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**THE EFFECTS OF MONOUNSATURATED FATTY ACID-RICH
DIETS AND PROCESSED TOMATOES ON
CARDIOVASCULAR DISEASE RISK FACTORS**

A Dissertation in

Nutrition

by

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ABSTRACT

Monounsaturated fatty acids have been shown to decrease risk of cardiovascular disease (CVD). Two studies were conducted to evaluate the effects of monounsaturated fatty acids on CVD risk factors. A meta-analysis of thirty-one selected controlled clinical trials was performed to compare the effects of moderate-fat versus lower-fat diets on lipids and lipoproteins in healthy subjects, and subjects with diabetes. The results of the study demonstrated that moderate-fat diets decreased triacylglycerol and increased high-density lipoprotein cholesterol (HDL-C) greater than lower-fat diets. However, the moderate-fat diets tested were higher than the amount recommended by the Institute of Medicine of the National Academies. Therefore, a second study was conducted. Twenty-five mildly hypercholesterolemic subjects participated in a 2-period randomized, crossover, controlled feeding study to compare the effects of a macadamia nut-rich (MAC) diet on CVD risk factors (e.g., lipids, lipoproteins, inflammatory markers and oxidative stress) compared to an Average American Diet (AAD). In individuals with baseline CRP levels < 1 mg/L only total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and non-HDL-C were reduced following the consumption of the MAC diet versus the consumption of the AAD. Furthermore, lipid hydroperoxide, a marker of oxidative stress, decreased after the consumption of the AAD and MAC diets compared to baseline in subjects with CRP < 1 mg/L while it did not differ in the subjects with CRP ≥ 1 mg/L. In addition to traditional cardiovascular risk factors, oxidative stress and inflammation are important emerging risk factors. Macadamia nuts contain a range of antioxidants including vitamin E as tocopherols and tocotrienol, the amino acids methionine and cysteine and selenium. A macadamia-nut rich diet is high in

monounsaturated fat and antioxidants. The antioxidants may exert a beneficial effect on oxidative stress and inflammation aside from the monounsaturated fatty acids. Thus, we conducted the tomato study to investigate the effects of antioxidants on multiple CVD risk factors. A randomized, treatment-controlled, 2-center (the Pennsylvania State University and University of California, Davis), 2-arm, parallel design was employed to evaluate the effect of chronic and acute consumption of processed tomatoes on LDL oxidation and paraoxonase (PON1) activity. In the run-in period, oxidized LDL increased at 6 H compared to 0 H after the high fat meal without processed tomatoes but did not change after the high fat meal with processed tomatoes. After six weeks of high processed tomato consumption, PON1 activity increased at 6 H compared to 3 H ($P < 0.05$) after the high fat meal without processed tomatoes. Furthermore, in the high tomato group, participants showed a trend for greater PON1 activity ($P < 0.07$) compared to those in the low tomato group in response to the high fat meal without processed tomatoes (for QR/RR carriers only). In conclusion, consumption of processed tomato products decreased oxidative stress; when consumed with a meal, postprandial LDL oxidation was decreased, and daily consumption of tomatoes for six weeks increased PON1 activity which would decrease LDL oxidation. Collectively, the results of the three studies show that a monounsaturated fatty acid-rich diet, and processed tomato products elicit beneficial effects on traditional and emerging CVD risk factors.

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Chapter 1
INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the United States for both men and women. Numerous people in the United States have one or more types of CVD including high blood pressure, coronary heart disease (CHD), stroke, congenital cardiovascular defects, rheumatic heart disease, and congestive heart failure. Traditional CVD risk factors have been identified. Major traditional risk factors such as dyslipidemia [elevated plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triacylglycerol (TG), and reduced high-density lipoprotein cholesterol (HDL-C)], cigarette smoking, hypertension, overweight and obesity, diabetes mellitus, and physical inactivity can be modified by diet intervention or lifestyle modification. Emerging risk factors also have been identified recently which include elevated high sensitive C-reactive protein (CRP), homocysteine, lipoprotein (a) [Lp(a)], fibrinogen, collagen vascular disease, coronary artery calcification, and endothelial dysfunction (1).

Numerous clinical trials have confirmed that diet intervention is an important tool for modifying CVD risk factors to further reduce CVD risk. In the mid -1960s, Keys et al (2) and Hegsted et al (3) developed blood cholesterol predictive equations and demonstrated that saturated fatty acid (SFA) raised blood TC level while polyunsaturated fatty acid (PUFA) lowered it whereas monounsaturated fatty acid (MUFA) had a neutral effect. Since then, little research has been done regarding the metabolic effect of MUFA until Mattson and Grundy (4) conducted a study using a liquid formula diet to compare the effects of PUFA and MUFA on plasma lipids and lipoproteins in humans. The results indicated that MUFA lowered LDL-C levels similarly to PUFA in normotriglyceridemic patients while MUFA did not reduce HDL-C levels as much as PUFA did (4). The most recent Dietary Guideline for Americans demonstrated that the total fat (TF) should be

kept between 20 to 35 percent of calories with the majority of fats coming from MUFA and PUFA (e.g., fish, nuts, and vegetable oils) (5). Numerous clinical trials have been done to examine the effects of MUFA and PUFA on lipids and lipoproteins. A meta-analysis that was done by Gardner and Kraemer to compare the effects of MUFA and PUFA on lipids and lipoproteins (6). They concluded that MUFA and PUFA did not differ with respect to their effects on LDL-C and HDL-C levels.

Nuts are low in SFA and high in unsaturated fatty acids including MUFA, omega-6 and omega-3 fatty acids which account for their beneficial effects on lipids and lipoproteins. Several major epidemiologic studies have demonstrated beneficial effects of nut consumption on coronary disease risk (7-9). Macadamia nuts, typically eaten as a snack, often with chocolate, are very rich in MUFA. In addition, macadamia nuts also contain antioxidants, vitamins, dietary fiber, minerals, phytochemicals and plant protein. The antioxidants in macadamia nuts include vitamin E as tocopherols and tocotrienol, epicatechin, selenium, methionine and cysteine. Several studies (10-12) have been conducted with macadamia nuts and the results consistently show that lipid profiles were improved after consumption.

Oxidation modification of low-density lipoprotein (LDL) plays an important role in the development of atherosclerotic lesion (13). Prevention of LDL oxidation is thought to reduce CVD risk. High-MUFA diets have been shown to reduce susceptibility of LDL to oxidation (14-16). Furthermore, antioxidants can interfere with LDL oxidation and attenuate the development of atherosclerosis. HDL-associated paraoxonase (PON1) activity has been shown to be inversely associated with CVD risk in humans as well as in mice (17-19). Processed tomato products contain antioxidants such as lycopene, ascorbic

acid, vitamin E, and other carotenoids. Therefore, processed tomato products might attenuate atherosclerosis by reducing LDL oxidation and increasing PON1 activity.

Diet is the cornerstone of intervention strategies for the prevention and treatment of CVD. Dyslipidemia, characterized by elevated TG and low HDL-C increases CVD risk. Low-fat diets worsen dyslipidemia, whereas moderate-fat diets favorably affect TG and HDL-C. We conducted a meta-analysis of 31 selected controlled-feeding studies (n = 1213) and calculated the effects of moderate-fat (MF) versus lower-fat (LF) diets on lipids and lipoproteins in healthy subjects and subjects with diabetes. The MF diets provided 30.2 to 50 % of energy (median 38.4 %) TF while the LF diets provided 18.3 to 30.2 % (median 27.9 %). The results showed that the estimated increase in HDL-C after MF diets compared to LF diets was 2.28 mg/dL. The estimated decreases in TG, TC, and LDL-C were -9.36 mg/dL, - 0.82 mg/dL, and - 0.72 mg/dL, respectively, after MF compared to LF diets. Compared to subjects without diabetes, subjects with type 2 diabetes had a similar increase in HDL-C (2.28 mg/dL) and a greater reduction in TC (- 4.09 mg/dL) and TG (- 24.79 mg/dL) after MF versus LF diets. The MF diets tested were higher than the amount recommended by the National Academies [DRI (Dietary Reference Intakes) (20 - 35 %) and ATP III (Adult Treatment Panel III) (25 -35 %)]. We were interested in assessing the lipid and lipoprotein responses to a MF diet that met the upper range of the TF recommendation. Thus, the second study was conducted.

Twenty-five mildly hypercholesterolemic men (n = 15) and women (n = 10) participated in a 2 - period randomized, crossover, controlled feeding study to compare the effects of a macadamia nut-rich (MAC) diet (33 % TF, 7 % SFA, 18 % MUFA, 5 % PUFA) on lipids, lipoproteins and CRP levels, to an average American Diet (AAD)

(33 % TF, 13 % SFA, 11 % MUFA, 5 % PUFA). There was no main effect of diet on levels of CRP. TC, LDL-C, and non-HDL-C following the MAC diet were decreased compared to baseline in individuals with low baseline CRP levels (< 1 mg/L). In addition, TC and non-HDL-C were reduced compared to the AAD in subjects with low CRP baseline. TC, LDL-C and non-HDL-C were not different across the two test diets in individuals with higher baseline CRP levels (> 1 mg/L). Oxidative stress (measured by lipid hydroperoxide) was decreased after the AAD and MAC diets compared with baseline in all subjects. In addition to traditional cardiovascular risk factors, oxidative stress and inflammation are important emerging risk factors. Macadamia nuts contain a range of antioxidants including vitamin E as tocopherols and tocotrienol, the amino acids methionine and cysteine and selenium. A macadamia-nut rich diet is high in MUFA and antioxidants. The antioxidants may exert a beneficial effect on oxidative stress and inflammation aside from the MUFA. Thus, we proposed the third study to evaluate effects of antioxidants on multiple CVD risk factors.

Many of the observational studies support the hypothesis that foods rich in carotenoids and antioxidants are associated with reduced risk of CVD. However, intervention trials with beta-carotene or vitamin E have not supported this hypothesis. In the third study, we focused on processed tomato products which are rich in lycopene and other carotenoids (vitamin C and polyphenolic compounds including flavonoids). Lycopene is the most potent antioxidant among various common carotenoids. The study employed a randomized, treatment-controlled, 2-arm, parallel design to investigate the effect of chronic intake of processed tomato products as well as acute intake of processed tomatoes (postprandial) on CVD risk factors.

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Chapter 2
LITERATURE REVIEW

2.1 TRADITIONAL AND EMERGING RISK FACTORS FOR CARDIOVASCULAR DISEASE

2.1.1 Lipids and lipoproteins

It has been demonstrated that elevated LDL-C, TC, TG and decreased HDL-C are associated with increased risk of coronary artery disease (CAD) (1, 2). Classification of TC, LDL-C, HDL-C and TG by ATP III is shown in **Table 2-1** (3). The Framingham Study was one of the first U.S. epidemiologic studies to demonstrate that the TC is significantly associated with risk of CHD (2). Anderson et al demonstrated a 9% increase in CVD death for every 10 mg/dL increase in TC (4).

Data from epidemiological studies and clinical trials clearly showed that reduction of LDL-C levels decreases the morbidity and mortality associated with CHD. The Scandinavian Simvastatin Survival Study demonstrated that every 1% decrease in LDL-C is associated with an approximate 1% decrease in CHD-related mortality (5). On the other hand, 1% reduction in CHD risk for every 1% decrease in LDL-C levels in middle-aged men and women was observed in the Framingham Heart Study (6).

ATP III identifies LDL-C as the primary treatment target. It has been suggested that LDL-C is physiologically sufficient as low as 25-60 mg/dL (7). The LDL-C concentration in the newborn infant is approximately 30 mg/dL, indicating that such low levels are safe. Moreover, persons who have extremely low levels of LDL-C throughout life due to familial hypobetalipoproteinemia have documented longevity. Statins are HMG CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors. The effect of decreasing cholesterol levels, as well as reducing the occurrence of CHD mortality and morbidity with statins has been evaluated in several large trials (8-10). The Reversal of

Atherosclerosis with Aggressive Lipid Lowering trial demonstrated that for patients with CHD, intensive lipid-lowering treatment (80 mg of atorvastatin) reduced progression of coronary atherosclerosis compared with moderate lipid-lowering therapy (40 mg of pravastatin) (8). Similarly, the Pravastatin or Atrovastatin Evaluation and Infection Therapy trial demonstrated that more aggressive statin therapy towards LDL-C lowering resulted in a greater protection against major cardiovascular events than a standard regimen (9). In addition, a 17% reduction in risk of nonfatal myocardial infarction in the statin group was seen compared with patients in the usual care group and it was due to a reduction in LDL-C to less than 80 mg/dL (10). These clinical findings suggest that the optimal LDL-C level may be well below the current National Cholesterol Education Program (NCEP) target level. Therefore, persons at high risk for coronary disease due to elevated LDL-C level should be treated by the most aggressive therapy.

A significant portion of patients at risk for CHD present without elevated LDL-C. Moreover, despite effectively treated for elevated LDL-C, many patients continue to develop CHD. Clearly, there are other CVD risk factors need to be identified. Strong epidemiological evidence demonstrated that HDL-C was independently and inversely associated with the risk of CHD (11, 12). Gordon et al analyzed data from four prospective Americans studies and demonstrated that a 1% decrease in HDL-C is associated with a 2-3% increase in CHD risk (13). ATP III defined low HDL-C (<40 mg/dL) as one of several major risk factors used to modify the therapeutic goal for LDL-C (3). Increasing physical activity, quitting smoking, certain medications (e.g., nicotinic acid, statins, and fibrates) can increase HDL-C levels.

Elevated serum TG has long been considered a risk factor by some investigators. Many prospective epidemiological studies have reported a positive relationship between serum TG levels and incidence of CHD. Hokanson and Austin indicated that 75% increase in CVD risk in women and 30% in men were associated with every 89 mg/dL increase in TG (14). In addition, Assmann et al demonstrated that fasting TG, irrespective of LDL-C or HDL-C, was an independent risk factor for CHD events (15). ATP III defined normal TG <200 mg/dL and Borderline-high TG is 200-399 mg/dL (3) (**Table 2-1**).

There is little doubt that decreased HDL-C is a potent risk factor for CHD, independent of other known risk factors while there are more debates with regards to the independent association of TG levels with CHD mortality. However, much evidence suggests that elevated fasting TG level is, in fact, an independent risk factor of CHD. Lifestyle changes such as smoking cessation, weight loss, increased physical activity and pharmacologic treatments (e.g., statins, fibrates, and niacins) have been shown to improve this dyslipidemia. Elevated TG, low HDL-C and small LDL particle size are referred to atherogenic dyslipidemia by ATP III and it is often seen in the metabolic syndrome (3). Although statin can decrease CHD risk by about one-third, it only increased HDL-C levels by only 5% to 15% and decreased TG by 7% to 30% (3). Therefore, statins only moderately modify this dyslipidemia. In the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT), CHD patients had low HDL-C but normal LDL-C (16). After gemfibrozil treatment, patients had a 6% increase in HDL-C, 31% decrease in TG, as well as a 22% reduction in the incidence of major coronary events (16).

It has been proposed that non-HDL-C is a risk marker for CHD. The measure of non-HDL-C incorporates LDL-C, IDL-C, and VLDL-C and therefore displays the cholesterol content of all apolipoprotein B (apo B)-containing lipoproteins. Apo B has been suggested to provide extra information aside from the conventional lipids and lipoproteins and predict CHD events (17-19). Liu et al indicated that there was no association between LDL-C and CHD risk within non-HDL-C levels (**Figure 2-1**) (20). On the contrary, within each level of LDL-C, there was a strong positive relationship between non-HDL-C and the risk for incident CHD (20). They further concluded that non-HDL-C is a stronger predictor of CHD risk than LDL-C (20).

Accumulating evidence from epidemiologic studies has shown beneficial effects of single nutrients as well as dietary patterns on CVD morbidity and mortality by modification of lipids and lipoproteins. SFA, MUFA, PUFA, *trans* fatty acids, dietary cholesterol, dietary sterols and stanols, and soluble fiber are important dietary factors that affect lipids and lipoproteins.

2.1.2 Oxidative stress

2.1.2.1 Free radicals and antioxidant defenses

Free radicals have been defined as atoms or molecules that contain one or more unpaired electrons (21). Common free radicals that generated in human bodies are hydroxyl radical ($\text{OH}\cdot$), superoxide ($\text{O}_2^{\cdot-}$) and nitric oxide ($\text{NO}\cdot$). The chemical reactivity of free radicals is increased because of the unpaired electrons. Free radicals can react with either other free radicals or non-radicals which may result in production of new radicals and then further damage cells or tissue in the body (22). Among damage that is caused by free radicals, lipid peroxidation has been studied most thoroughly.

PUFAs have more than one double bond which weakens the carbon-hydrogen bond at the adjacent carbon atom. When free radical abstracts a hydrogen atom from a methylene group of the fatty acids, a chain reaction is initiated (23). Lipid hydroperoxide is formed and it is unstable. The final breakdown products are aldehyde and polymerization products which can damage cell membrane, protein and other cell components.

Humans have developed antioxidant defenses to protect against free radicals. Cellular antioxidant defenses such as superoxide dismutases (SOD), glutathione peroxidases and catalase enzyme can remove superoxide, hydrogen peroxide (22); they are not consumed during the reactions. Vitamin E, beta-carotene, coenzyme Q, membrane structural organization are important membrane antioxidants and can break lipid peroxide chain reactions, quench singlet oxygen and maintain membrane integrity (23). In addition, vitamin E, beta-carotene, lycopene, along with many other proteins act as extracellular antioxidants and protect the extracellular fluids from the radicals. Moreover, Uric acid, vitamin E, vitamin C, beta-carotene, carotenoids are the major components of plasma antioxidants (24).

Despite large amount of antioxidant defenses that are present in the body to protect cells and tissue from injury, the formation of free radicals and the defenses may not be balanced which results in oxidative stress (25).

2.1.2.2 Oxidized LDL and atherosclerosis

Human low-density lipoprotein (LDL) is a spherical particle with a density of 1.019-1.063 g/dL and a diameter of 22-28 nm. Cholesterol esters and triglycerides form the central lipophilic core of a LDL and it is surrounded by a monolayer of phospholipids. On the surface of the LDL particle is the large protein, apo B (26).

Among the different lipid classes in LDL, PUFA accounts for about half of which the main one is linoleic acid with small amount of arachidonic acid and docosahexaenoic acid also present. The major antioxidant of LDL is α -tocopherol while gamma-tocopherol, carotenoids, oxycarotenoids, and ubiquinol-10 are the minor ones. These antioxidants and PUFA are the major defense that protects LDL against free radical attack and oxidation (26).

Monocyte-derived macrophages, the precursor of cholesterol-laden foam cells in atherosclerotic lesions, can not take up native LDL to cause lipid loading (27). Furthermore, macrophages do not accumulate cholesterol in vitro when incubated with a very high concentration of native LDL (28). This leads to the hypothesis that native LDL undergoes some form of modification and then can be taken up by the macrophages which cause the formation of a foam cell. It has been reported that acetylated LDL can cause cholesterol accumulation in macrophages (28). In addition, oxidative modification of LDL is taken up by macrophages quickly and induces cellular cholesterol accumulation (29, 30). This evidence demonstrated that LDL modification is the key step in the development of atherosclerotic lesions and accumulative evidence had shown that the atherogenicity is enhanced as the LDL undergoes oxidative modification (31). The modified LDL is taken up by the macrophages via a receptor, called scavenger receptor A, which is not down-regulated by intracellular cholesterol content (32). Therefore, macrophages take up modified LDL continuously and eventually cause the formation of foam cell.

LDL can be oxidized by transition metal ions and cultured cells such as macrophages, smooth muscle cells and endothelial cells in vitro. In general, LDL

oxidation involves free radical attack on the cholesterol, phospholipids, fatty acids and apo B-100. There are three consecutive phases (33, 34). The first is a lag-phase where the antioxidant (e.g. alpha-tocopherol, beta-carotene, lycopene, and other carotenoids) in the LDL is depleted while oxidation begins on the LDL surface and diene absorption increases. The duration of this phase depends on the amount of antioxidants within the LDL particle. During the propagation phase, PUFA in LDL are rapidly oxidized and degraded in a lipid peroxidation process and various reactive products such as lipid hydroperoxides are formed (35). Finally, the lipid hydroperoxides are converted to smaller fragments (e.g., malondialdehyde, 4-hydroxynonenal, etc) which then bind covalently to apo B (36). The modified apo B then can be recognized by the scavenger receptor so the oxidized LDL is taken up by the macrophage and the foam cell is formed as cholesterol accumulates within the macrophage. Accumulation of foam cells then leads to the generation of the atherosclerotic lesion.

