploratory Secondary Analyses

The main hypotheses of the present study were to examine the effect of pistachio consumption on the dietary intakes and serum concentrations of putative cardioprotective nutrients, that is γ-tocopherol, magnesium, and on serum lipid profiles. However, it has been hypothesized that other compounds such as arginine and copper may also be cardioprotective. Pistachios are a good source of arginine and copper, thus a secondary analysis was performed to examine the effect on pistachio consumption on dietary intake and serum concentrations of the nutrients.

Arginine

Previous studies with pistachios did not report the effect of pistachio consumption on dietary intake of arginine. No significant differences were seen for dietary arginine intake between the intervention group compared to the control group at any of the time periods. However, in the intervention group, arginine intake was higher at weeks 3 and 4 compared to weeks 1 and 2. Alper and Mattes (2003) reported a significant increase in the arginine/lysine ratio with supplementation of peanuts, but dietary arginine intake was not reported by these authors.

Arginine is the amino acid precursor of the endogenous vasodilator nitric oxide, and increased intakes of arginine may have a favorable influence on endothelial function (Ros, 2009). Short-term feeding studies that assessed atherogenic events taking place in the postprandial period demonstrated the potential of food for improving endothelial function (Ros). Thus improving intakes of dietary arginine through nut consumption may improve endothelial dysfunction.

Copper

This is the first feeding study using pistachios to report dietary intakes of copper. No significant differences were seen for dietary copper intake at any of the time periods between the intervention group compared to the control group. A pistachio-enriched diet significantly increased dietary copper intakes in the intervention group at weeks 3 and 4 and weeks 5 and 6 compared to weeks 1 and 2. Similar results were seen in the intervention group for total copper intake (diet + supplements) between the same time periods. Morgan and Clayshulte (2000) conducted a randomized, controlled study using

a pecan-enriched diet (68 g/day) compared to a control diet (the nutrient content of copper is similar for both pistachios and pecans [see Table 13]). These authors reported higher intakes of dietary copper when pecans were incorporated into the diet, similarly the present study found significantly higher intakes of copper with consumption of pistachios.

Copper may play a beneficial role in both the risk reduction of CVD and lung cancer. Aliabadi (2008) suggested that improving copper levels in the diet by appropriate food selection is warranted. This author reported that copper deficiency has been shown to be involved in atrial thrombosis, systolic and diastolic hypertension, enhancement of inflammation, reduced blood clotting, changes in lipid metabolism, and, possibly, atherosclerosis. Mahabir et al. (2006) investigated intake of dietary trace metals and lung cancer risk in a case-control study. These authors reported that with increasing intakes of dietary copper, there were 41, 49, and 66% reductions in risk of lung cancer for all subjects. Mahabir et al. (2007) also examined the association of copper intake with DNA repair capacity (DRC) and reported that individuals with high copper intake + suboptimal DRC, low copper + proficient DRC, and low copper + suboptimal DRC had 33, 66, 154% increased risk of lung cancer when compared with individuals with high dietary copper intake + proficient DRC. Hence, consumption of a diet with foods rich in copper, such as pistachios, may play a role in lowering the risk of CVD and lung cancer.

BMI

This current study reported no significant effect on BMI during the course of the study. Likewise, all previous feeding studies with pistachios reported no significant

differences in body weight and/or BMI with consumption of a pistachio-enriched diet. These data are consistent with findings in other feeding studies with nuts. Bes-Rastrollo et al. (2009) investigated long-term association between nut consumption and weight change in a free living population. These authors evaluated the dietary intake of nuts and subsequent weight change in 51,188 women in the Nurses' Health Study II and found that higher nut consumption was not associated with greater body weight gain during eight year follow-up in healthy middle-aged women. Women that ate nuts $\geq$ 2 times per week (1 ounce servings) had a lower mean weight gain than did women who rarely ate nuts.

The results of the present study suggest that incorporating nuts into a normal diet does not lead to a significant weight gain or a significant increase in energy intake; however, further research is warranted to determine if body weight will be affected by incorporating approximately 2-ounces of pistachio nuts per day into a normal diet for a longer period of time greater than four weeks. Using the same experiment design as for the present study, analyses of foods consumed by the intervention group during the intervention period could be compared to weeks 1 and 2 (baseline) to determine if participants in the intervention group would decrease the intake of other foods, such as snack foods, or eat less at the following meal after consuming the nuts, allowing their total energy intake to remain unchanged.

Limitations

There were limitations associated with this study. First, physical activity of the participants was not recorded for the duration of the study. Therefore, it could not be

determined if changes in physical activity played a role in there being no significant weight gain during the intervention period. Second, no data were collected on the menstrual cycle or on the pre- or post menopausal status in the women . Lanza et al. (1998) demonstrated that fluctuations of plasma α-tocopherol concentrations occur by phase of the menstrual cycle in premenopausal women, specifically with α–tocopherol being lower during menses than during the luteal phase. This current study was unable to determine if phases of the menstrual cycle influenced α-tocopherol concentrations in the female participants.