Minimally modified LDL is indistinguishable from native LDL and is not recognized by the scavenger receptor. However, it causes endothelial cells to secrete high level of chemotactic factor leading to increased monocyte adhesion (37) (**Figure 2-2**). Oxidized LDL is cytotoxic to endothelial and other cells and could damage arterial cells directly (38). Products of oxidized LDL are involved in monocyte and T-cell recruitment directly or by stimulating the secretion of chemokines and endothelial cell adhesion molecules (37, 39). Recruitment of monocytes eventually leads to their differentiation into macrophages and foam cell formation. It is also hypothesized that oxidized LDL may alter gene expression in arterial wall cells leading to cytokine or

growth factor stimulation. The mechanism that oxidized LDL is atherogenic is very complex and has been summarized by Witztum and Steinberg (**Table 2-2**) (40).

The hypothesis that links antioxidant vitamins and CVD is based in part on the oxidative modification of LDL, and its role in the development and progression of atherosclerosis (40, 41). Antioxidant vitamins have been shown to inhibit LDL oxidation (42, 43), decrease reactive oxygen species (44-46), and decrease LDL oxidative susceptibility (47). Epidemiologic and observational studies (48) have reported that diets rich in antioxidants (e.g., high amount of fruits and vegetables), as well as specific antioxidants (i.e. alpha-tocopherol, vitamin C, and beta-carotene), may have a beneficial effect on CVD risk. However, the data from clinical trials (both primary and secondary prevention studies) fail to support a beneficial effect of antioxidant vitamin supplements on CVD events as reviewed in (49).

2.1.3 C-reactive protein

Accumulating evidence has shown that atherosclerosis is as an inflammatory disease. Individuals with high risk of CVD had higher acute phase response markers, among which CRP has been studied more thoroughly (50). CRP is the prototype acute phase protein in humans with five identical polypeptides (each has a molecular of 23 kDa) (51). It is synthesized by the liver in response to inflammatory cytokines (52). Yasojima et al showed that CRP could be detected in human atherosclerotic plaques which indicated that it is synthesized in the lesion (53). Furthermore, human coronary artery smooth muscle cells have been shown to produce CRP in response to inflammatory cytokines (54). In addition, CRP can be synthesized by human adipocytes and modulated by selected pharmacologic intervention (55). Vascular and extravascular sources of

inflammation stimulate pro-inflammatory cytokines [e.g., Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF- α)] which induce the production of Interleukin-6 (IL-6). IL-6 stimulates acute-phase reactants production including CRP via increasing the specific hepatic genes (**Figure 2-3**) (56).

In adults, CRP levels were categorized to <1 mg/L (low risk), 1-3 mg/L (average risk), and >3.0 mg/L (high risk) with regard to their future cardiovascular events (57). Elevated baseline CRP levels have been linked to future risk of coronary events (50, 58). Similarly, elevated CRP levels had a role in predicting prognosis and recurrent events in patients with stroke and peripheral arterial disease (59, 60). In the Womens' Health Study, CRP was a better CVD predictor than soluble vascular cell adhesion molecule-1 (sVCAM-1), Lp(a), homocysteine, even LDL-C (**Figure 2-4**) (61). Ridker et al conducted analyses to estimate the relative risk of CVD associated with lipids and CRP (62, 63). The top quintiles of CRP and TC: HDL-C in both men and women corresponded to a very-high-risk group compared with those that had both parameters in the lowest quintile (**Figure 2-5**) (62). Moreover, CRP presents prognostic information at all levels of LDL-C (64). The Women's Health Study confirmed the interactive effect between serum CRP and TC: HDL-C ratio on CVD risk assessment (65). Ridker et al concluded that after adjustment for age, blood pressure, smoking, diabetes, and obesity, high-sensitivity CRP added supplementary prognostic information than that carried by lipid measures only (65).

Statins, exercise, weight loss, blood pressure control, quitting smoking, and blood cholesterol lowering diets have been reported to lower CRP levels. Dietary modifications of CRP have been studied by some investigators. A mediterranean diet is high in fruits

and vegetables and adherence to it has been associated with decreased CRP levels (66). In a randomized trial, participants not only lost 2.1-3.3 kg but also had decreased CRP levels after four weight loss diets (Atkins, Zone, Weight Watchers, and Ornish) for a year (67). It was further showed that the reduced CRP levels were significantly associated with weight loss (67). Diet with a low dietary glycemic load or diets high in soluble fibers and n-3 fatty acids are associated with reduced CRP levels (68-70). Several studies have demonstrated that baseline CRP levels may influence lipid and lipoprotein responses to a dietary intervention (71-73). However, how CRP interferes with lipid and lipoprotein responses to the diet remains unclear.

2.2 THE EFFECT OF MONOUNSATURATED FATTY ACIDS ON CARDIOVASCULAR DISEASE RISK

2.2.1 The effect of monounsaturated fatty acids on lipids and lipoproteins

Dietary MUFA is present in many fat-containing foods, especially in vegetable oils and nuts (**Table 2-3**) (74). The recommended intake of MUFA in therapeutic lifestyle changes (TLC) diet is up to 20% of the total calories (3). Mediterranean diets are characterized by a low intake of SFA and a relatively high intake of MUFA (75). The Seven Countries Study followed 15 cohorts in southern Europe and northern Europe for 5-15 years (76). The results showed that all-cause death rate was negatively associated with MUFA intake and the lower all-cause and CHD death rates were found in cohorts in which olive oil was the primary source of dietary fat (76).

2.2.1.1 Monounsaturated fatty acids versus saturated fatty acids

Population recommendations aimed at reducing the incidence of CHD have previously emphasized the need for reduction in SFA intakes. SFA calories are typically replaced either with dietary carbohydrates or unsaturated fatty acids which results in a low-fat, high-carbohydrate diet or a moderate-fat (higher in MUFA or PUFA) diet. National Cholesterol Education Program/American Heart Association Step I or step II diets are usually recommended for lowering blood cholesterol concentrations (3). Step I and step II diets have 8-10% and <7% of energy from SFA, 300 mg/d and 200 mg/d cholesterol, and <30% of energy from TF, respectively. A Step I diet lowers total cholesterol and LDL-C by about 5-7% and a Step II diet lowers TC and LDL-C by additional 3-7%. These low-fat, high-carbohydrate diets have beneficial effects on TC and LDL-C. However, progress towards achieving these population targets through reductions in TF has been slow and may partly reflect consumer resistance to low-fat products. Furthermore, although low-fat high-carbohydrate diets have been extensively recommended, their overall benefit remains controversial because of their potentially deleterious triglyceride-raising effect and their negative effect on LDL peak particle diameter and HDL-C levels. In addition, a low-fat, high-carbohydrate diet also can cause persistent deterioration in glycemic control, exaggerate hyperinsulinemia in type 2 diabetes. In contrast to low-fat, high-carbohydrate, diets rich in MUFA, in which SFA energy is replaced by MUFA, have been reported to reduce total plasma and LDL-C levels without raising plasma TG or lowering HDL-C concentrations. Keys et al reported that the serum TC decreased by 2.7 mg/dL for every 1% of total dietary energy where oleic acid is substituted for SFA (77). In a prospective study, 80,082 relatively healthy

women were followed for 14 years (78). It was found that compared with equivalent energy from carbohydrates, the relative risk of CHD for a 5% increment in energy from MUFA was 0.81 [95% confidence interval (CI), 0.65-1.00; $p=0.05$] (78).

Controlled clinical studies have been conducted to investigate the effects of high-MUFA diet on lipid and lipoproteins in the mid-1980s. Grundy et al (79) conducted a controlled feeding study on 11 hypercholesteremia patients with a mean plasma TC level of 251 mg/dL for three dietary periods, each lasting four weeks. The High-Sat (diet rich in SFA) and High-Mono (diet rich in MUFA) diets contained 40% of their total calories as TF and 43% as carbohydrate; the Low-Fat diet (diet low in fat) had 20% fat and 63% carbohydrate. Both the High-Mono and Low-Fat diets lowered plasma TC (by 13% and 8%, respectively) and LDL-C (by 21% and 15%, respectively) when compared with the High-Sat diet. In addition, the Low-Fat diet raised TG levels and significantly reduced plasma HDL-C while the High-Mono diet had no effect on levels of TG or HDL-C when compared with the High-Sat diet. This indicated that a diet rich in MUFA was at least as effective in lowering TC and LDL-C as a diet low in fat and high in carbohydrate and a diet rich in MUFA had no adverse effect on HDL-C and TG. Forty-eight healthy men and women were recruited and assigned to either a high-carbohydrate, high fiber diet (6.7% of SFA, 9.3% of MUFA, 22.1% of TF) or an olive-oil-rich diet (9.8% of SFA, 24.0% of MUFA, 40.6% of TF) for 36 days (80). Similar to the study conducted by Grundy et al (79), there were similar reduction in TC and LDL-C after both diets. Moreover, the olive-oil-rich diet did not cause HDL-C to decrease or TG to increase as in the high-carbohydrate, high fiber diet. Numerous controlled feeding studies have been performed to further examine the effects of high-MUFA diet on lipid profile (79-108).

More recently, Williams et al. (109) performed two randomized crossover studies on 30 healthy middle-aged men and 23 young men with a family history of CHD. In both studies subjects were randomized to receive either a high-MUFA diet (38% energy as TF, with 18% energy as MUFA and 10% as SFA) or the control diet (13% energy as MUFA and 16% as SFA) first for 8 weeks. Following a 4-6 weeks washout period they were crossed over to the opposing diets for a further period of 8 weeks. In middle-aged men, there was a mean reduction in LDL-C of 11% on the high-MUFA diet with no change on the control diet. A 7.8% decrease of LDL-C concentration after the high-MUFA diet and a 6.2 % increase after the control diet were reported in the 23 young men. These results demonstrated that high-MUFA diets (partial replacement of SFA) can achieve significant reductions in TC and LDL-C concentrations with TF and energy intakes remaining unchanged. In another randomized, double-blind, crossover designed study (93), 22 subjects consumed five different diets: AAD [34% fat; 16% SFA, 11% MUFA], Step II (25% TF; 7% SFA, 12% MUFA), OO (olive oil) (34% fat; 7% SFA, 21% MUFA), PO (peanut oil) (34% fat; 7% SFA, 17% MUFA), and PPB (peanut and peanut butter) (36% fat; 8% SFA, 18% MUFA). The last three diets were defined as high-MUFA diet. The high-MUFA diets lowered TC by 10% and LDL-C by 14%. In addition, TG concentrations were 13% lower after the high-MUFA diets and were 11% higher after the step II diet. HDL-C was maintained the same with the high-MUFA diets while the Step II diet lowered it by 4 % compared with the AAD. The investigators further calculated the CVD risk reduction and reported that CVD risk were reduced by 25%, 16%, and 21%, respectively, after olive oil (OO), peanut oil (PO), and peanut and peanut butter

(PPB) diets (**Figure 2-6**). Therefore, a diet that is high in MUFA may have more favorable effects on the lipid profile than a low-fat diet.

2.2.1.2 Monounsaturated fatty acids versus polyunsaturated fatty acids (n-6)

Early studies demonstrated that serum cholesterol concentrations were reduced significantly as PUFAs was substitute for SFA (110-112). Keys et al. (113, 114) and Hegsted et al. (115, 116) developed predictive equations to examine the effects of fatty acids on the serum cholesterol level. One of the equations is expressed as following:

$$\Delta \text{TC} = 2.32 \text{ SFA} + 0.32 \text{ MUFA} - 1.46 \text{ PUFA} + 6.51 \text{ cholesterol} + 0.83 \text{ (116)}$$

The equations suggested that SFA raise serum cholesterol whereas MUFA are relatively neutral and PUFA lower plasma cholesterol compared to complex carbohydrates. In a meta-analysis, Mensink et al (117) performed multiple regression analysis using isocaloric exchanges of SFA, MUFA, and PUFA versus carbohydrates as the independent variables. The results indicated that all fatty acids elevated HDL-C when substituted for carbohydrates and MUFA increased HDL-C more than PUFA while less than SFA. The coefficient for PUFA was negative and significantly different from zero, but that for MUFA was negative but not significant which suggested that MUFA might have a neutral or a lowering effect on LD-C. With regards to TG, all these three fatty acids had negative coefficients which indicated their TG lowering effects. Therefore, a high-MUFA diet may not be as beneficial as a high-PUFA diet in terms of cholesterol-lowering effect. However, Mattson and Grundy reported that in patients with normal TG, MUFA was as effective as PUFA with regards to lowering LDL-C and PUFA decreased HDL-C more than MUFA. Furthermore, a meta-analysis conducted by Gardner and

Kraemer (118) demonstrated that diet high in MUFA elicited similar effects as diet high in PUFA on LDL-C and HDL-C levels.

In a study that was conducted on 31 women and 27 men, subjects were placed on a diet rich in saturated fat (19.3%) for 17 days (119). For the next 36 days, they received a diet with the same TF content, but enriched with olive oil and sunflower oil (MUFA diet) or with sunflower oil alone (PUFA diet). The serum LDL-C level decreased by 17.9 % in those on the MUFA diet and by 12.9 % in those on the PUFA diet. In men, the HDL cholesterol level fell slightly but not significantly with both diets. In women, the HDL cholesterol level did not change with either. The results demonstrated that a diet rich in MUFA was as beneficial as a diet rich in (n-6) PUFA in lowering LDL cholesterol. In two randomized crossover trials 71 (Trial I, n=29; and trial II, n=42) healthy free-living nutrition students at the University of Otago were asked to follow for two and half weeks a diet high in saturated fat yet with a total fat content that conformed to nutrition recommendations (30-33% energy) (120). During the two and half week comparison diet, SFA rich foods were replaced with foods rich in n-6 PUFA (trial I) whereas in trial II the replacement foods were rich in MUFA. By replacing SFA with MUFA (trial II) TC decreased by 12 %, LDL-C by 15 %, and HDL-C by 4 % (this is less than the reduction of HDL-C by high-PUFA diet which is 14%), respectively. The result suggests that the high-MUFA diet was effective in lowering TC and LDL-C and had a better effect on HDL-C than the high-PUFA diet.

2.2.2 The effect of monounsaturated fatty acid on oxidative stress

Accumulated evidence suggests that LDL may undergo oxidative modification which then initiates the development of atherosclerosis (34). Some studies demonstrated

that MUFA reduces susceptibility of LDL to oxidative modification. The initial step in the oxidation is the peroxidation of PUFAs so decreasing the concentration of PUFA should lead to a reduction on the susceptibility of LDL to oxidation. Therefore, there is a possibility that diets enriched in oleate might result in LDL that is less susceptible to oxidative modification. In one study (121) the investigators compared LDL between subjects consuming oleate-enriched diet and subjects consuming linoleate-enriched diet [contained significantly more oleate (28.7 % vs. 11.5 %) and less linoleate (31.9 % versus 50.9 %)]. The results showed that generation of conjugated dienes was significantly lower in the LDL from the oleate group. Furthermore, after incubation with endothelial cells, LDL from the oleate group underwent less degradation by macrophages. An oleate-rich diet is reported to lower or not effect LDL cholesterol concentrations, and will decrease the susceptibility of LDL to oxidative modification. The investigators tested the effect of olive oil supplementation (50 g/day) to the diet of 10 healthy male subjects, during a 2-week period, on macrophage uptake of their LDL and on the propensity of their LDL to oxidation (with copper ions) (122). Olive oil supplementation resulted in LDL enrichment with oleic acid (C18:1) and sitosterol. Macrophage uptake of LDL was studied by analysis of cellular cholesterol content and by analysis of the macrophage cholesterol esterification rates. The LDL resistance to oxidation was shown by a reduction in its peroxide, malondialdehyde and conjugated diene content by 73, 28 and 32%, respectively. LDL incubation with oleic acid with copper ions demonstrated a dose-dependent inhibition of lipoprotein oxidation by up to 72% as opposed to linoleic and arachidonic acids (50 microM) which increased LDL oxidation by 22 and 72%, respectively.

2.3 THE EFFECT OF NUTS ON CARDIOVASCULAR DISEASE RISK

Nuts are a nutrient dense food with high amount of protein and fat, especially MUFA and PUFA. In addition, nuts also contain many other nutrients such as plant protein, fiber, plant sterols, vitamins (e.g., folic acid, niacin, vitamin E, vitamin B6) minerals (e.g., copper and magnesium), and other phytochemical compounds (e.g., flavonoids and phenolic compounds) (123). Hu and Willett (124) demonstrated that nuts are a component of optimal diets for the prevention of CHD. Results from epidemiological studies have supported the positive association between frequent nut consumption and reduced risks of CHD. The Adventist Health Study (AHS) demonstrated that compared with the group eating nuts less than one serving/week, those who consume more than four servings/week had a 48% reduction in definite fatal CHD events and 51% reduction in definite myocardial infarction (MI) (125). In Nurses' Health Study, 86016 relatively healthy women were followed for 14 years (126). Women who consumed nuts more than five oz/week had a 35% reduction in total CHD compared with those who rarely ate nuts after adjustment for other risk factors. Albert et al (127) conducted the Physicians' Health Study among 21454 male participants. After 17 years follow-up, there were 201 sudden deaths. Subjects who consumed nuts more than two times per week had a 47% reduction in sudden death when compared to those who were rarely or never consumed nuts.

Numerous clinical trials have been conducted to look at the lipid-lowering effects of nuts including almonds (128-133), walnuts (129, 134-138), pecans (97, 139), hazelnuts (140), and macadamia nuts (141). The studies demonstrated consistent results with regard to LDL-C reduction (**Figure 2-7**) (142). Accumulating evidence has demonstrated

that nuts have beneficial effects on lipids and lipoproteins due to a favorable fatty acid profile (143). In addition, other dietary components in nuts could also contribute beneficial effects on CVD lipid and lipoprotein risk factors.

2.3.1 Vitamins

2.3.1.1 Vitamin E

The Cambridge Heart Antioxidant Study (CHAOS) (144) reported that vitamin E supplementation reduced risk by 77% (95% CI, -89 to -53%; $p=0.005$) and 47% (95% CI, -66 to -17%; $p=0.005$) for MI and all cardiovascular events (including nonfatal MI and cardiovascular death) in patients with established coronary artery disease. Similarly, in the SPACE Study, the vitamin E supplementation in 196 haemodialysis patients with history of CVD resulted in a 64% significant reduction (RR, 0.46; 95% CI, 0.27-0.78; $p=0.014$) in the composite CVD endpoint (fatal and nonfatal MI, ischaemic stroke, peripheral vascular disease, and unstable angina). In the Alpha Tocopherol Beta Carotene (ATBC) Cancer Prevention Trial (145), of 1862 men with a previous MI, there was a significant reduction in nonfatal MI (38%; 95% CI, -4 to -59%) although no effects were shown for fatal coronary end points. Likewise, a subsequent report (146) which examined the effect of antioxidants on the incidence of angina pectoris reported a 9% (95% CI, -1 to -17%; $p=0.04$) reduction in the vitamin E supplement group. The Heart Protection Study (HPS) (147) and the HDL-Atherosclerosis Treatment Study (HATS) (148) used three antioxidant vitamins (alpha-tocopherol, vitamin C, and beta-carotene) in combination to assess effects on CVD. There was no effect on all cause mortality [Relative Risk (RR), 1.04; 95% confidence interval (CI), 0.97-1.12] and CVD mortality (RR, 1.05; 95% CI, 0.95-1.15) in the supplement group (600 mg/d of vitamin E, 250

mg/d of vitamin C, and 20 mg/d of beta-carotene) in the HPS study (147). In the HATS study, the average stenosis progression in the antioxidant alone group (800 IU/d RRR-alpha-tocopherol, 1000 mg/d of vitamin C, 25 mg/d of beta-carotene, and 100 µg/d selenium) and the placebo group were not significantly different (1.8% vs. 3.9%, p=0.16) (148).

2.3.1.2 Folate

In 1975, McCully and Wilson (149) introduced the homocysteine theory to explain, in part, the pathogenesis of arteriosclerosis. Many subsequent studies have reported that elevated homocysteine levels are associated with an increased risk of coronary atherosclerosis, cerebrovascular disease, peripheral vascular disease, and thrombosis (150, 151).

A marked inverse relationship between folate status and plasma homocysteine concentration was reported in the Hordaland Homocysteine study (152). A meta-analysis (153) of 12 randomized controlled trials showed that 0.5-5.0 mg/d supplements of folic acid significantly reduced homocysteine by 25% (95% CI, 23-28%); adding 0.5 mg/d vitamin B12 resulted in a further 7% (3-10%) reduction in blood homocysteine. There was no difference in homocysteine lowering within the range of 0.5-5.0 mg/day folic acid. Patients with CVD may require more folate than is recommended for the general population to achieve normal homocysteine levels. In a randomized controlled trial conducted by Wald et al. (154), 151 patients with ischemic heart disease were randomized to 1 of 5 doses of folic acid (0.2, 0.4, 0.6, 0.8, and 1.0 mg/d) for 3 months. In this study, 0.8 mg/d of folic acid was needed to maximally reduce serum homocysteine.

To put this in context, the Recommended Dietary Allowance (RDA) for folate for healthy men and women 19 years of age and older is 400 µg/day.

Based on a case-cohort study of 36,000 Dutch adults who did not have cardiovascular disease at baseline (with a mean follow-up of 10.3 years) (155), the RR for the highest tertile of plasma folate concentration (9.5 nmol/l in women) versus the lowest tertile (6.1 nmol/l in women) was 0.22 (95% CI, 0.06-0.87) for fatal CHD. This study demonstrated the inverse association between a higher plasma folate concentration and the risk of CHD mortality in women but not men. He et al. (156) found that subjects in highest quintile of folate intake (936 µg/d) had a lower risk of ischemic stroke than those in the lowest quintile (237 µg/d) (RR, 0.68; 95% CI, 0.50-0.92; p=0.03). However, the same association was not found for folate intake and hemorrhagic stroke.

Liem et al. (157) studied the effect of folic acid supplementation in a secondary prevention study of patients with existing CAD. Subjects (n=300) were randomly assigned to 0.5 mg/d folic acid for a 24 months follow-up. The relative risk for all-cause mortality and a composite of vascular events was 1.05 (95% CI: 0.63-1.75) for the folic acid supplementation group which indicated no treatment effect on recurrent events.

2.3.1.3 Vitamin B6

The question has been raised about the role that vitamin B₆ has on homocysteine levels relative to that of folic acid and vitamin B₁₂. In a meta-analysis (the Homocysteine Lowering Trialists' Collaboration), vitamin B₆ did not affect homocysteine levels perhaps because the effect is masked by the greater effects of folic acid and vitamin B₁₂ (153). In a recent randomized trial, healthy elderly subjects were given 1.6 mg/d of vitamin B₆ in conjunction with a folic acid supplement (400 µg/d) for 6 weeks. Supplementation

with vitamin B₆ resulted in an additional 7.5% reduction (p=0.008) in homocysteine levels beyond that observed for the folic acid supplement (which reduced homocysteine levels by 19.6%; p<0.001) (158). It also has been reported that a low level (<20 nmol/L) of vitamin B₆ [Odds Ratio (OR), 4.6; 95% CI, 1.4 to 15.1; P<0.001] rather than elevated homocysteine (OR, 0.92; 95% CI, 0.4 to 2.1) was strongly associated with the risk of stroke and transient ischemic attack (TIA) in folate-repleted population (159). Kelly et al. (160) found a strong inverse relationship between blood levels of vitamin B₆ status and increased quartiles of CRP distribution both in case (new ischemic stroke) and control cohorts (p=0.001 for each cohort).

2.3.1.4 Niacin

Numerous studies have shown that niacin (nicotinic acid) in gram doses increases HDL-C and decreases TG, LDL-C, and Lp(a) (3, 161-166). Studies in both healthy subjects with hyperlipidemia (163-166) and patients with type 2 diabetes with dyslipidemia (167) have shown that 500 to 3000 mg/d of extended-release niacin is generally well tolerated and has a dose-related effect on the lipid profile [LDL-C was significantly decreased 6% and 14% while HDL-C was increased 17% and 23% by 1000 mg/d and 2000 mg/d, respectively (165); TG was decreased more as the dosage of extended release-niacin increased: 5% at 500 mg/d; 11% at 1000 mg/d, and 44% at 3000 mg/d (163). Greater lipid effects have been noted in some studies. For example, a 21% reduction in LDL-C levels has been reported by Goldberg et al. (163) and other investigators (164-166) with niacin alone. Goldberg et al. (163) also reported that 1-3 g/d of extended release-niacin can increase HDL-C levels by 30%. Capuzzi et al. (166) reported that niacin can decrease Lp(a) by as much as 40%. Backes and Gibson (168)

demonstrated that niacin decreases small-dense LDL-C, especially in patients with mixed dyslipidemia or hypertriglyceridemia. The mechanism by which niacin exerts its effects on triglyceride levels involves an inhibition of hepatic production of very-low-density lipoprotein (VLDL) (169). The increase in HDL-C is due to a decrease in HDL degradation (170).

2.3.2 Minerals

2.3.2.1 Calcium

Meta-analyses of epidemiologic studies have shown that an increase in dietary calcium is associated with a blood pressure (BP) lowering response (171, 172). Systolic blood pressure (SBP) decreased by 0.34 mmHg and 0.15 mmHg for each 100 mg per day increase in calcium intake for men and women, respectively. For diastolic blood pressure (DBP), the decrease was 0.22 mmHg and 0.051 mmHg for men and women, respectively.

In a meta-analysis on 42 studies (9 dietary and 33 supplement studies), there was a significant reduction in SBP by 1.44 mmHg and in DBP by 0.84 mmHg with a > 1g/d calcium intake after 2 weeks of intervention (173). In a comparison of dietary versus supplemental calcium sources, SBP tended to decrease by 2.10 mmHg and 1.09 mmHg (P=0.14), respectively; and DBP by 1.09 mmHg and 0.87 mmHg (P=0.67), respectively. Collectively, the data show that dietary and supplemental sources of calcium have comparable hypotensive effects.

Data from a cross-sectional study (174) conducted in Spain also supported the combination use of minerals for BP lowering. The results showed that the BP change was related to calcium and sodium intake and to the ratio of sodium to potassium. Controlling sodium intake (below 2400 mg/d) in combination with a calcium intake of

more than 800 mg/d, reduced BP by 44% in hypertensive patients on hypotensive drug therapy. In addition, BP was decreased by 30% and 52%, with moderate reduction sodium intake (< 2,400 mg/day) in normotensive and non-medicated hypertensive subjects.

2.3.2.2 Magnesium

In the Atherosclerosis Risk in Communities (ARIC) Study (175), Ma et al assessed the association between dietary/serum magnesium levels and cardiac risk among different population groups. SBP were significantly inversely related to serum magnesium (except for African American women; correlation coefficients were -0.06 to -0.08 , $P \leq 0.01$). However, no significant associations were observed between serum magnesium and DBP levels. Dietary magnesium was inversely associated with both SBP and DBP among women (correlation coefficients were -0.05 to -0.07 , $P \leq 0.01$). Dietary magnesium was only significantly inversely related with DBP among African American men (correlation coefficient was -0.09 , $P \leq 0.01$). In another analysis of data from the ARIC Study (176), blood pressure was inversely related to serum magnesium but not dietary magnesium. Compared with subjects in the lowest quartile of serum magnesium (≤ 1.5 meq/L), women in the highest quartile (≥ 1.8 meq/L) had an OR of incident hypertension of 0.70 (P for trend 0.01). No inverse association was found between incident hypertension and either serum or dietary magnesium levels among men. In NHANES (National Health and Nutrition Examination Survey) III (177), there was no association between magnesium intake and BP.

In a review conducted by Jee et al (178) of 20 clinical studies, increasing magnesium intake had a very modest BP-lowering effect among hypertensive and

normotensive subjects. The median magnesium dose was 15.4 mmol/d with a study intervention duration of 8.5 weeks. Magnesium supplementation resulted in a small reduction in BP. The pooled net estimates of BP change were -0.6 mmHg (P = 0.051) for SBP and -0.8 mmHg (P = 0.142) for DBP. However, there was an apparent dose-dependent effect of magnesium when limiting the analysis to data from double blind studies. For each 10-mmol/d magnesium increase, SBP decreased significantly (-4.3 mmHg, P<0.001) however, DBP did not change significantly (-2.3 mmHg, P = 0.09).

Dietary magnesium is positively related to glucose homeostasis and improved insulin sensitivity. In the Women's Health Study (179), a significant inverse association was found between magnesium intake and risk of type 2 diabetes, independent of age and Body Mass Index (BMI). This inverse relationship also was reported in an analysis of data from the Health Professionals' Follow-up Study and the Nurses' Health Study (180). In the ARIC Study (181), the adjusted relative odds of incident type 2 diabetes rose progressively across the lower serum magnesium categories. In another analysis of data from the Nurses' Health Study (182), a higher magnesium intake was associated with lower fasting insulin concentrations among women without diabetes.

2.3.2.3 Potassium

In an analysis of randomized controlled trials (RCTs), potassium intake was found to be inversely related with BP (183). Potassium supplementation (31 trials with dose of at least 60 mmol/d and a median intervention of 5 weeks) was associated with a significant reduction in SBP (-3.11 mmHg) and DBP (-1.97 mmHg), respectively. The effects of treatment were enhanced in studies when participants were concurrently exposed to a high sodium intake.

When calcium intake was increased in combination with magnesium and potassium in conjunction with a reduction in sodium intake, the hypotensive effect was more pronounced. In the Dietary Approaches to Stop Hypertension Study (DASH) (184), participants were allocated to one of three treatments: a typical American diet, a diet rich in fruits and vegetables and a combination diet (i.e., “DASH” rich in fruit, vegetables and low-fat dairy products, characterized by higher amounts of calcium, magnesium, potassium and fiber). Compared to the control diet, the DASH diet and the fruits and vegetable diet reduced SBP by 5.5 mmHg and 2.8 mmHg, respectively; and DBP by 3.0 mmHg and 1.1 mmHg, respectively. The DASH diet reduced SBP in the hypertensive participants by 11.4 mmHg and DBP by 5.5 mmHg. When comparing the DASH diet to the fruits-vegetables diet in hypertensive subjects, the DASH diet further decreased SBP by 4.1 mmHg and DBP by 2.6 mmHg.

2.3.3 Dietary Fiber

Several large cohort studies (185-192) have shown that dietary fiber intake is associated with a reduction in CHD risk. These epidemiologic studies were conducted typically with thousands of participants and followed up for several years. The end points included CHD, CVD, MI, coronary death and others. The majority of the results showed that higher dietary fiber intake was inversely associated with risk of cardiac events, although this trend generally was attenuated or non-significant after multivariate adjustment. When comparing the different sources of fiber intake, fiber from cereal and fruits usually was associated with a lower risk of cardiac events than fiber derived from vegetable sources. Soluble fiber seemed to lower heart disease risk more than did insoluble fiber.

A number of studies have been conducted to evaluate the effects of dietary fiber on risk factors for CVD. Many studies have demonstrated cholesterol lowering effects of dietary fiber. The response depends mainly on the type and amount of fiber in the diet. Also, there is some evidence of a blood pressure lowering effect.

Brown et al (193) conducted a meta-analysis of 67 studies that evaluated the blood cholesterol lowering effects of oat products, psyllium, pectin, and guar gum. The average dose of soluble fiber was 9.5 g/d which was given for an average of 49 days. Over a range of 2-10 g of soluble fiber per day, net reductions of TC and LDL-C were -0.045 mmol/L and -0.057 mmol/L per gram soluble fiber. Over a wider dose response range (2-30 g/d), the reductions were -0.028 mmol/L and -0.029 mol/L per gram soluble fiber. High fiber diets also significantly reduced HDL-C, but to a lesser extent (0.002 mmol/L per gram soluble fiber). The soluble fiber from oats, psyllium, pectin, or guar gum all significantly decreased TC and LDL-C levels; none significantly affected TG. One gram of soluble fiber from oats, psyllium, pectin, or guar gum elicited changes in TC of -0.037, -0.028, -0.070, and -0.026 mmol/L, respectively, and for LDL-C of -0.032, -0.029, -0.055, and -0.033 mmol/L, respectively.

In a meta-analysis conducted by Anderson et al (194), 10.2 g/d of psyllium lowered serum TC by 4%, LDL-C by 7%, and the ratio of apolipoprotein (apo) B to apo A-I by 6%, compared to subjects in placebo group. No significant effects on serum HDL-C or TG concentrations were observed.

Two recent meta-analyses (195, 196) reported blood pressure lowering effects of dietary fiber. Streppel et al. (196), reported that fiber supplementation (average dose, 11.5 g/d) decreased SBP by 1.13 and DBP by 1.26 mmHg. The average blood pressure

reduction in these studies tended to be greater among older populations (>40 years) and in patients with hypertension (SBP -5.95 mmHg, DBP -4.20 mmHg), respectively.

2.3.4 Plant stanol/sterol

Plant-derived sterols are structurally similar to cholesterol. The most abundant plant sterols are sitosterol, campesterol and stigmasterols. Stanols are saturated sterols that lack the delta 5 double bond in their B-ring. Vegetable oils, nuts, soybeans, and seeds contain sterols. Plant sterols exist either in the free form, or in combination with glycosides or as esters with fatty acids. They displace intestinal cholesterol from the micelles thereby reducing intestinal cholesterol absorption, and blood cholesterol levels. To achieve a clinically meaningful cholesterol lowering effect, 2 g/day is needed. It is important to note that this level of intake is attainable only by consuming stanol/sterol-supplemented foods. The current recommendation of the NCEP ATP III is 2 g/d plant sterols/stanols as a therapeutic option to enhance LDL-C lowering. This recommendation is based on studies that show no further significant cholesterol lowering at doses higher than 2-3 g/d (197-202). Moreover, the majority of studies (203-220) have shown that the LDL-C and total cholesterol lowering effects are comparable irrespective of whether stanol or sterol is fed in either the free or esterified form.

There is some evidence that stanols and/or sterols intake further improve cholesterol lowering response to statin therapy. In a multicenter study (221) subjects with primary hypercholesterolemia (206 mg/dl) were randomized to four treatment groups: placebo plus regular margarine 25 g; placebo plus sterol-ester margarine (25 g/day; 2 g of plant sterol); cerivastatin (400 µg plus 25 g/day regular margarine); and cerivastatin (400 µg plus 25 g/day sterol-ester margarine with 2 g of plant sterol). After

4-weeks, LDL-C was significantly reduced by 32% in cerivastatin group (versus placebo) and by 8% in the sterol-ester margarine group (versus regular margarine). The combination of sterol-ester margarine plus cerivastatin lowered LDL-C by 39% which is approximately additive to the independent treatment effects observed. Two other small studies (360, 365) also demonstrated additive effects of stanol and statin on LDL-C lowering.

In the study conducted by Blair et al (222) with a larger population [67 women and 100 men with elevated LDL-C (≥ 130 mg/dl) on statin therapy (doses were not stated)], subjects were given 5.1 g/day of plant stanol esters or placebo. Plant stanol esters reduced TC by 12% and LDL-C by 17% compared to 5% and 7% for the placebo, respectively. These results demonstrate that stanols can further reduce LDL-C beyond that achieved by statin therapy.

2.4 THE EFFECT OF PARAOXONASE (PON1) ON CARDIOVASCULAR DISEASE RISK

2.4.1 HDL bound paraoxonase

Human serum PON1 is a glycoprotein that consists of 354 amino acids with a molecular mass of 43-45 kDa (223). Although it is believed that PON1 is synthesized and secreted by the liver, the regulation of these process remains unclear (224). After synthesis in the liver, a portion of PON1 is secreted into the plasma and it binds to high-density lipoprotein (HDL) by high affinity desorption where apolipoprotein A (apo A) and phospholipids are required (225, 226). PON1 has a hydrophobic N-terminal end which makes it anchor to the lipoproteins. However, it only attaches to HDL rather than

LDL or very-low-density lipoprotein (VLDL). It is speculated that apo A and PON1 are closely associated because they tend to stick together during the serum purification of PON1 (227). The name of this enzyme, PON1, is paraoxon, which is a toxic metabolite of the insecticide parathion (228). PON1 also hydrolyze other organophosphate and arylesters; thus, it has both arylesterase and paraoxonase activities.

2.4.2 Paraoxonase polymorphisms

There are three members (PON1, PON2, and PON3) in the PON gene family and they present on chromosome 7 very closely. Human PON1 gene is located at the chromosome 7 q21-22 (229). There are two major polymorphisms in the PON1 coding sequence: replacement of glutamine (Q or A) by arginine (R or B) at position 192 and substitution of leucine (L) by methionine (M) at position 55 (230). Mackness et al showed that polymorphisms of PON1 modified the efficacy of the antioxidant action in preventing LDL oxidation (231). It has been demonstrated that PON1 192 Q/R polymorphism is the major determinant of the wider inter-variation in the capacity of hydrolyzing organophosphates (224, 229). Although the enzyme catalytic efficiency does not influenced by the L/M polymorphism at position 55, the PON1 levels are lower in individuals with PON1 55M (232, 233). It has been suggested that the L/M isozymes were responsible for the quantitative differences in the enzyme concentration (234) while the Q/R isozymes were associated with the marked qualitative differences (235, 236).

It has been indicated that the Q allele is more abundant than the R allele and the Q allele is responsible for the anti-atherosclerosis effects of PON1 while R allele is related to the CHD risk (237, 238). PON1 allozymes have different activities when catalyze different substrates (239). Aviram et al (240) demonstrated that the PON1 active site

requirements for its arylesterase/paraoxonase activities and its protective role in LDL oxidation are not identical. Furthermore, they also suggested that the Q allele had a greater effect with regards to protect LDL oxidation from copper ion than the R allele (240).

The Q homozygote represented those individuals with lower activity towards paraoxon while the QR heterozygote and R homozygote polymorphism persons had higher activity (224, 229, 236) (**Table 2-4**). However, the R homozygote showed a low activity hydrolyzing lipid hydroperoxides. It has been demonstrated that the phenylacetate substrate activity is not affected by the PON1-192 genotype (241). The relationship between the PON1 192Q/R polymorphisms and the incidence of CHD has been investigated by several research groups (**Table 2-5**) (237, 238, 242-247). Results from four of those studies showed that the R allele was positively associated with the presence of CHD although R allele has high activity towards paraoxon. Serrato et al determined the PON1-192 genotype in 223 patients with angiographically documented CAD and in 247 subjects in the general population in the United States (243). An increased frequency of R allele was found in patients with CAD as compared with that in the general population (0.44 vs. 0.31, respectively; OR, 1.7; 95% CI: 1.3 - 2.2; P=0.0001) was found (243). One hundred and fifteen healthy volunteers and 75 patients in Japan with CAD were studied to investigate the correlation of the PON1-192 polymorphism with the incidence of CAD (247). There was a significant difference in R allele frequency between patients (0.74) and control individuals (0.59) ($P < 0.002$). The logistic regression model demonstrated that PON1-192 polymorphism is an independent risk factor for CAD (OR, 3.02; 95% CI, 1.02 – 8.98; $P < 0.05$; adjusted for age, sex,

PON1-55 polymorphism, smoking, hypertension, diabetes mellitus, and serum levels of cholesterol, TG, and HDL-C) (247). Similar studies were also done in subjects with diabetes mellitus (237, 242). Odawara et al recruited 164 Japanese patients with noninsulin-dependent diabetes mellitus (NIDDM) (42 with CHD and 122 without CHD) to determine the significance of PON1-192 with CHD (237). The proportion of R allele carriers was higher than that of Q carriers in diabetic patients with CHD compared to those without CHD ($\chi^2=7.68$, $P = 0.003$). Furthermore, R allele carriers showed an increased odds ratio for CHD (OR, 8.82; 95% CI, 1.13 – 68.73; $P = 0.037$) independent of other risk factors of CHD (age, sex, smokers, blood pressure and total cholesterol) (237). Similarly, in a case-control study, 434 NIDDM patients (171 with CAD; 262 without CAD) were investigated (242). The odds ratio of CHD for the R allele carriers was 2.5 (95% CI, 1.2 - 5.3; $P = 0.003$) compared with the Q allele carriers. In the further multivariate analysis, the R allele still was significantly associated with CHD (OR, 1.94; 95% CI, 1.08 – 3.50; $P = 0.03$) (242). A meta-analysis reported that PON1-192R was associated with CHD while no association was found between PON1-55M and CHD (248).