Conclusions

In conclusion, this is the first study to report the favorable effects of pistachio consumption on dietary intakes of putative cardioprotective nutrients, that is, γ-tocopherol, magnesium, arginine, and copper and serum concentrations of γ-tocopherol and magnesium. Additionally, this study demonstrated a favorable effect on the serum concentration of total cholesterol in participants. Findings from this study may provide a foundation for dietary recommendations for clinicians counseling patients on the incorporation of nuts, specifically pistachios, into a healthy diet. Furthermore, pistachios in the amount of 68 g/ day could be incorporated into dietary strategies designed to reduce the risk of CVD and lung, prostate, and colon cancer without significant weight gain. Previous studies have shown the potentially beneficial effects of pistachios in preventing CVD. As pistachios are one of the best dietary sources of phytosterols, a recommendation for a future study would be to determine the effect of pistachio

consumption on the intake and serum concentration of these compounds, which are cholesterol-lowering and may be protective against certain cancers, such as lung and prostate.

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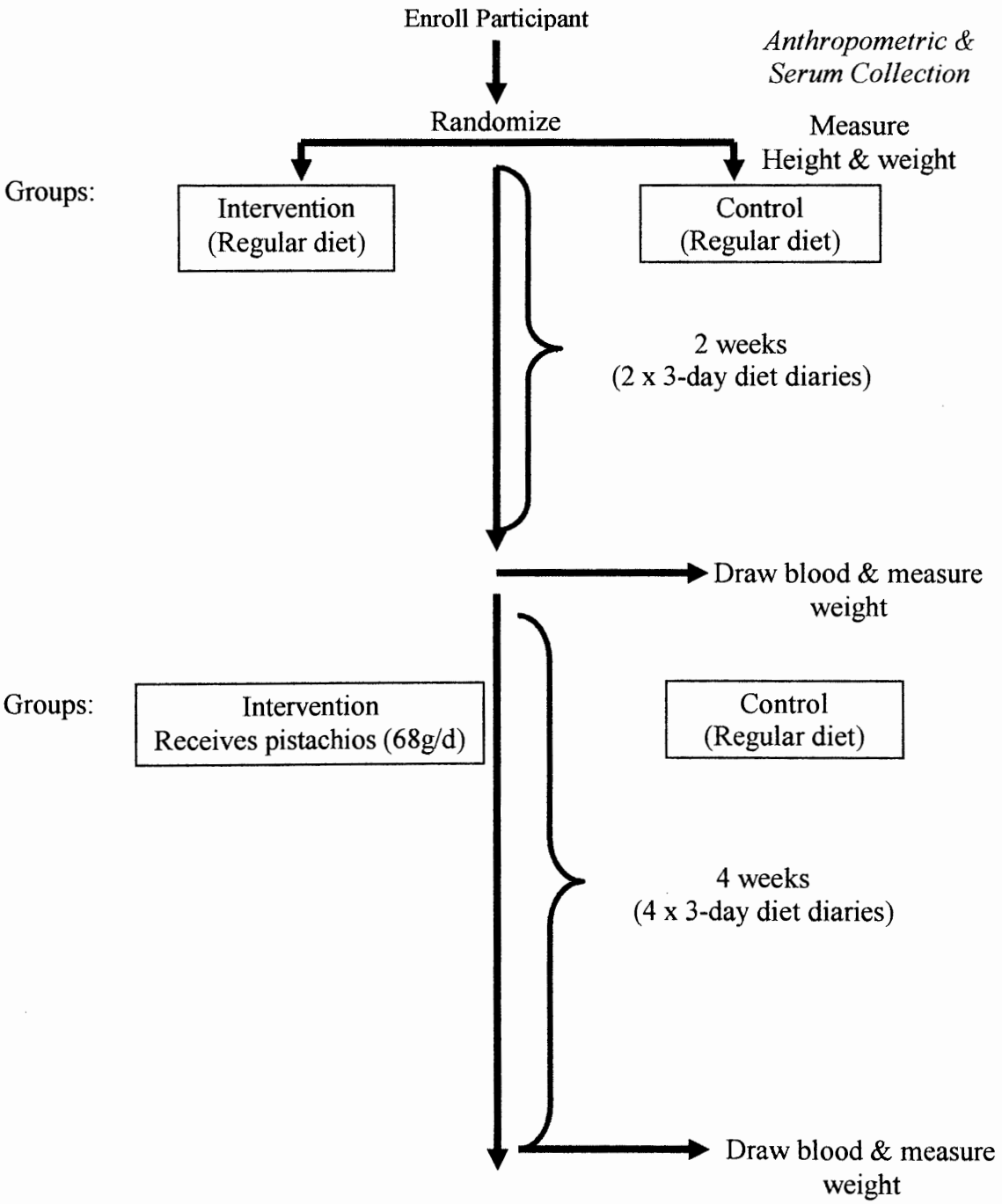
APPENDIX A

Diagram of Study Design

STUDY DESIGN



APPENDIX B

Determination of Serum Cholesterol

Stanbio Cholesterol LiquiColor® Procedure No. 1010

Principle

Free cholesterol is oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. Cholesterol ester is converted to free cholesterol by the action of cholesterol esterase. A quinoneimine chromogen, with absorption maxima at 500 nm, is produced when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase with hydrogen peroxide. The intensity of the final red color is proportional to the total cholesterol concentration.