Hegele et al demonstrated that PON1-192Q homozygote had a less atherogenic lipid profile [e.g., TC (log TC: 1.62 vs. 1.65; $P = 0.024$), LDL-C (log LDL-C: 1.01 vs. 1.07; $P = 0.007$), non-HDL-C (log non-HDL-C: 1.22 vs. 1.28; $P = 0.001$), TG (transformed TG: -0.92 vs. -0.89; $P = 0.052$), and apolipoprotein B (log apolipoprotein B: 0.06 vs. 0.11; $P = 0.0003$) were significantly lower and HDL-C was significantly higher (log HDL-C: 0.41 vs. 0.36; $P = 0.003$)] compared to the PON1-192 R homozygote and the heterozygote (249). They further concluded that the PON1 polymorphism accounted

for 1% of the variation in TC and related lipoprotein trait in the Hutterites (249). In a study conducted in 163 healthy Chinese subjects, the PON1 genotype was not measured directly but rather by inferring from the plasma PON1 activity (250). Consistent with the previous study, subjects had low plasma PON1 activity (inferred to PON1 192QQ) had lower plasma apolipoprotein B and TG and higher HDL-C. However, plasma TC and LDL-C were higher in PON1-192QQ as well. Discrepancy may be caused by the variation by the indirect measurement of PON1 genotype in the latter study.

The frequencies of the PON1 alleles vary across human populations. Several groups had reported the genotype (PON1-192) distribution in the United States and United Kingdom. Serrato et al reported that in 247 (104 male and 143 female) healthy Caucasian individuals in the general population of United States, 120 (49%) had PON1-192QQ, 99 (40%) had PON1-192QR and 28 (11%) had PON1-192RR (243). Similarly, 46% of 82 apparently healthy people in the United Kingdom had PON1-192QQ, 42% and 12% had PON1-192QR and PON1-192RR, respectively (251). Therefore, in the White population, PON1-192QQ accounts for about 40-50% while PON1-192RR+QR accounts for about 50%-60% of PON1-192 genotype distribution (243, 251-256).

2.4.3 Paraoxonase and atherosclerosis

HDL-C is inversely associated with the risk of developing CHD. However, the mechanism for the protective effect of HDL-C is unknown. It has been suggested that the inverse cholesterol transport and several enzymes that are related to HDL-C may be involved in this protective process. PON1 is one of the enzymes that may elicit a beneficial effect. Serum PON1 activity was reduced and it was inversely correlated with serum lipid oxidative stress in the atherosclerotic apolipoprotein E (apo E)-deficient mice

(257). Similarly, rabbits had a 50% reduction in PON1 activity after a high cholesterol diet intake for 14 weeks (258). PON1 activity was decreased by 52% in atherosclerosis-susceptible mice (strain C57BL/6J) after an atherogenic diet, whereas it remained unchanged in atherosclerosis-resistant mice (C3H/HeJ) (259). McElveen et al reported a lower PON1 activity in patients who survived a myocardial infarction compared to healthy control subjects (260).

Shih et al produced PON1-knockout mice by disrupting exon1 of the PON1 gene to examine the character of PON1 in vivo (261). After a high-fat, high-cholesterol diet, PON1-knockout mice were more susceptible to atherosclerosis than the wild type mice (261). Similarly, combined PON1 knockout/apo E knockout mice and apo E knockout mice were produced to compare atherogenesis. The study found that the PON1 deficiency promotes atherogenesis in mice (262). Human PON1 transgenic mice were generated to investigate the effects of PON1 in atherosclerosis. The transgenic mice had 2- to 4-fold increased PON1 levels in plasma and the atherosclerotic lesion sizes were decreased significantly (263).

Mackness et al (264) showed that PON1 activity was lower while the HDL concentration remained unchanged in subjects with insulin-dependent diabetes and familial hypercholesterolemia. In addition, the PON1 activity was also lower in subjects with diabetes compared to control subjects (251). Navab et al reported that in a subset of patients with coronary atherosclerosis (n = 5), the PON1 activity was significantly lower compared to the control subjects (48 ± 29 vs. $98 \pm 17 \mu\text{g/ml}$, $P < 0.01$) while the HDL concentrations were not different (50 ± 6.1 vs. $48.3 \pm 5.3 \text{ mg/dL}$) (265). In addition, the HDL with normal PON1 activity protected against LDL-induced monocyte migration

efficiently while the low PON1 activity HDL failed (265). Therefore, PON1 may partly explain the inverse relationship between the HDL level and risk of atherosclerotic events. A recent study examined the PON1 activity in patients with coronary artery disease (CAD) and healthy individuals (266). CAD patients had significant lower PON1 activity and HDL-C compared to the healthy subjects (266).

Mackness et al (267) examined PON1 activity, mass concentration and gene distribution in 417 subjects with angiographically proven CAD. CAD patients had lower PON1 activity and concentration than the healthy subjects regardless of PON1 genotype. Whether there is an association between certain polymorphisms of the genes that encode PON1 and CHD risk remain controversial. In contrast, serum PON1 concentration and activity may be a better predictor for CHD risk than PON1 genotype (267).

2.4.4 Paraoxonase and oxidized stress

LDL oxidation is involved in the initial stage of the development of atherosclerosis. In the late 1980s, Mackness first proposed that serum PON1 was protective against LDL oxidation (268). Most studies have supported the theory that PON1 reduces oxidative stress then further impedes atherogenesis. Avian HDL lacks PON1 activity and when incubated with human LDL, it failed to protect Cu^{2+} - induced accumulation of lipid peroxides on LDL (269). The investigators demonstrated that PON1 has an important role in the antiatherogenic/anti-inflammatory effects of HDL (269). HDL isolated from PON1-knockout mice failed to protect LDL against oxidation in a co-cultured cell model of the artery wall (261). In addition, HDL and LDL from PON1-knockout mice were more susceptible to oxidation in co-cultured cells compared to those from wild-type mice (261). These studies demonstrated that PON1 is the major

component of HDL responsible for protecting LDL against oxidation. PON1 was also shown to protect HDL from oxidation. HDL peroxide and aldehyde formation were reduced significantly and HDL oxidation lag phase was prolonged when adding PON1 to human HDL (257).

To investigate the effect of PON1 on macrophage oxidative stress, arterial and mouse peritoneal macrophages (MPM) from two kinds of mice - PON1⁰ and PON1⁰/E⁰ (C57BL/6J and apo E knockout genetic background, respectively) were harvested (270). Both PON1⁰ and PON1⁰/E⁰ mice had significantly lower PON1 activity compared to their controls. In PON1⁰ mice, lipid peroxide content increased by 84% in serum compared to the control mice, and also increased by 3.2 fold in LDL. Peroxide content increased by 71% in arterial macrophages from PON1⁰/E⁰ mice compared to control mice. LDL oxidation by MPM was increased by 40% and 19%, respectively, in PON1⁰ and PON1⁰/E⁰ mice, compared to the LDL oxidation by MPM from control mice (270). Using isolated HDL (0.25 mg/mL) from PON1 transgenic mice, lipid hydroperoxide levels were significantly reduced by 33% and 13% when incubating with LDL for 3 and 6 hours, respectively, compared to the lipid hydroperoxide in LDL plus wild type HDL (263). Thus, LDL oxidation was prevented efficiently by PON1 in transgenic mice. Watson et al also demonstrated that purified PON1 may reduce the ability of mildly oxidized LDL to induce proinflammatory effects when incubated in a vascular cell culture system by destroying the active lipids in the oxidized LDL (271). In sum, PON1 can hydrolyze not only oxidized lipids in oxidized LDL but also those in macrophages.

Fuhrman et al demonstrated that oxidized macrophages increase uptake of oxidized LDL because LDL receptor activity was increased and LDL underwent cellular

modification (272). A reduced uptake of oxidized LDL and expression of CD36 scavenger receptor mRNA were observed following decreased lipid peroxide in MPM harvested from E⁰ mice injected with human PON1 (273). Rozenberg et al showed that PON1⁰/E⁰ mice had a 35% greater cholesterol content compared to the control E⁰ mice (274). When incubated with human PON1, macrophage cholesterol biosynthesis was inhibited in a dose-dependent manner. A PON1 phospholipase A₂-like activity on MPM was also demonstrated by Rozenberg et al (274). Because of this activity, lysophosphatidylcholine (LPC) was released and it inhibited cholesterol biosynthesis in MPMs (274).

Excess free cholesterol from peripheral cells is transported back to liver for degradation by HDL via reverse cholesterol transport. Multiple mechanisms for the efflux of cholesterol have been explored. Aqueous diffusion and scavenger receptor class-B type I (SR-BI)-mediated cholesterol effluxes are bidirectional and the direction depends on the cholesterol gradient (275). The adenosine triphosphate (ATP)-binding cassette transporter AI (ABCAI) mediated cholesterol and phospholipids efflux uses ATP as energy source and is unidirectional (275). HDL oxidation has been shown to reduce the ability of transporting cholesterol. PON1 not only decreased oxidized HDL but also increased the ability of HDL to promote macrophage cholesterol efflux (257). Aviram and Rosenblat demonstrated that PON1 causes increased HDL-mediated cholesterol efflux via the ABCAI receptor and this may be caused by PON1 induced particle conformational changes of HDL and the changes of its affinity to the receptors (276).

Avoram and Rosenblat proposed that PON1 can hydrolyze oxidized lipids in oxidized LDL and macrophages, reduce macrophage-mediated LDL oxidation, and

reduce oxidized LDL uptake by the macrophage (**Figure 2-8**) (276). Therefore, PON1 exerts beneficial effects in the development of atherosclerosis.

2.4.5 Paraoxonase and dietary fat intervention

Limited research has been conducted to investigate the association between diet and PON1 activity. Shih et al (259) demonstrated a reduction in serum PON1 activity and liver PON1 mRNA levels in C57BL/6J atherosclerosis-susceptible mice after a high fat, cholesterol-rich diet (15.8 g/100 g SFA, 1.25 g/100 g cholesterol) for three months. In contrast, the C3H/HeJ atherosclerosis-resistance mice had increases in both PON1 activity and liver mRNA levels. Apolipoprotein A-I transgenic rabbits had a higher PON1 activity and HDL-C compared to the nontransgenic littermates. After a pro-atherogenic diet for 14 weeks, PON1 activity and HDL-C were reduced in both transgenic and nontransgenic rabbits (258). Rats were randomized to four groups receiving control diet, control diet supplemented with triolein, tripalmitin or fish oil for 20 days. The triolein supplemented group had a greater PON1 activity while fish oil had a lower PON1 activity. There was no effect in the tripalmitin supplemented diet (277). Kudchodkar and colleagues concluded that the expression of serum PON1 in rats can be regulated by dietary fat (277).

Twelve healthy men were randomized to receive a milkshake (46 g test fat, 100 g ice-cream with 10 g fat, 150 ml low-fat milk, 50 ml evaporated milk, 10 g yogurt, 12 g egg yolks, 30 g yolk whites, 50 g canned apricots without the syrup, and chocolate flavoring) either rich in fat used for deep-frying or rich in unused fat (278). The used fat was higher in SFA compared with the unused fat. Serum PON1 activity was reduced by 27 % for up to 8 hours and it returned to normal by 12 hours after the used fat meal. In

contrast, the unused fat meal led to a significant increase in the PON1 activity. Fourteen patients with type 2 diabetes were fed meals (milkshake: 60 g oil, 50 g tinned apricots, 10 g natural yoghurt, 1 egg, 200 ml skimmed milk, two tablespoons Weight Watcher mousse; a commercial instant desert containing no fat and no sugar) rich in thermally stressed safflower or olive oil. Postprandial serum PON1 activity was increased in women mainly after the olive oil enriched meal. In contrast, ten healthy men consumed a high fat, high carbohydrate meal (45 % fat, 37 % carbohydrate, 22 % protein, 193 mg cholesterol, 25 g SFA, 19 g MUFA and 2.5 g PUFA) and the PON1 activity did not change after three hours in the postprandial state (279). De Roos et al reported that replacement of saturated fat by trans fat in 32 men and women decreased HDL-C and the PON1 activity (280). Fourteen familial combined hyperlipidemia patients were given 1.88 g of EPA and 1.48 g of DHA per day for 8 weeks. A modest increase in PON1 concentration was seen in these patients along with an increase in HDL₂-C (281).

2.4.6 Paraonase and antioxidants

Apolipoprotein E deficient mice were supplemented with placebo, catechin or quercetin (50 µg/d), or red wine (0.5 mL/d) for up to 6 weeks (282). LDL-associated lipid peroxides decreased by 39 %, 48 %, and 49 %, respectively, and the atherosclerotic lesion area was smaller by 39 %, 46 %, and 48 %, respectively, in the mice treated with catechin, quercetin, or red wine compared to the mice treated with placebo (282). The consumption of catechin, quercetin, or red wine resulted in 14 %, 113 %, and 75 % greater PON1 activity, respectively, compared to placebo group (282). Pomegranate juice contains pectin, ascorbic acid and polyphenolic flavonoids with antioxidant activity. Pomegranate juice concentrate was diluted in water and given to apo E knockout mice

with advanced atherosclerosis for two months (283). PON1 activity had a 43 % increase in mice that consumed pomegranate juice compared to the placebo controlled mice (283). Additionally, PON1 activity increased by 18 % in 13 healthy, nonsmoking men after supplemented with 50 ml pomegranate juice per day for two weeks (284).

Kleemola et al demonstrated that PON activity was negatively correlated with intake of vegetables (285). Similarly, serum PON1 activity was lower after high vegetable diet intake compared to the low vegetable diet (252). The reduction of PON1 activity is also associated with the reduction in HDL-C ($r = 0.35$, $p < 0.05$). Additionally, no correlations were found between serum PON1 levels and vitamin C, vitamin E, and beta-carotene intake (286). In contrast, Jarvik et al reported that vitamin C or E intakes were significant positive predictors of PON1 activity (287). Therefore, how antioxidants in the diet modulate PON1 activity needs further investigation.

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Table 2-1 Lipid and lipoproteins classification (3)

LDL Cholesterol (LDL-C) <100 100-129 130-159 160-189 ≥190	Optimal Near optimal/above optimal Borderline high High Very high
Total Cholesterol (TC) <200 200-239 ≥240	Desirable Borderline high High
HDL Cholesterol (HDL-C) <40 ≥60	Low High
Triglycerides (TG) <150 150-199 200-499 >500	Optimal Borderline high High Very high

Source: Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III), 2001

Table 2-2 Potential mechanisms by which oxidized LDL may be atherogenic (34)

- 1. It has enhanced uptake by macrophages leading to cholesteryl ester enrichment.**
 - 2. It is chemotactic for circulating monocytes.**
 - 3. It inhibits the motility of tissue macrophages.**
 - 4. It is cytotoxic.**
 - 5. It can alter gene expression of neighboring cells such as induction of MCP-1 and colony-stimulating factors.**
 - 6. It is immunogenic and can elicit autoantibody formation.**
 - 7. It can adversely alter coagulation pathways.**
 - 8. It can adversely alter vasomotor properties of coronary arteries.**
-

Source: Witztum and Steinberg, 1991

Table 2-3 Fat and fatty acids composition of foods high in MUFA (1-oz portion) (74)

	Calories	Total Fat, g	SFA, g	MUFA, g	PUFA, g
Vegetable oil					
Canola oil	248	28.0	2.0	16.4	8.2
Olive oil	238	27.0	3.6	20.0	2.2
High oleic (>70%) safflower oil	240	27.2	1.7	20.4	3.8
High oleic (>70%) oil/sunflower oil	248	28.0	2.8	23.4	1.1
Nuts and seeds*					
Mixed nuts	168	14.6	2.0	8.9	3.1
Almonds	166	14.6	1.4	9.5	3.1
Cashews	163	13.1	2.6	7.7	2.2
Hazelnuts	188	18.8	1.4	14.7	1.8
Macadamia nuts	199	20.9	3.1	16.5	0.4
Peanuts	166	14.1	2.0	7.0	4.4
Peanut butter (smooth), 2 Tbsp	190	16.3	3.3	7.8	4.4
Pistachios	172	15.0	1.9	10.1	2.3
Pecans	187	18.3	1.5	11.4	4.5
Sesame butter (tahini) from kernels, 2 Tbsp	169	15.2	2.1	5.8	6.7
Sesame seeds	160	13.9	2.0	5.2	6.1
Walnuts (English)	182	17.5	1.6	4.0	11.1
Walnuts (black)	172	16.0	1.0	3.6	10.6
Fruits					
Avocado, raw	45	4.3	0.7	2.7	0.5
Olives	32	3.0	0.4	2.2	0.3

*All nuts and seeds are dry roasted.

Source: Kris-Etherton, 1999

Table 2-4 The PON1 substrate activity polymorphism [4]

B alloenzyme more active with:	Paraoxon Methylparaoxon Chlorthion oxon EPN oxon Armin
Similar activity of alloenzymes with:	Phenyl acetate Chlorpyrifos oxon 2-Naphthyl acetate
A alloenzyme more active with:	Diazoxon Sarin Soman Phospholipid hydroperoxides?

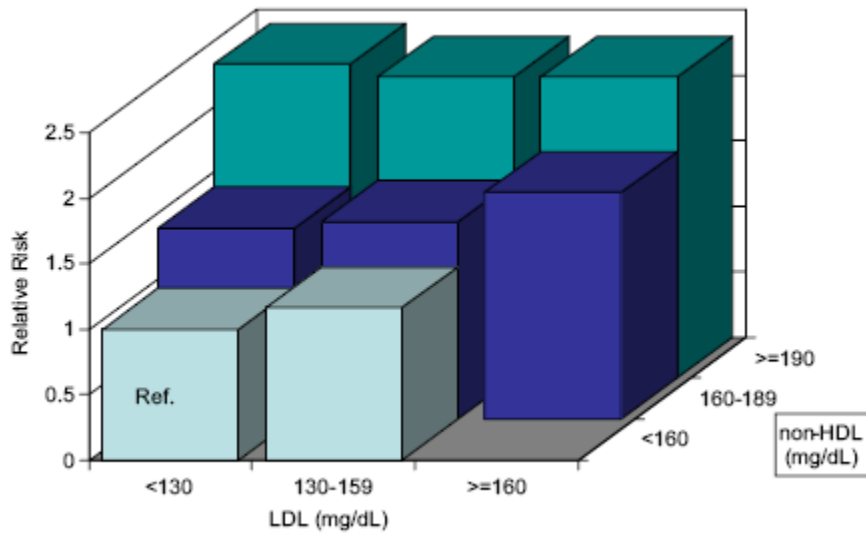
A alloenzyme: PON1 Q allozyme; B alloenzyme: PON1 R allozyme. Source: Anderson et al., 1987

Table 2-5 Frequency of the PON1-192R polymorphism in CHD (288)

	+CHD	-CHD				
Ruiz et al. (1995)	0.35	0.26	NIDDM	263	+CHD	
				171	-CHD	
Serrato and Marion (1995)	0.44	0.31	Angiography	233	+CHD	
				247	-CHD	
Hermann et al. (1996)	0.31	0.30	ECTIM (mixed countries)	642	+CHD	
				701	-CHD	
Antikainen et al. (1996)	0.26	0.26	CABG (4 years)	380	+CHD	HDL ≤ 1.1 mmol/l
						LDL ≤ 4.5 mmol/l
						TG ≤ 4.0 mmol/l
Suehiro et al. (1996)	0.60	0.62	Japanese	169	-CHD	
				134	+CHD	(44 NIDDM)
				252	-CHD	
Sanghera et al. (1997)	0.43	0.33	Singapore Indians	122	+CHD	
				165	-CHD	
	0.59	0.53	Chinese	246	+CHD	
				244	-CHD	
Odawara et al. (1997)	0.69	0.58	Japanese NIDDM	42	+CHD	
				122	-CHD	
Zama et al. (1997)	0.74	0.59	Japanese	75	+CHD	
				115	-CHD	

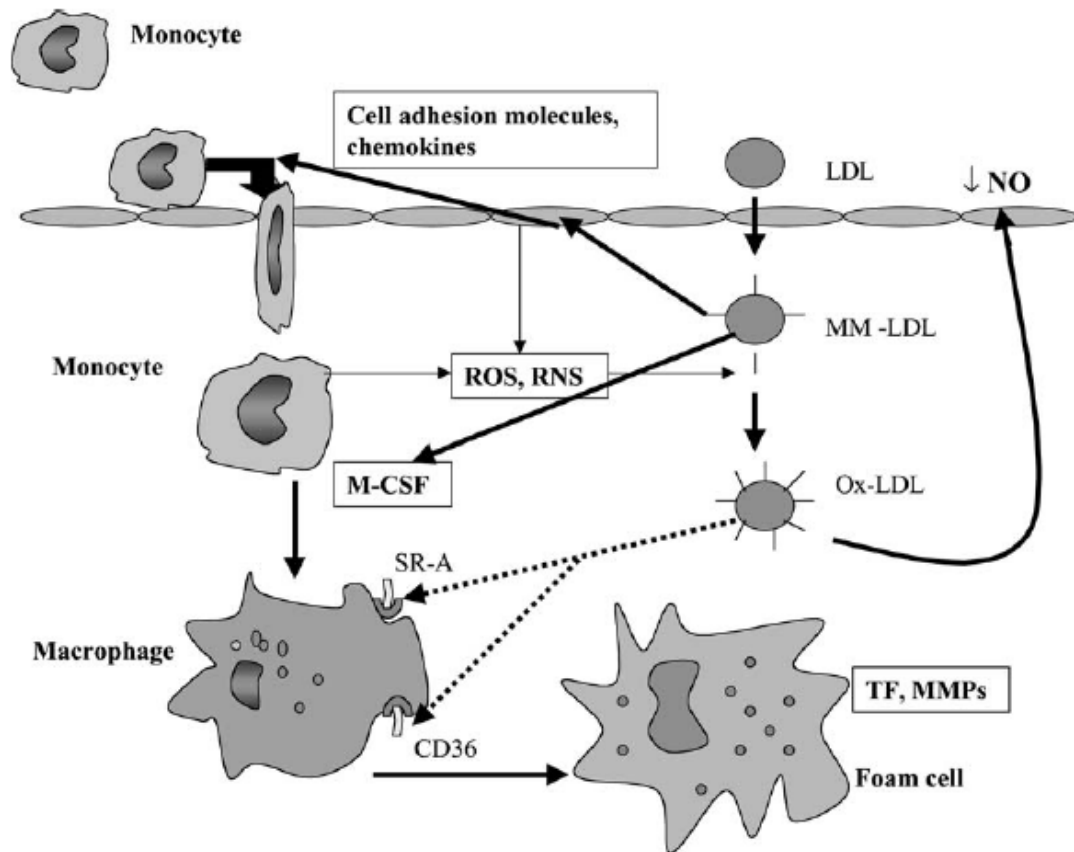
Source: Mackness et al., 1999

Figure 2-1 Risk of CHD incidence for the joint distribution of non-HDL and LDL-C (20)



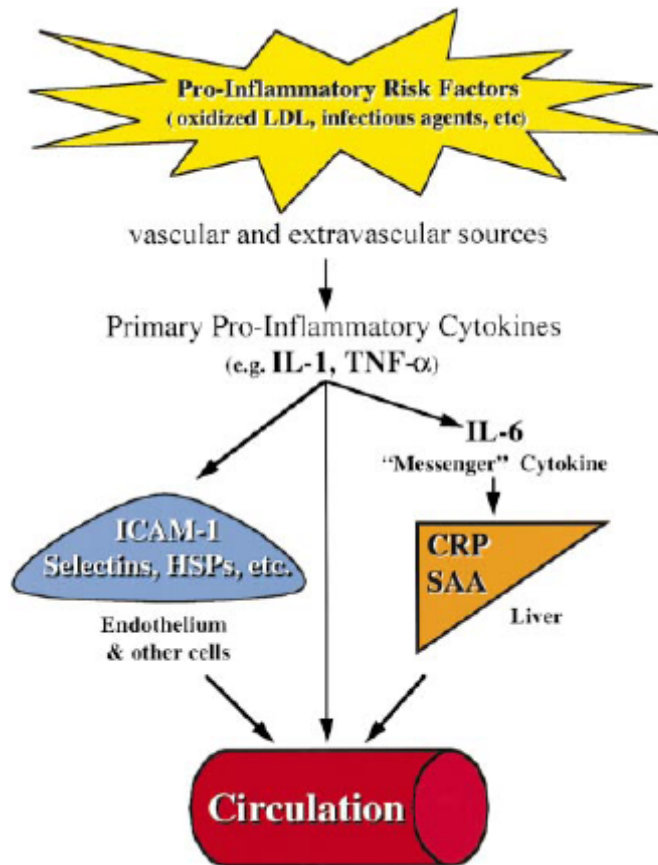
Source: Liu et al., 2006

Figure 2-2 The role of oxidized LDL in atherosclerosis (289)



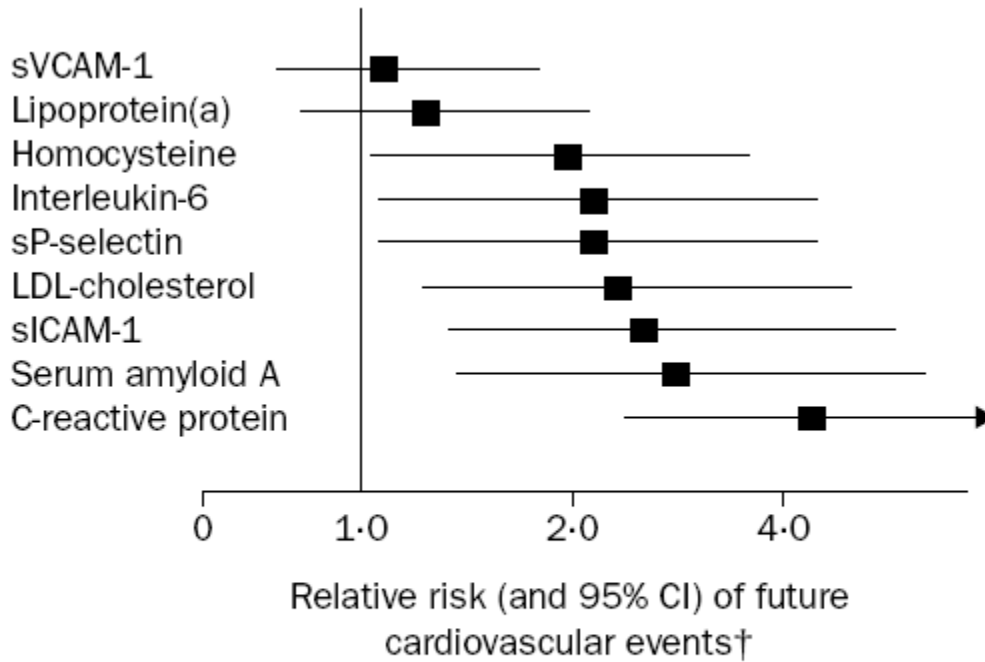
Source: Singh et al., 2005

Figure 2-3 The inflammatory response (56).



Source: Libby and Ridker, 1999

Figure 2-4 Prognostic value of various cardiovascular biomarkers in healthy women (61)

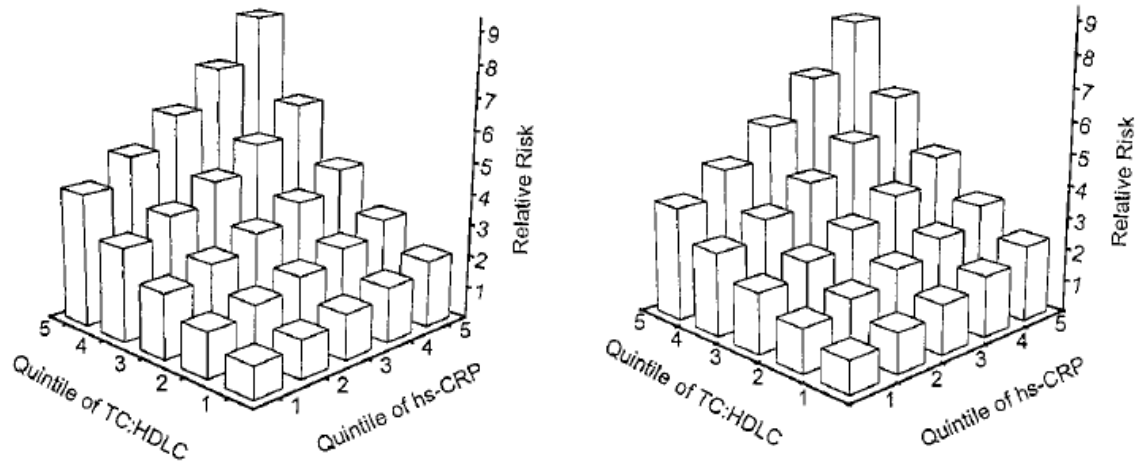


*Data from Women's Health Study. †Top vs bottom quartile after adjustment for age and smoking.

sVCAM-1 = soluble vascular-adhesion molecule-1; sICAM = soluble intracellular adhesion molecule-1.

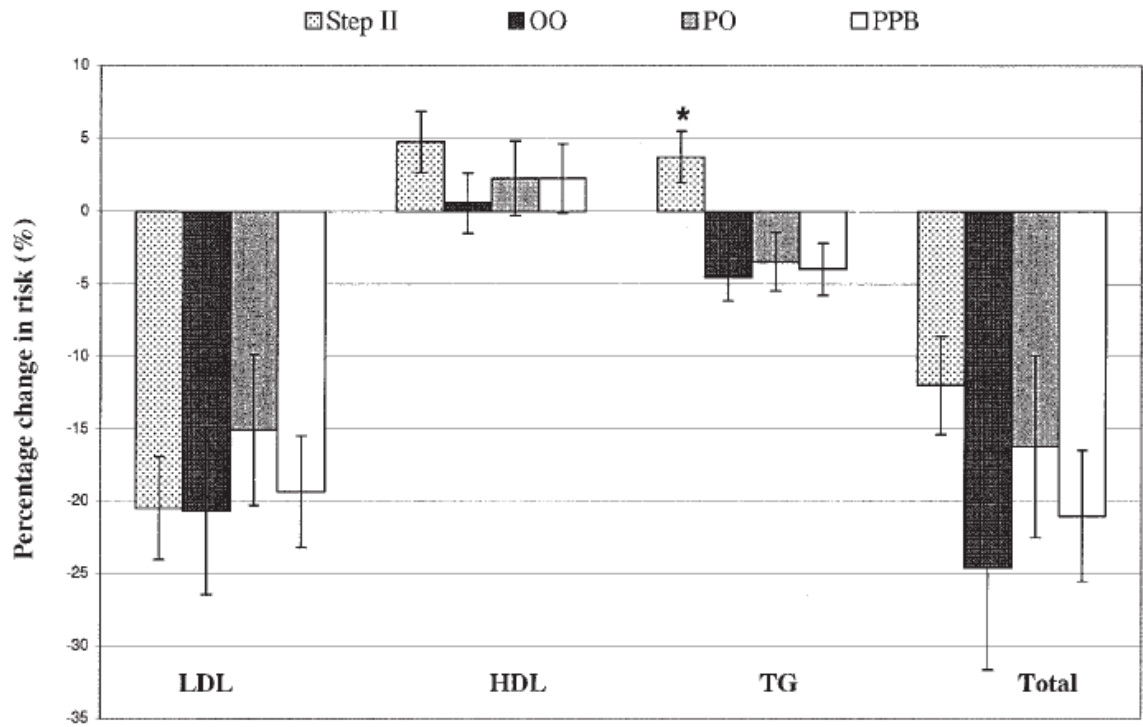
Source: Ridker, 2001

Figure 2-5 Interactive effects of CRP and lipid testing in men (left) and women (right) (62)



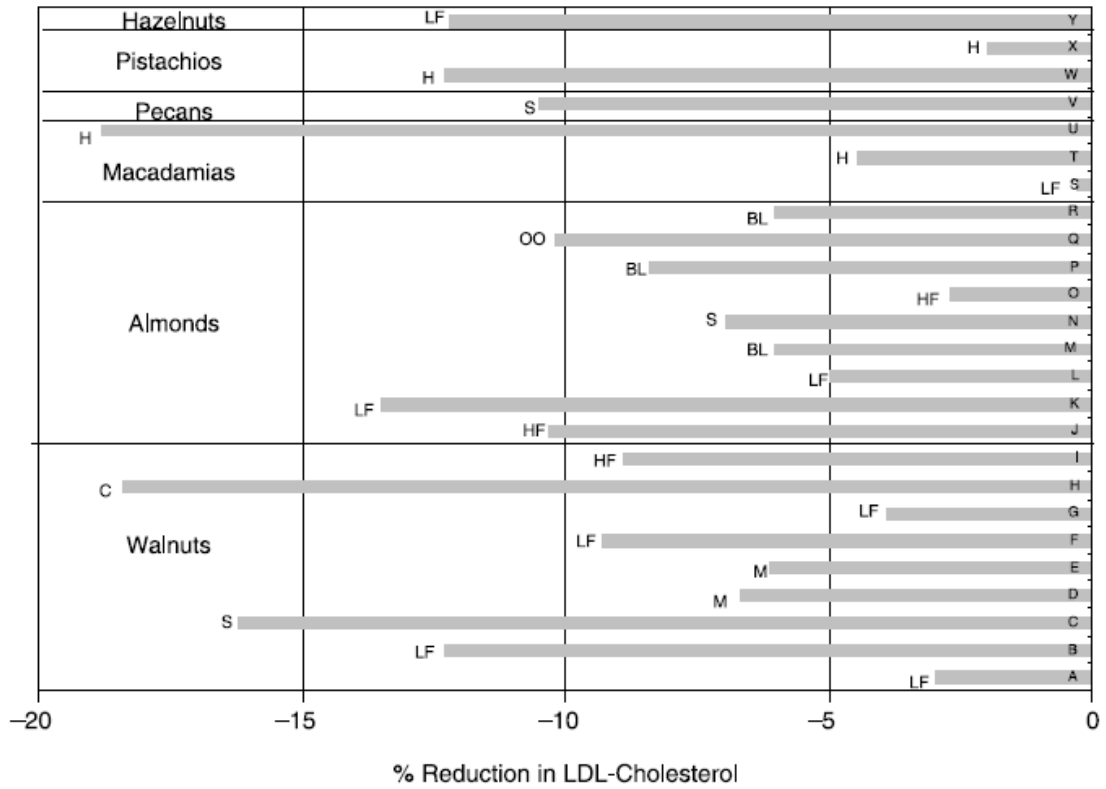
Source: Ridker, 2001

Figure 2-6 Effects of LDL-C, HDL-C, and TG concentrations on CVD risk reduction in response to Step II, olive oil (OO), peanut oil (PO), and peanut and peanut butter (PPB) diet (n=22) (93)



*Significantly different from the other three diets, $P = 0.005$. Values are least-squares means \pm SEs (93). Source: Kris-Etherton et al., 1999

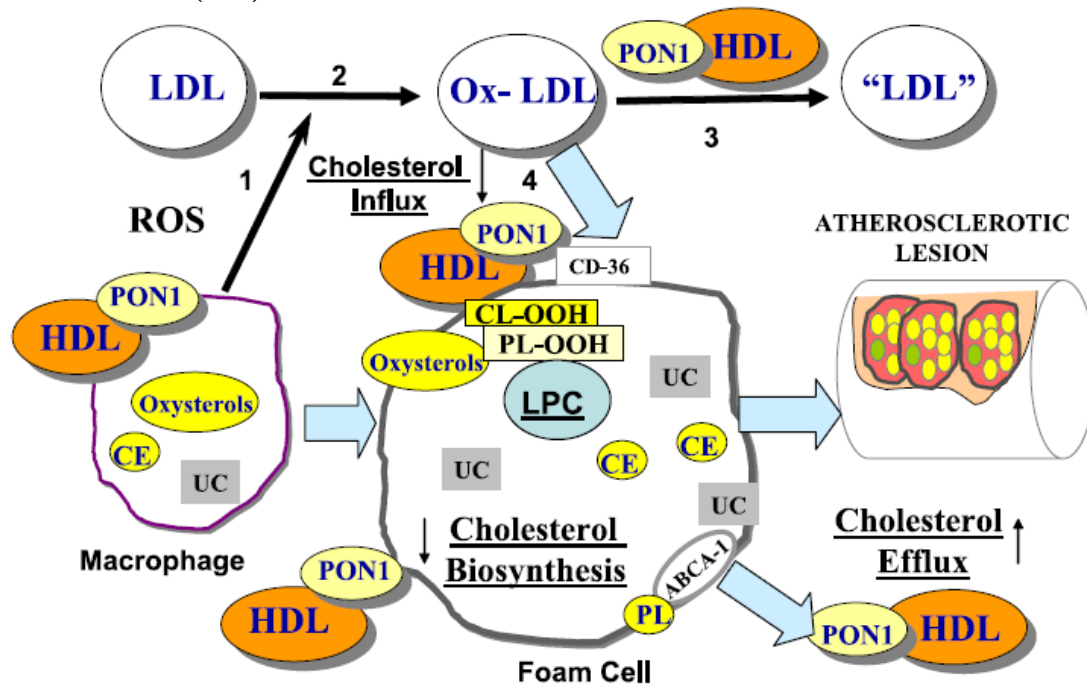
Figure 2-7 LDL-C lowering reported in tree nut studies (142)



Note: Comparison reference diets at left of each column; LF = low-fat, H = habitual, S = Step I, BL = Baseline, OO = olive oil-based diet, HF = high-fat, M = Mediterranean, C = Control; See Tables I and II for study details.

Source: Griel and Kris-Etherton, 2006

Figure 2-8 Paraoxonase inhibits macrophage foam cell formation and attenuates atherosclerosis (276)



Source: Aviram and Rosenblat, 2004

Chapter 3

MODERATE-FAT VERSUS LOWER-FAT BLOOD CHOLESTEROL LOWERING DIETS ON LIPIDS AND LIPOPROTEINS IN HEALTHY SUBJECTS AND PERSONS WITH DIABETES: A META-ANALYSIS OF RANDOMIZED CONTROLLED CLINICAL TRIALS

3.1 ABSTRACT

Dyslipidemia increases coronary heart disease (CHD) risk, and often presents in diabetes, which amplifies risk of CHD. Lower-fat (LF) diets increase TG and decrease HDL-C; moderate-fat (MF) diets decrease TG and lower HDL-C less. The objective of the present study is to quantify the magnitude of lipid and lipoprotein responses to MF versus LF cholesterol-lowering, weight maintenance diets in subjects with and without diabetes. A meta-analysis of 30 controlled-feeding studies (n = 1213 subjects) was conducted to evaluate LF versus MF diets on lipids and lipoproteins in subjects with and without diabetes. In all subjects, MF and LF diets decreased LDL-C similarly. MF diets decreased HDL-C less versus the LF diets. The estimated increase in HDL-C after MF diets versus LF diets was 2.28 mg/dL (95% CI: 1.66 - 2.90 mg/dL, $P < 0.0001$). The MF diets decreased TG whereas LF diets increased TG. The decrease in TG was - 9.36 mg/dL (- 12.16, - 6.08 mg/dL, $P < 0.00001$) for MF versus LF diets. In subjects with diabetes, there was a similar increase in HDL-C (2.28 mg/dL) versus subjects without diabetes, however, there was a greater reduction in TG (- 24.79 mg/dL, $P < 0.05$) on the MF diet. Subjects with diabetes had greater reductions in the TC to HDL-C (TC: HDL-C) ratio (- 0.62, $P < 0.0001$) and non-HDL-C (- 5.39 %, $P < 0.06$) after MF versus LF diets. Based on greater reductions in TG, the TC: HDL-C ratio and non-HDL-C especially in subjects with diabetes, the CHD risk reduction would be greater for a MF versus a LF weight maintenance, cholesterol-lowering diet. Moreover, both men and women had greater estimated reductions (6.37 % and 9.34 %, respectively) in predicted CHD risk after the MF diets compared to the LF diets.

3.2 INTRODUCTION

Diet is the cornerstone of intervention strategies for the prevention and treatment of coronary heart disease (CHD). The National Cholesterol Education Program (NCEP) recommends the Therapeutic Lifestyle Changes (TLC) diet [low in saturated fatty acids (SFA), < 7%; trans fatty acids (TFA), as low as possible; and dietary cholesterol, < 200 mg/day] to reduce low density lipoprotein cholesterol (LDL-C) and risk of CHD. The total fat (TF) recommendation of 25 - 35 % of energy [to be provided primarily by monounsaturated fat (MUFA), ≤ 20 %; and polyunsaturated fat (PUFA), ≤ 10 %] (1) is individualized. In addition to lowering LDL-C levels, the goal of medical nutrition therapy for the treatment of dyslipidemia is to increase or prevent decreases in HDL-C, and decrease or prevent increases in TG (2, 3). Improving calorie balance to achieve desirable weight is the major change for the management of dyslipidemia, characterized by elevated triacylglycerol (TG) levels (≥ 150 mg/dL) and low levels of high density lipoprotein cholesterol (HDL-C) (men < 40 mg/dL and women < 50 mg/dL) (1). These nutrition goals are consistent with those made by the American Diabetes Association (ADA) (4), and the American Heart Association (AHA) (5).