Procedure

Pipet 40 µL of sample into 800 µL of reagent into a disposable culture tube; pipet 40 µL of standard (200 mg cholesterol/dL) into 800 µL or reagent; pipet 40 µL of normal saline into 800 µL of reagent (blank). Incubate samples, standard, and blank at 37°C for 5 minutes. Transfer to a quartz cuvet and read optical densities at 500 nm within 60 minutes.

Calculation

Values are derived by the following equation

$$\text{Serum Total Cholesterol (mg/dL)} = \frac{Au}{As} \times 200$$

Au = absorbance value of the unknown

As = absorbance value of the standard

200 = concentration of the standard (mg/dL)

Reagent

4-aminophenazone, 0.25 mmol/L; phenol, 25.0 mm/L; peroxidase, 5.0 U/mL; cholesterol esterase, 0.15 U/mL; cholesterol oxidase, 0.1 U/mL; buffers and stabilizers

Source: Stanbio Laboratory, San Antonio, TX

APPENDIX C

Determination of Serum Triglycerides

Stanbio Triglyceride LiquiColor® Procedure No. 2200

Principle

Serum triglycerides are converted to glycerol and free fatty acids by the action of lipase. Glycerol is then phosphorylated by adenosine-5'-triphosphate to produce glycerol-3-phosphate and adenosine-5'-diphosphate in a reaction catalyzed by glycerol kinase. The glycerol-3-phosphate is oxidized by glycerylphosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide, which reacts with 4-aminoantipyrine and 4-chlorophenol under the influence of peroxidase to form quinoneimine. The intensity of the final red color is proportional to the glycerol concentration of triglycerides.

Procedure

Pipet 40 µL of sample into 800 µL of reagent into a disposable culture tube; pipet 40 µL of standard (200 mg triglyceride/dL) into 800 µL or reagent; pipet 40 µL of normal saline into 800 µL of reagent (blank). Incubate samples, standard, and blank at 37°C for 5 minutes. Transfer to a quartz cuvet and read optical densities at 500 nm within 60 minutes.

Calculation

Values are derived by the following equation

$$\text{Serum Triglyceride (mg/dL)} = \frac{Au}{As} \times 200$$

Au = absorbance value of the unknown

As = absorbance value of the standard

200 = concentration of the standard (mg/dL)

Reagent

ATP, 2.0 mM; magnesium salt, 15.0 mM; 4-aminoantipyrine, 0.5mM; 4-chlorophenol, 4mM; glycerophosphate oxidase, 1500U/L, sodium azide, 0.01%, lipase 4000 U/L; glycerol kinase, 400 U/L; peroxidase, 2000 U/L; good's buffer 50mM, pH 6.7±0.1

Source: Stanbio Laboratory, San Antonio, TX

APPENDIX D

Determination of Serum HDL-C

Stanbio HDL Cholesterol Procedure No. 0599

Principle

Both LDLs and VLDLs are precipitated from serum using a magnesium chloride/dextran sulfate reagent.

Procedure

Pipet 50 µL of precipitating reagent into 500 µL of serum in a microfuge tube. Mix well and allow to stand for 5 minutes. Centrifuge for 10 minutes at 1000 x g. Remove clear supernatant containing HDL cholesterol. Determine HDL cholesterol using Stanbio procedure 1010.

Reagent

Magnesium sulfate, 1185 mmol/L; dextran sulfate, 1.1% w/v

Source: Stanbio Laboratory, San Antonio, TX

APPENDIX E

Determination of Serum Magnesium

Stanbio Magnesium LiquiColor® Procedure No. 0130

Principle

Serum magnesium and Xylidyl-Blue-1 combine under alkaline conditions to form a water soluble re-purple chelate with an absorption maximum at 520nm. The intensity of the final color is proportional to the magnesium concentrations.

Procedure

Pipet 10 µL of serum into 1000 µL of reagent; pipet 10 µL of standard (2.0 mEq/L) into 1000 µL of reagent; pipet 10 µL of normal saline into 1000 µL of reagent (blank).

Calculation

Values are derived by the following equation

$$\text{Serum Magnesium (mEq/L)} = \frac{Au}{As} \times 2.0$$

Au = absorbance value of the unknown

As = absorbance value of the standard

2.0 = concentration of the standard (mEq/L)

Reagent

Tris buffer, 0.2 mmol/L; potassium carbonate, 70 mmol/L; xylidyl blue, 0.1 mmol/L; sodium azide, 0.1%

Source: Stanbio Laboratory, San Antonio, Tx