Typically, energy from SFA is decreased by replacement with carbohydrate (resulting in a reduced-fat diet) or unsaturated fatty acids (resulting in a moderate-fat diet). Alternatively, SFA can be reduced without energy replacement, resulting in a lower-fat diet. Although a moderate-fat (MF) diet that is low in SFA typically lowers LDL-C to the same extent as a lower-fat (LF) diet, it decreases HDL-C less than a LF diet, and decreases TG, whereas an LF diet frequently increases TG (6). Numerous

studies have compared the effects of MF versus LF diets on lipids and lipoproteins (7-38).

The objective of our meta-analysis is to quantify the magnitude of the changes in lipids and lipoproteins in response to a MF blood cholesterol-lowering diet rich in unsaturated fat versus a LF diet in subjects with and without diabetes. We conducted this analysis to ascertain whether substitution of SFA with carbohydrate versus unsaturated fat resulted in differential effects on the lipid and lipoprotein profile in subjects with and without diabetes. We also conducted a regression analysis to examine the relationship between the TF, SFA, MUFA, and PUFA content of the diets and the magnitude of change observed in the lipid and lipoprotein parameters. This is important because identifying a more optimal quantity of TF and fatty acid composition will be useful in clinical practice to achieve beneficial changes in the lipid and lipoprotein profile, and the greatest reduction in CHD risk. For subjects with diabetes this is particularly germane because of their increased risk for CHD due, in part, to a high prevalence of dyslipidemia. We believe the current study is important because it is the first meta-analysis to compare responses to MF and LF diets in blood cholesterol-lowering, weight maintenance diets in subjects with and without diabetes.

3.3 METHODS

3.3.1 Selection of Studies

Studies evaluating the effects of MF versus LF diets were identified by a literature search (MEDLINE, National Library of Medicine, and Bethesda) of articles published between 1987 and 2007. Key words in the search included: moderate-fat diet, low-fat diet, controlled trial, cardiovascular disease, type 2 diabetes mellitus, lipoproteins, and

lipids. Studies also were chosen by examining bibliographies of review articles. Thirty-two published studies (7-38) were included that met the following criteria: (1) controlled-feeding with a crossover or parallel design comparing MF and LF diets; (2) designed to lower plasma total cholesterol (TC) and LDL-C with the primary purpose of reducing the risk of cardiovascular disease; (3) comparison diets were isoenergetic; (4) participants maintained a constant weight during the study; (5) dietary protein, and cholesterol were kept constant between diets; (6) diet periods lasted ≥ 2 weeks to stabilize plasma cholesterol concentration; and (7) studies were published in English.

Two articles (12, 13) provided similar data because they were conducted with the same subjects. Therefore, only one was included. Seven studies (30-34, 36, 37) were conducted on subjects with diabetes and were included in the analysis. However, one article (37) did not report data for lipid and lipoprotein concentrations for the initial and after-diet periods and therefore was excluded. Six studies (7-12) utilized a crossover design without randomization. Data were analyzed with and without these studies. Since the results did not differ, these studies were included.

3.3.2 Data Abstraction

Study characteristics and data from each paper were extracted and inputted into the database. The following information was extracted: (1) subjects' characteristics, including sample size, number of male and female participants, age, and body mass index (BMI); (2) study design, including type of study (crossover or parallel), presence or absence of run-in period, duration of diet intervention, and wash-out period; (3) macronutrient composition of MF and LF diets, including cholesterol content and percentage of energy derived from TF, SFA, MUFA, and PUFA; (4) measurement of

lipids and lipoproteins (TG, TC, LDL-C, and HDL-C) at baseline and after diet intervention, and standard deviations of means; (5) Non-HDL-C was calculated by subtracting HDL-C values from TC values and the ratio of TC: HDL-C was determined using the mean values for TC and HDL-C presented in each group.

3.3.3 Statistical Analyses

To allow for direct comparisons between outcome variables and between studies, the results from each study were quantified as effect size (δ), defined as $\delta = (\mu_{MF} - \mu_{LF})/\sigma$, where μ_{MF} represents is the average effect of the MF diet, μ_{LF} represents the average effect of the LF diets and σ represents the pooled standard deviation (39). Some variables [e.g., TG and Apolipoprotein A-I (Apo A-1)] are not directly comparable because they are measured on different scales. Effect sizes are unit-less and thus permit direct comparisons between variables. An effect size of 1.0 approximates one standard deviation between diets for each endpoint variable. In the current meta-analysis, one standard deviation for TC, LDL-C, HDL-C, and TG can be converted to 27.3, 24.1, 10.4, and 46.8 mg/dL, respectively.

Effect sizes were estimated differently for trials with parallel versus cross-over designs. For parallel design studies, the average diet effects, μ_{MF} and μ_{LF} , were estimated separately as the difference between the reported post-diet and baseline means, and the effect difference, $\mu_{MF} - \mu_{LF}$, was then calculated. The standard deviation was estimated from the reported between-subject standard deviation and the effect size was estimated as the effect difference divided by the standard deviation. For cross-over design studies, the effect difference, $\mu_{MF} - \mu_{LF}$, was estimated directly as the difference between the reported MF period and LF period means. The standard deviation was estimated from the

reported within-subject standard deviation and the effect size was estimated as the effect difference divided by the standard deviation. An effect size (mean changes in endpoints) greater than zero indicates that endpoints were greater after the MF diet intervention, whereas an effect size less than zero indicates the endpoints were greater after the LF diet intervention. Not all studies reported all endpoints and therefore, the number of studies included in the analyses varied (**Table 3-1**).

Meta-analysis entails estimation of a pooled effect size across studies. In this analysis, the random effects model described by Hedges and Olkin (39) was used to estimate the pooled effect size for each variable. The results were weighted by taking the reciprocal of the estimated effect size variance for that study (39). This method gives more weight to larger studies, because they have less variation, and less weight to smaller studies. 95% confidence intervals for the pooled effect size estimates were also calculated based on the random effects model.

Not all of the studies implemented the same experimental diets, resulting in variation in the levels of TF, SFA, MUFA, and PUFA across studies. Meta-regression analysis was performed in order to assess the possibility of dose-dependent diet effects. This approach is analogous to a simple linear regression analysis in which the studies are the cases, the effect size is the dependent variable and the dietary content is the independent variable.

Forest plots were used to present the meta-analysis results (**Figures 3-1, 3-2, 3-3, 3-4**). Each line in the plot represents one study. The midpoint of the line indicates the calculated estimated effect size and the size of the square denotes the relative weight that study received in the analysis. The ends of the line indicate the calculated 95 %

confidence interval for the effect size. In general, studies with wider confidence intervals have smaller weights because those estimates are more variable. The diamond at the bottom of the plot represents the pooled effect size estimate and the width of the diamond indicates the 95 % confidence interval for the pooled estimates. The vertical stripe denotes an effect size of zero so that study lines that cross the stripe denote non-significant results. Even if all of the individual studies are not significantly different from zero, the pooled effect size estimate can be significantly different from zero.

Analyses were conducted with or without studies involving subjects with diabetes, and results were reported separately. Student's t tests were used for comparisons of the lipid and lipoprotein results between subjects with and without diabetes. Analyses were performed using the SAS statistical software package version 9 (SAS Institute, Cary, NC). $P < 0.05$ was used to denote statistical significance. P values < 0.10 and > 0.05 were considered statistical trends.

A standard measure of publication bias has not yet been set (although several tests exist) and funnel plots have been used widely (40). Therefore, funnel plots were used to detect publication bias in the current meta-analysis (data not shown). The variables evaluated were continuous therefore sample size was plotted against effect size. The plot was relatively symmetric for HDL-C. The plots showed some asymmetry for TC, LDL-C and TG. As noted previously, not all studies reported all four endpoints (TC, LDL-C, HDL-C, and TG) which may contribute to the asymmetry of the plots.

3.4 RESULTS

3.4.1 Characteristics of the Studies

Thirty studies that met the inclusion criteria were included in the analysis. The study participants (n = 1213) ranged in age from 20 to 64 years and had a BMI of 21.1 - 30.2 kg/m². Sample size ranged from 8 to 161 subjects and the length of diet intervention ranged from 2 to 12 weeks. Selected characteristics of the studies and participants are presented in **Table 3-2** and **Table 3-3**. Macronutrient composition of the diets is shown in **Table 3-4**. Ninety individuals had diabetes (average age 58.8, range 52.7 to 63 years). The mean BMI for subjects with diabetes was 28.4 (range 26.7 - 30.0 kg/m²). A parallel design was used in six trials and the remaining 24 were crossover studies.

The MF diets provided 30.2 to 50 % of energy while the LF diets provided 18.3 to 30.2 %. Mean intakes of SFA, MUFA, and PUFA were 8.8 % of energy (4 - 11), 23.6 % (10.9 - 33), and 7.1 % (3.5 - 20.8), respectively, for the MF diets. For the LF diets, the mean intakes of SFA, MUFA, and PUFA were 8.2 % (3 - 12.9), 11.4 % (6 - 15.5), and 6.5 % (2 - 12), respectively.

3.4.2 Diet Composition and Lipid, Lipoproteins, and Apolipoproteins

Study effect sizes, pooled effect sizes, and the 95 % confidence intervals for LDL-C, HDL-C, and TG are shown in **Figures 3-1, 3-2, 3-3**. One study effect size was significantly different from zero for HDL-C, however, most of the studies showed a similar pattern (favoring MF diets). Two studies had significant negative effect sizes for TG while the majority of studies showed a consistent pattern (favoring MF diets). HDL-C concentrations were significantly higher on the MF diets ($\delta = 0.22 \pm 0.03$, $P < 0.00001$ vs. the LF diets). TG was significantly lower on the MF diets ($\delta = -0.20 \pm 0.03$, $P <$

0.00001 vs. the LF diets). There were no significant differences in TC and LDL-C between diets.

The forest plot for apoA-1 is shown in **Figure 3-4**. The majority of the studies favored the MF diet compared to the LF diet. The pooled effect size was 0.19 ± 0.07 (0.04 ± 0.01 g/L, $P = 0.006$), which suggests that apoA-1 increased in the MF diet compared to the LF diet. The effect size for apoB was 0.05 ± 0.05 (0.007 ± 0.007 g/L, $P = 0.26$), which indicates that there was no difference between the MF and LF diets.

3.4.3 Studies in Subjects with or without Diabetes

In studies of subjects without diabetes, HDL-C increased significantly ($\delta = 0.22 \pm 0.10$, $P < 0.000001$, approximately 2.28 mg/dL) while TG decreased significantly ($\delta = -0.17 \pm 0.04$, $P < 0.000001$, approximately 7.95 mg/dL) after MF diets compared to LF diets. TC and LDL-C were reduced similarly for the MF and LF diets. Studies of subjects with diabetes showed a similar increase in HDL-C ($\delta = 0.22 \pm 0.11$, $P = 0.04$, approximately 2.28 mg/dL), a nonsignificant reduction in TC ($\delta = -0.15 \pm 0.03$, $P = 0.16$, approximately - 4.09 mg/dL), and a significant reduction in TG ($\delta = -0.53 \pm 0.11$, $P < 0.000001$, approximately - 24.79 mg/dL) after the MF diets compared to LF diets. Subjects with diabetes had a greater reduction in TG (-0.26 , approximately 12.16 mg/dL, $P < 0.05$) compared to subjects without diabetes on the MF diets (**Figure 3-5**). The number of studies examining subjects with diabetes was relatively small [6 for LDL-C and HDL-C (30-34) ($n = 90$) and 7 for TC and TG (30-34, 36) ($n = 108$)]. Therefore, only the increase in HDL-C and the decrease in TG reached significance, and the effect sizes had relatively larger standard errors.

Non-HDL-C decreased by 12.0 % from baseline for subjects on MF diets and by 9.4 % on LF diets. The decrease on the MF diets was 2.6 % greater than on the LF diets ($P = 0.05$) (**Figure 3-6**). A trend ($P < 0.06$) was found for a greater reduction (5.4%) in subjects with diabetes compared to subjects without diabetes in the non-HDL-C differences between MF and LF diets (**Figure 3-6**). The TC: HDL-C ratio was reduced by - 0.36 compared to baseline in subjects on the MF diet and - 0.06 on the LF diet. The difference for this ratio between these two diets was significant (-0.30 ± 0.09 , $P = 0.001$) (**Figure 3-7**). A greater reduction (0.6, $P < 0.0001$) was seen in subjects with diabetes compared to subjects without diabetes in the TC: HDL-C ratio between MF and LF diets (**Figure 3-7**).

3.4.4 Weighted Averages of the Changes in Lipid and Lipoproteins

The weighted averages show that subjects with diabetes had greater decreases in TC and TG after the MF diets compared to LF diets (**Table 3-5**). In addition, in subjects with diabetes, HDL-C increased by 1.01 mg/dL in response to the MF whereas subjects without diabetes had decreases on both diets.

3.4.5 Regression Analyses

TG changes from baseline were greater with increases in TF ($R^2 = 0.31$, $P < 0.0001$), SFA ($R^2 = 0.20$, $P = 0.0012$), and MUFA ($R^2 = 0.21$, $P = 0.0007$). However, stepwise regression analysis indicated that only increments in TF ($P < 0.0001$) and SFA ($P < 0.05$) were associated with decrements in TG. The changes in TG in subjects without diabetes and subjects with diabetes were in the same direction but not of the same magnitude [e.g., at the lower limit of recommended TF (25 %), TG increased by 6.2 % and 2.4 % from baseline for subjects with and without diabetes; at the higher limit of

recommended TF (35 %), TG decreased from baseline by 3.1 % and 12.2 % for subjects without diabetes and subjects with diabetes, respectively] (**Figure 3-8**).

Increasing TF ($R^2 = 0.11$, $P = 0.008$) and MUFA ($R^2 = 0.13$, $P = 0.007$) resulted in greater decreases in TC from baseline. These dietary factors were not significant when entered into a multiple regression model. Percent HDL-C change from baseline was reduced with increases in TF ($R^2 = 0.26$, $P < 0.0001$) and MUFA ($R^2 = 0.23$, $P = 0.0003$). The changes in HDL-C in subjects without diabetes and subjects with diabetes were similar at the higher limit of recommended TF (decreased by 1.42 % and 1.32 %) but not the same at the lower limit of recommended TF (decreased by 7.83 % and 4.81 % for subjects without diabetes and subjects with diabetes, respectively) (**Figure 3-9**). TF was the only dietary factor positively associated with HDL-C percent change from baseline in the stepwise regression analysis. We also included unsaturated fat (UNSAT) (MUFA + PUFA) in the model to explore the relationship between unsaturated fat and lipid and lipoprotein percent changes from baseline. HDL-C percent changes from baseline were positively correlated with UNSAT ($R^2 = 0.24$, $P = 0.003$) while changes of TG were negatively correlated with UNSAT ($R^2 = 0.24$, $P = 0.002$).

3.4.6 Predicted Changes in CHD Incidence in Men and Women

On the basis of the changes in LDL-C, HDL-C and TG, we calculated the predicted changes for CHD risk (**Table 3-6**) using a model presented by Sacks and Katan (6). Men had 13.73 % reduction in predicted CHD incidence after MF diets and 7.36 % reduction after LF diets (**Figure 3-10**). Similarly, women had 12.95 % and 3.61 % reduction in predicted CHD risk after MF diets and LF diets (**Figure 3-10**), respectively.

3.5 DISCUSSION

Our meta-analysis demonstrated similar TC and LDL-C lowering effects of both diets in subjects and without diabetes and different effects on TG and HDL-C. However, there was a marked TG-lowering in subjects with diabetes versus subjects without diabetes on the MF diet versus LF diet. Despite this, subjects with diabetes still had elevated TG levels on the MF diet, indicating the necessity for further treatment. In contrast, subjects without diabetes with normal TG levels still had a modest decrease in TG on the MF diet. HDL-C decreased similarly in subjects with and without diabetes on both diets (which likely was due to the decreased SFA intake), but the decrease from baseline was less on the MF versus LF diet. Based on predicted changes in CHD incidence, using the prediction model developed by Sacks and Katan, both diets would lower CHD incidence, however, the MF diet would reduce risk more. A MF diet would have 5 % and 6.5 % greater reduction compared to LF diet in predicted CHD incidence in men and women, respectively. The predicted reduction in CHD risk for persons with diabetes would be 12.6 % for men and 12.0 % for women on a LF diet, 17.6% (men) and 18.5% (women) for the MF diet.

Non-HDL-C and apo B are more potent predictors versus other risk factors for CHD incidence among men with diabetes, and the TC: HDL-C ratio is considered to be the best predictor of CHD (41). In our study, the TC: HDL-C ratio and non-HDL-C were decreased significantly in subjects with diabetes after MF diets versus LF diets. Furthermore, the decreases were greater in subjects with diabetes than those without diabetes. Considering these other lipid risk factors, our results, in conjunction with other

reports (6, 42) indicate that an MF diet evokes a greater CHD risk reduction than the LF diet than would be predicted by changes in only LDL-C, HDL-C and TG.

Our findings agree with those reported previously (30-34, 36, 37), and have extended those of others who have compared MF versus LF diets in either subjects with diabetes or healthy subjects. Garg conducted a meta-analysis of high-monounsaturated-fat diets for subjects with diabetes mellitus and reported a reduction in plasma TG [- 32.04 mg/dL; 95 % CI: - 38.27, - 25.81 mg/dL], TC [- 5.85 mg/dL; 95% CI: - 9.36, - 2.34 mg/dL], very low density lipoprotein cholesterol (VLDL-C) [- 7.8 mg/dL; 95 % CI: - 9.36, - 5.85 mg/dL] and a modest increase in HDL-C [1.95 mg/dL; 95% CI: 1.17, 2.73 mg/dL] compared with high carbohydrate (low-fat) diets. LDL-C reduction [- 0.39 mg/dL; 95 % CI: - 3.9, 3.12 mg/dL] was similar on both blood cholesterol-lowering diets (42). Collectively, our results, which are a summary of many studies with both healthy individuals and subjects with diabetes, and those of other investigators, demonstrate that a MF diet elicits more favorable effects on TG and HDL-C in both healthy subjects and subjects with diabetes. This TG-lowering response has been reported to be due to a decrease in hepatic secretion of VLDL triacylglycerol (43).

It seems clear that less carbohydrate and additional unsaturated fat has a positive influence on the lipoprotein levels but this does not address the optimal ratio of MUFA to PUFA. Mediterranean diets are characterized by a low intake of SFA and a relatively high MUFA intake (44). The Seven Countries Study (45) reported that all-cause death rate was negatively associated with MUFA intake and all-cause and CHD death rates were lower in cohorts that consumed olive oil as the primary source of dietary fat (45). Despite evidence from observational studies showing that MUFA may be beneficial,

results from experiments with African Green Monkeys seem to be disparate (46-48). Monkeys were fed a diet that was similar in calories (35 %) from fat but differed in SFA, MUFA, and PUFA. After 5 years, the monkeys fed the high MUFA and PUFA diets had significantly lower LDL-C than the monkeys fed a high SFA diet. HDL-C was higher in the monkeys fed MUFA and SFA versus PUFA. However, the monkeys fed MUFA had the greatest LDL particle enrichment with cholesteryl oleate, as well as the largest LDL particle size (46, 48). Coronary artery atherosclerosis was similar in monkeys fed MUFA and those fed SFA (48). It has been suggested that increased amounts of cholesterol oleate proportional to an increase in LDL-C particle size in nonhuman primates is associated with an increase in coronary artery atherosclerosis (49-52). Although these findings have been reported in other animal models (53), there are no supportive data from human studies.

There are several strengths and limitations of our meta-analysis. An extensive literature search was conducted to identify eligible studies. Well controlled feeding studies were selected in which body weight was maintained. We included 30 clinical trials with 1213 subjects (both healthy subjects and subjects with diabetes), which is a relatively large meta-analysis. However, only seven studies were conducted with subjects with diabetes. Some studies did not report all lipid and lipoprotein data at baseline or after diet intervention which is probably a bias of publication. Furthermore, the studies analyzed were of relatively short duration (2 to 12 weeks). The analysis therefore does not address effects that may take longer to occur. HDL-C for example may change relatively slowly after weight loss or pharmacotherapy requiring many months to achieve a new stable level.

A key question that arises from our analysis is: what is the clinical application of these findings? Of note is that the average fat content of the MF diets evaluated exceeded the upper range recommended for TF (e.g., 35% of calories). Since a major emphasis of nutrition recommendations for overweight persons including individuals with insulin resistant syndrome and diabetes is to decrease calories and lose weight, adding fat to the diet could be problematic if implemented incorrectly, resulting in a hypercaloric diet. Thus, a strategy for achieving a diet higher in TF is to decrease dietary carbohydrate calories (especially refined carbohydrates). Less dietary carbohydrate requires less insulin secretion for glucose homeostasis, thereby benefiting persons with insulin resistant syndrome (54). Moreover, decreasing carbohydrate (without adding fat to the diet) would result in a higher fat (on the basis of percent calories), hypocaloric diet that would favor weight loss. Additional diet composition changes can be made in the type of fat included in the diet. Decreasing dietary carbohydrate, and hence calories, and not adding fat calories should be evaluated in clinical practice for ease of application and health outcomes in different population groups, including healthy persons, and persons with diabetes and insulin resistant syndrome.

In conclusion, a MF blood cholesterol-lowering diet is preferred for healthy individuals and persons with diabetes for improving the lipid profile. Importantly, the predicted CHD risks are 6.37 % lower in men and 9.34 % in women after MF diet compared to LF diet as measured by lipid risk factors. It will be important that MF diets are implemented in a way where fat is not added, but rather, dietary carbohydrate (especially refined) is reduced (as are calories) with an accompanying increase in total fat as a percent of calories (i.e., by replacing cholesterol-raising fats with healthy,

unsaturated fats) and a decrease in calories leading to changes in lipids and lipoproteins reported herein as well as weight loss. By reducing energy intake to promote weight loss, together with modifications in the type and amount of fat in the diet, great strides can be made in decreasing CHD risk by favorably modulating lipid and lipoprotein risk factors.

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Table 3-1 Number of studies, sample sizes and endpoints selected for inclusion in analyses

Endpoints	Number of Studies	Subjects without Diabetes	Subject with Diabetes
TC	31	25	6
LDL-C	27	22	5
HDL-C	30	24	6
TG	29	24	6
Apo A-I	13	13	0
Apo B	13	13	0

TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C; high density lipoprotein cholesterol; TG: triacylglycerol; Apo A-I: apolipoprotein A-I; Apo B: apolipoprotein B;

Table 3-2 Characteristics of studies and study participants

References	Sample Size	Year ¹	Age (years)	BMI (kg/m ²)	Study Design ²	Duration (days)
Ginsberg et al (15)	36	1990	23	23.9	P	70
Berry et al (16)	17	1992	21	21.8	P	84
Baggio et al (7)	11	1988	20.9	24.0	C	21
Grundy et al (8)	10	1988	64	25.9	C	42
Mensink et al (14)	48	1989	27	22.6	P	36
Grundy et al (9)	11	1986	58	28	C	28
Garg et al (33)	10	1992	63	30	C	21
Garg et al (30)	10	1988	56	29	C	28
Kris-Etherton et al (21)	22	1999	34	24	C	24
Berglund et al (38)	85	N/A	35.5	27.6	C	49
Lopez-Segura et al (19)	21	1996	24.4	24.7	C	24
Jensen et al (12)	41	1998	20.6	23	P	28
Garg et al (34)	42	1994	58	28.1	C	42
Garg et al (32)	10	1992	61.5	27.7	C	28
Curb et al (22)	30	2000	35.3	23	C	30
Parillo et al (31)	10	1992	52.7	26.7	C	15
Castro et al (20)	21	2000	23	24.7	C	28
Perez-Jimenez et al (18)	22	1995	23	24.7	P	81
Lopez-Miranda et al (11)	90	1997	22.8	24.8	C	28
Perez-Jimenez et al (24)	59	2001	23.1	22.9	C	28
Perez-Jimenez et al (10)	25	1999	20.6	23.8	C	28
Nelson et al (17)	11	1995	32.9	23.1	C	50
Perez-Martinez et al (24)	59	2001	21	21.1	C	28
Perez-Martinez et al (26)	97	2003	20.1	23.6	C	28
Rajaram et al (25)	23	2001	38	N/A	C	28
Jansen et al (13)	41	2000	20.9	24.5	P	28
Appel et al (35)	161	2005	53.6	30.2	C	42
Moreno et al (27)	84	2004	23.8	22.2	C	28

Sanders et al (28)	29	2003	24.2	24.2	C	14
Bravo-Herrera et al (29)	41	2004	23.4	23	C	28
¹ N/A: not available.						
² P: parallel; C: crossover.						

Table 3-3 Baseline subject characteristics and fat content of diets by group¹

	All subjects (n = 1213)		Subjects without Diabetes (n = 1123)		Subjects with Diabetes (n = 90)	
	MF Diet	LF Diet	MF Diet	LF Diet	MF Diet	LF Diet
Diet groups	MF Diet	LF Diet	MF Diet	LF Diet	MF Diet	LF Diet
Age (years)	34.3±15.9	34.3±15.9	29.3±11.9 ³	29.3±11.9 ⁴	60.5±2.7	60.5±2.7
BMI (kg/m ²)	24.8±2.4	24.8±2.4	23.9±1.7 ³	23.9±1.7 ⁴	28.8±0.9	28.8±0.9
TF (%)	39.2±4.5 ²	26.3±3.7	37.7±2.7 ³	26.5±3.7	47.5±2.9	25.0±4.1
SFA (%)	8.8±1.7 ²	8.0±1.7	8.8±1.5	8.1±1.5	9.0±2.7	7.5±3.1
MUFA (%)	24.1±4.2 ²	11.8±2.5	22.9±3.2 ³	12.0±2.7 ⁴	30.3±3.6	10.5±1.3
PUFA (%)	6.5±2.2	6.2±1.3	6.1±2.1 ³	6.1±1.7	8.5±1.7	6.8±2.2
Cholesterol (mg/d)	253.1±71.3	253.5±72.5	267.5±63.7 ³	267.9±65.1 ⁴	166.7±57.7	166.7±57.7
TC (mg/dL)	190.3±29.3	189.9±30.0	184.5±26.9 ³	184.1±27.7 ⁴	224.6±16.8	224.6±16.8
LDL-C (mg/dL)	121.7±23.4	120.5±23.8	117.4±22.2	116.2±23.4	140.8±17.9	140.8±18.0
HDL-C (mg/dL)	45.2±7.0	45.2±7.0	48.0±11.7 ³	48.0±11.7 ⁴	32.0±2.0	32.0±2.0
TG (mg/dL)	121.9±75.7	121.9±75.7	93.5±11.7 ³	95.6±11.7 ⁴	273.2±71.2	273.2±71.2

¹ Data presented as mean ± SD

² P<0.05 compared to LF (lower-fat) diet in all subjects

³ P<0.05 compared to MF (moderate-fat) diet in subjects with diabetes

⁴ P<0.05 compared to LF diet in subjects with diabetes

Table 3-4 Macronutrient compositions (% of energy) of moderate-fat (MF) and lower-fat (LF) diets in studies selected for inclusion

Reference	% kcal CHO ¹		% kcal Protein		% kcal TF ²		% kcal from fatty acid classes (MF) ³			% kcal from fatty acid classes (LF)		
	MF	LF	MF	LF	MF	LF	SFA	MUFA	PUFA	SFA	MUFA	PUFA
Ginsberg et al. (15)	46.4	52.5	16.1	16.7	37.8	30.1	9.1	18.0	10.7	8.9	10.7	10.5
Berry et al. (16)	50.5	64.9	17.0	16.9	32.5	18.3	6.6	16.6	7.5	4.7	6.8	5.7
Baggio et al. (7)	46	56	16	16	38	28	9.7	24.6	3.5	11.7	12.6	4.1
Grundy et al. (8)	45	65	15	15	40	20	7	27	6	7	7	6
Mensink et al. (14)	46.0	62.2	12.2	14.1	40.6	22.1	9.8	24	5.1	6.7	9.3	5.2
Grundy et al. (9)	43	63	17	17	40	20	4	28	7.5	6.7	6.7	6.7
Garg et al. (33)	35	50	15	15	50	25	11	32	7	8	12	5
Garg et al. (30)	35	60	15	15	50	25	10	33	7	9	9	6
Kris-Etherton et al. (21)	50	59	16	16	34	25	7	21	6	7	12	6
Berglund et al (38)	48.8	54.9	15.5	16.1	35.7	29	8.7	20.8	6.2	8	15.5	5.5
Lopez-Segura et al. (19)	44.1	54.5	17.2	17.6	38.7	27.9	9.2	24.7	4.8	9.2	13.5	5.2
Jensen et al. (12)	44.1	54.5	15	15	38.4	27.9	9.2	24.4	4.8	9.2	13.5	5.2
Garg et al. (34)	40	55	15	15	45	30	10	25	10	10	10	10
Garg et al. (32)	38	65	17	15	45	20	5	31	10	3	11	6
Curb et al. (22)	48	54	17	16	35	30	9	20	6	9	15	7
Parillo et al. (31)	40	60	20	20	40	20	7	29	4	5	13	2
Castro et al. (20)	45.7	56	14.7	14.3	39.6	29.7	9.8	25.3	4.5	6.8	13.8	9.1
Perez-Jimenez et al. (23)	45.7	56	14.7	14.3	39.6	29.7	9.8	25.3	4.5	6.8	13.8	9.1
Lopez-Miranda et al. (11)	44.1	54.5	17.2	17.6	38.7	27.9	9.2	24.7	4.8	9.2	13.5	5.2
Perez-Jimenez et al. (24)	44.1	54.5	15	15	38	28	10	22	6	10	12	6
Perez-Jimenez et al. (10)	44.1	54.5	17.5	17.6	38.4	27.9	9.2	24.4	4.8	9.2	13.5	5.2
Nelson et al. (17)	45.7	61.9	15.7	15.9	38.7	22.2	10.6	15.5	12.6	6.4	9.2	6.6
Perez-Martinez et al. (24)	44.1	54.5	17.5	17.6	38.4	27.9	9.2	24.4	4.8	9.2	13.5	5.2
Perez-Martinez et al. (26)	44.1	54.5	17.7	17.5	38.1	27.5	9.1	24.1	4.9	9.1	13.2	5.2
Rajaram et al. (25)	47.2	56.8	13.1	14.5	39.6	28.3	8.1	18.9	10.7	8.2	11	6.3

Jansen et al. (13)	44.1	54.5	17.5	17.6	38.4	27.9	9.2	24.4	4.8	9.2	13.5	5.2
Appel et al. (35)	48	58	15	15	37	27	6	21	10	6	13	8
Moreno et al. (27)	44.1	54.5	17.7	17.5	38.1	27.5	9.1	24.1	4.9	9.1	13.2	5.2
Sanders et al. (28)	47	53	13	14	37.5	30.2	10.2	10.9	20.8	12.9	6.5	6.4
Bravo-Herrera et al. (29)	47	55	15	15	38	30	10	22	6	10	6	12

¹CHO: carbohydrate; MF: moderate-fat diets; LF: lower-fat diets.

²TF: total fat

³SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Table 3-5 Weighted averages of the lipid and lipoproteins in selected studies

	All subjects		Subjects without Diabetes		Subjects with Diabetes	
	MF Diets	LF Diets	MF Diets	LF Diets	MF Diets	LF Diets
TC (mg/dL)	-16.75	-15.99	-15.99	-15.71	-33.66	-22.44
LDL-C (mg/dL)	-15.29	-14.10	-15.52	-13.98	-15.56	-16.48
HDL-C (mg/dL)	-0.78	-3.21	-0.81	-3.29	1.01	-1.50
TG (mg/dL)	-9.06	2.04	-5.43	2.25	-79.21	-2.06
Apo A-I (g/L)	-0.03	-0.05	-0.03	-0.05	N/A	N/A
Apo B (g/L)	-0.06	-0.06	-0.06	-0.06	N/A	N/A

MF diets: moderate-fat diets; LF diets: lower-fat diets; N/A; not applicable. The averages of the endpoints were weighted by the sample sizes. The values are presented as the difference between the after diet treatment and baseline.

Table 3-6 Predicted changes in coronary heart disease (CHD) incidence

Serum Lipid and Lipoproteins	MF Diet	LF Diet
LDL-C ¹ concentration (mg/dL)	- 15.3	- 14.1
CHD incidence	- 15.3 %	- 14.1 %
HDL-C ² concentration (mg/dL)	- 0.8	- 3.2
CHD incidence		
Men	1.6 %	6.4 %
Women	2.3 %	9.6 %
TG ³ concentration (mg/dL)	- 9.1	2.0
CHD incidence		
Men	ND	0.3 %
Women	ND	0.9 %

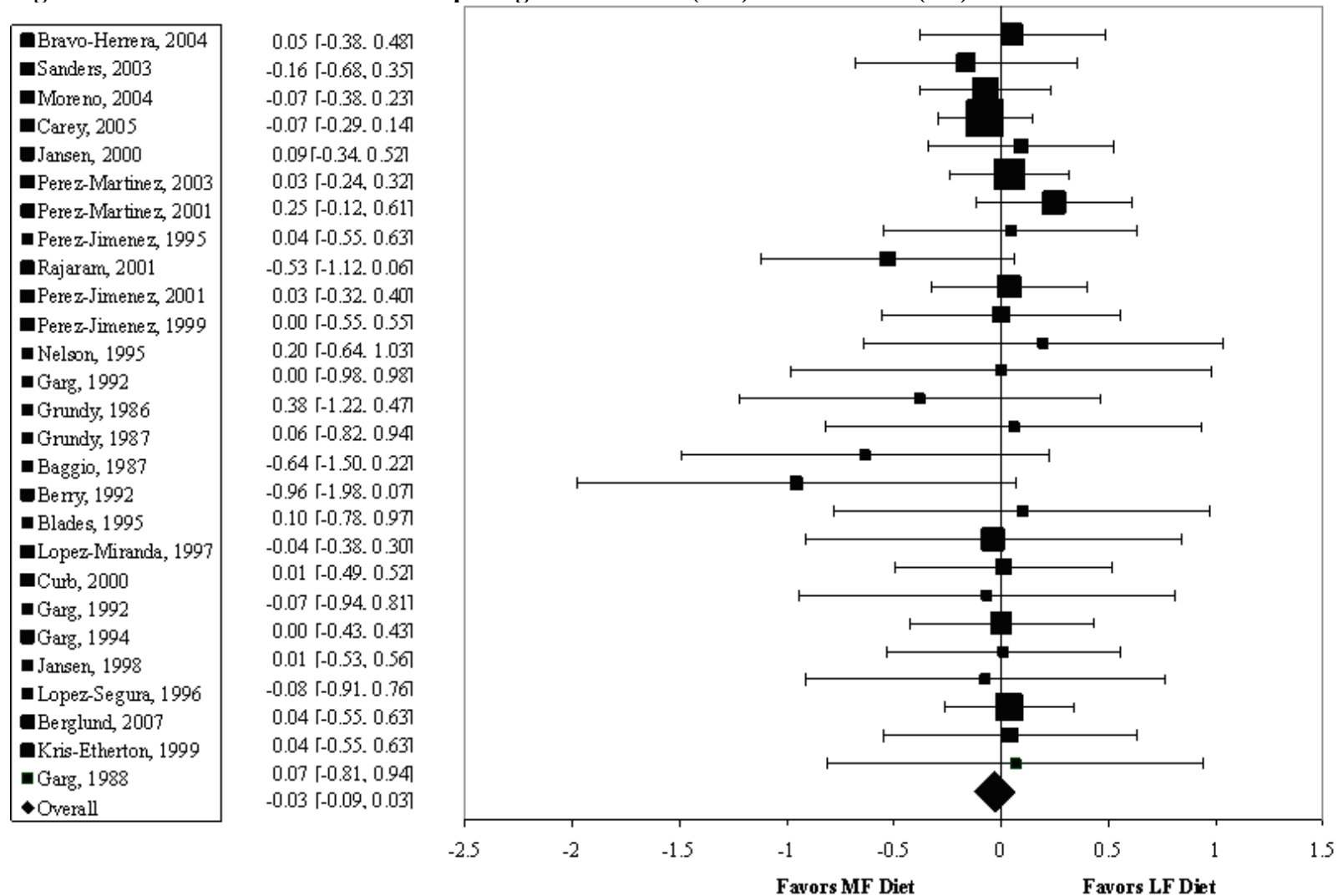
¹ Δ LDL: - 1 mg/dL leads to Δ Coronary Artery Disease (CAD): - 1 % (55).

² Δ HDL: - 1 mg/dL leads to Δ Coronary Artery Disease (CAD): + 2 % in men; + 3 % in women (56).

³ Δ TG: + 88 mg/dL leads to Δ Coronary Artery Disease (CAD): + 14 % in men; + 37 % in women (57).

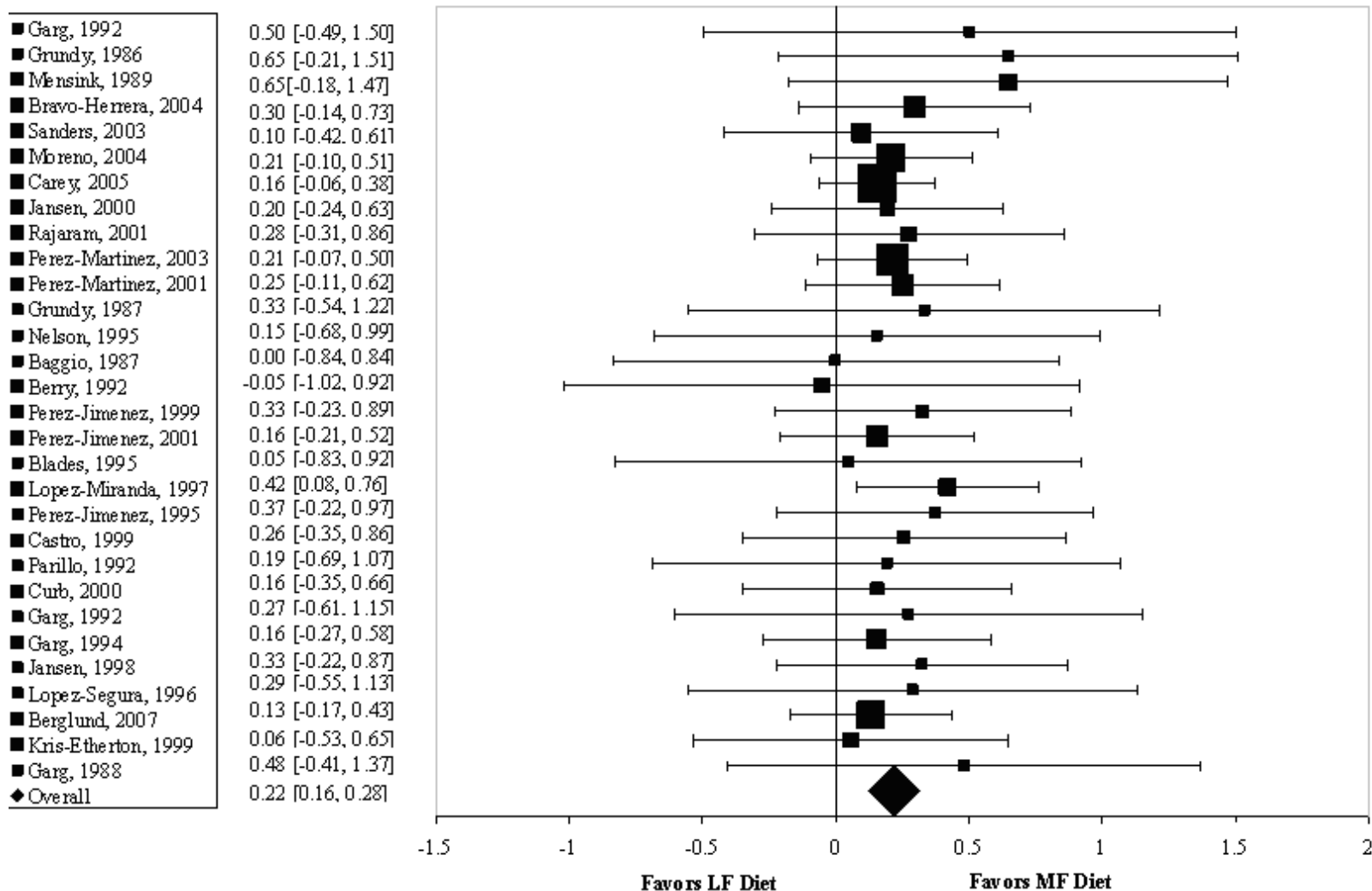
ND: not defined.

Figure 3-1 Effect sizes for LDL-C comparing moderate-fat (MF) and lower-fat (LF) diets



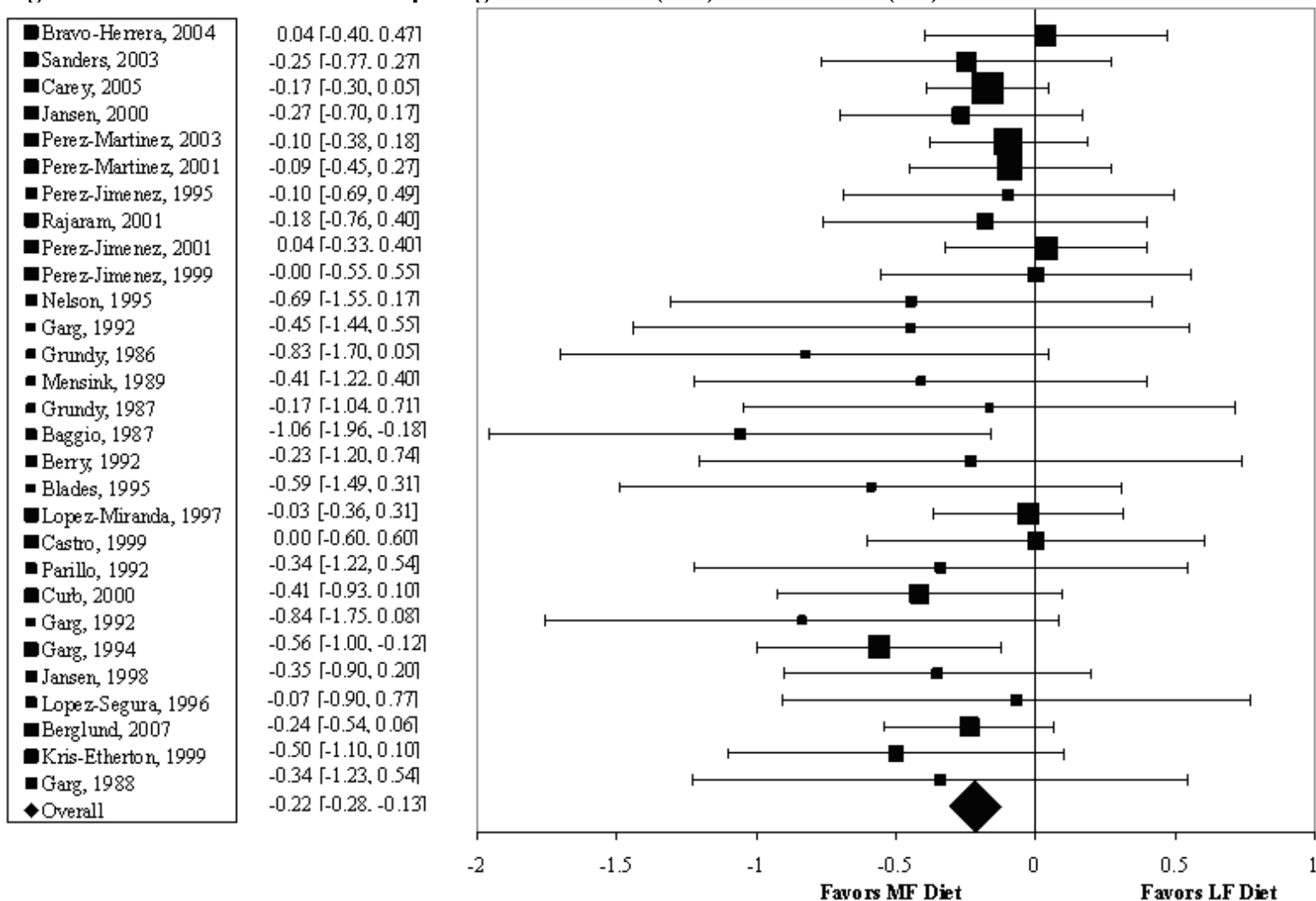
No differences were shown between these two diets on LDL-C.

Figure 3-2 Effect sizes for HDL-C comparing moderate-fat (MF) and lower-fat (LF) diets



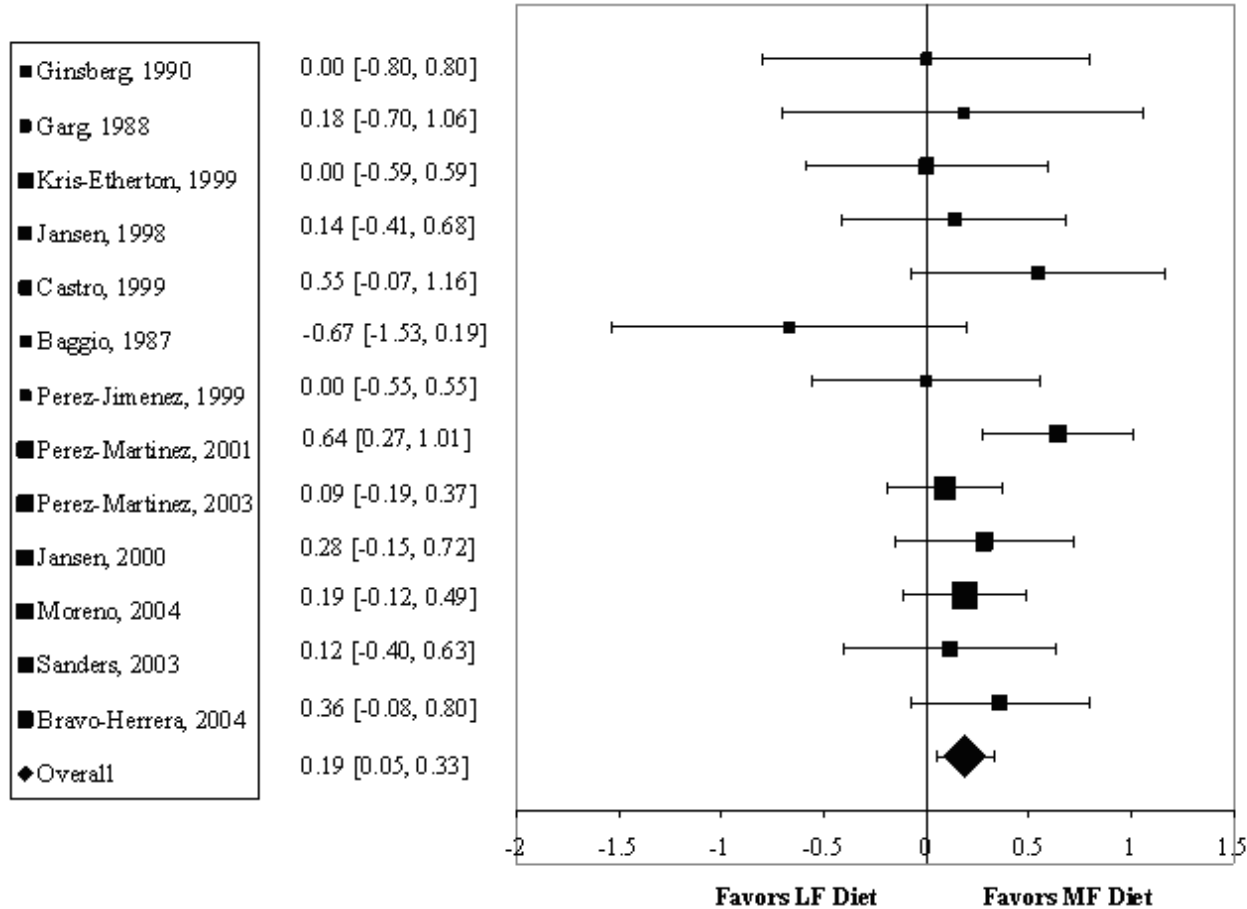
HDL-C concentrations were higher on the MF diets ($d = 0.22 \pm 0.03$, $p < 0.00001$; 2.28 mg/dL) compared to LF diets.

Figure 3-3 Effect sizes for TG comparing moderate-fat (MF) and lower-fat (LF) diets



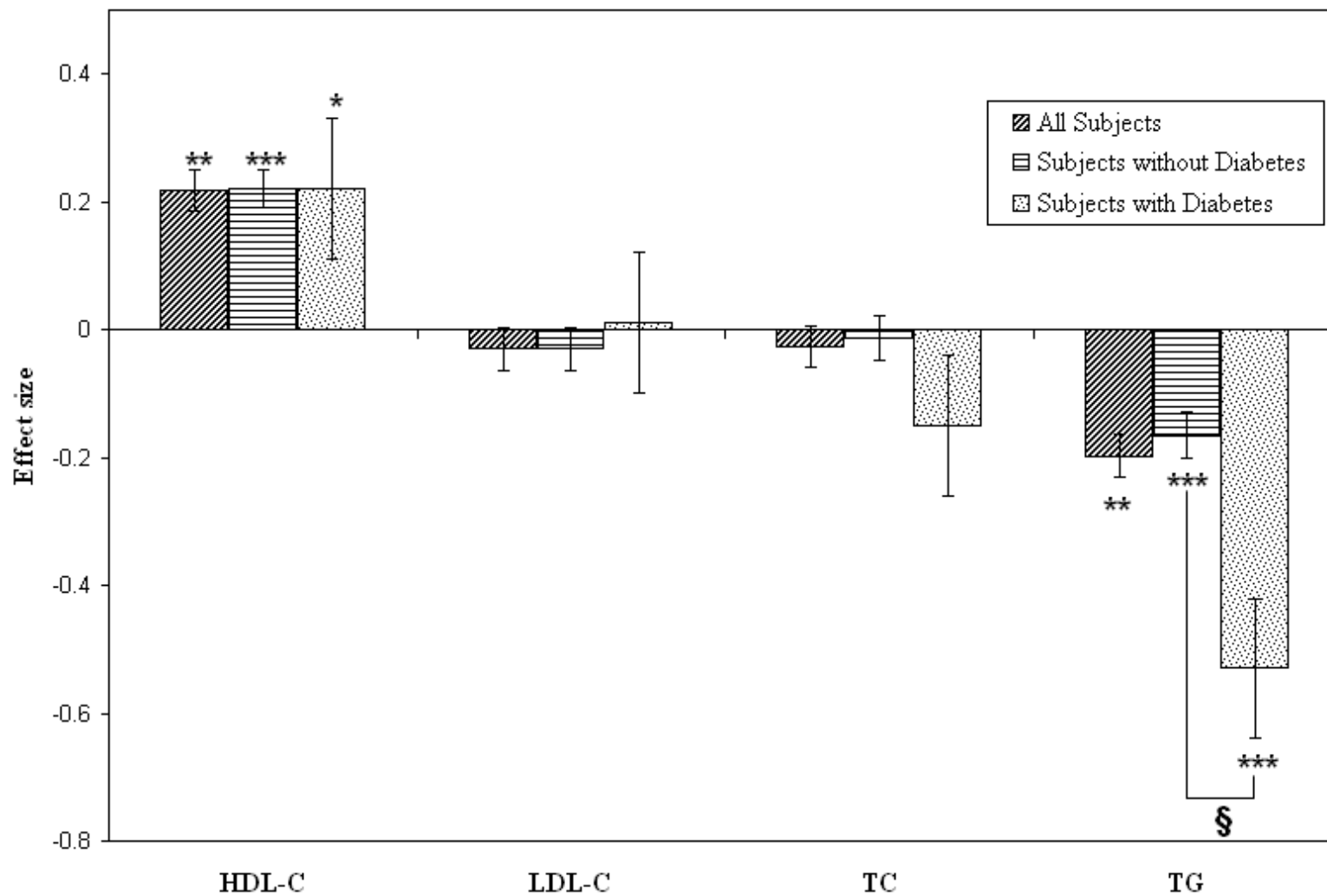
TG concentrations were reduced by the MF diets ($d = -0.20 \pm 0.03$, $P < 0.00001$; -9.36 mg/dL) compared to LF diets.

Figure 3-4 Effect sizes for Apo A-I comparing moderate-fat (MF) and lower-fat (LF) diets



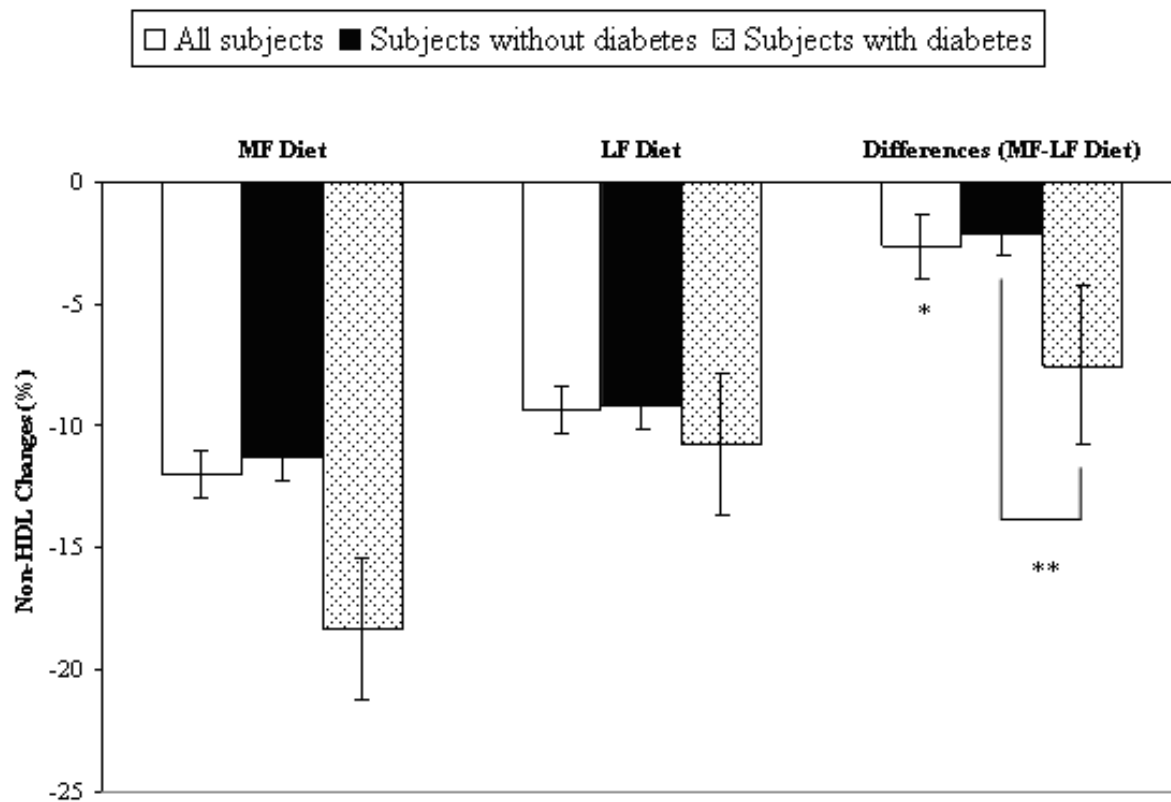
Apo A-I concentration was increased by the MF diets ($d = 0.19 \pm 0.07$, $P = 0.006$) compared to LF diets.

Figure 3-5 Effect sizes for changes in lipid and lipoproteins in subjects with and without diabetes



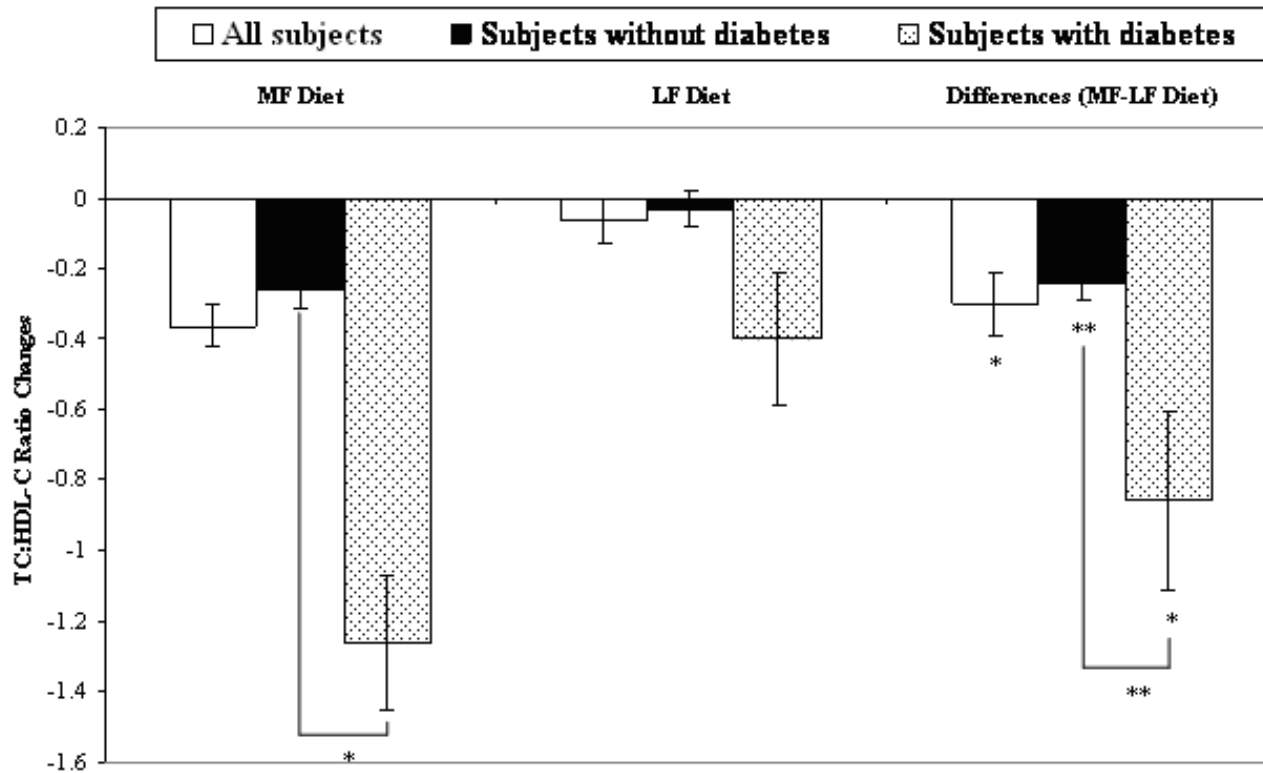
*** $p < 0.000001$, ** $p < 0.00001$, * $p < 0.05$: significant difference between MF Diet and LF Diet; § $p < 0.05$: significant difference between subjects without and with diabetes. Subjects with diabetes had a greater reduction in TG ($d = -0.51 \pm 0.17$, ≈ -23.86 mg/dL) compared to subjects without diabetes ($d = -0.17 \pm 0.04$, ≈ -7.95 mg/dL) ($P < 0.05$).

Figure 3-6 Non-HDL-C Changes from baseline for moderate-fat (MF) and lower-fat (LF) diets



* $P < 0.05$, ** $P < 0.06$ Non-HDL-C was decreased by 12.01 % from baseline in the MF diets and by 9.37 % in the LF diets. MF diets had a 2.64 % greater decrease. Subjects with diabetes had a 5.39 % greater reduction compared to subjects without diabetes ($P < 0.06$) in the differences between MF and LF diets.

Figure 3-7 TC: HDL-C ratio changes from baseline



*P < 0.0001, **P < 0.001 TC: HDL-C ratio was reduced by - 0.36 compared to baseline in MF diets and - 0.06 in the LF diets.

The difference between MF and LF diets was - 0.30 ± 0.09 (P < 0.001). Subjects with diabetes had a greater reduction (0.62, P < 0.0001) compared to subjects without diabetes in the differences between MF and LF diets.

Figure 3-8 The correlation between TG percent changes from baseline and total fat

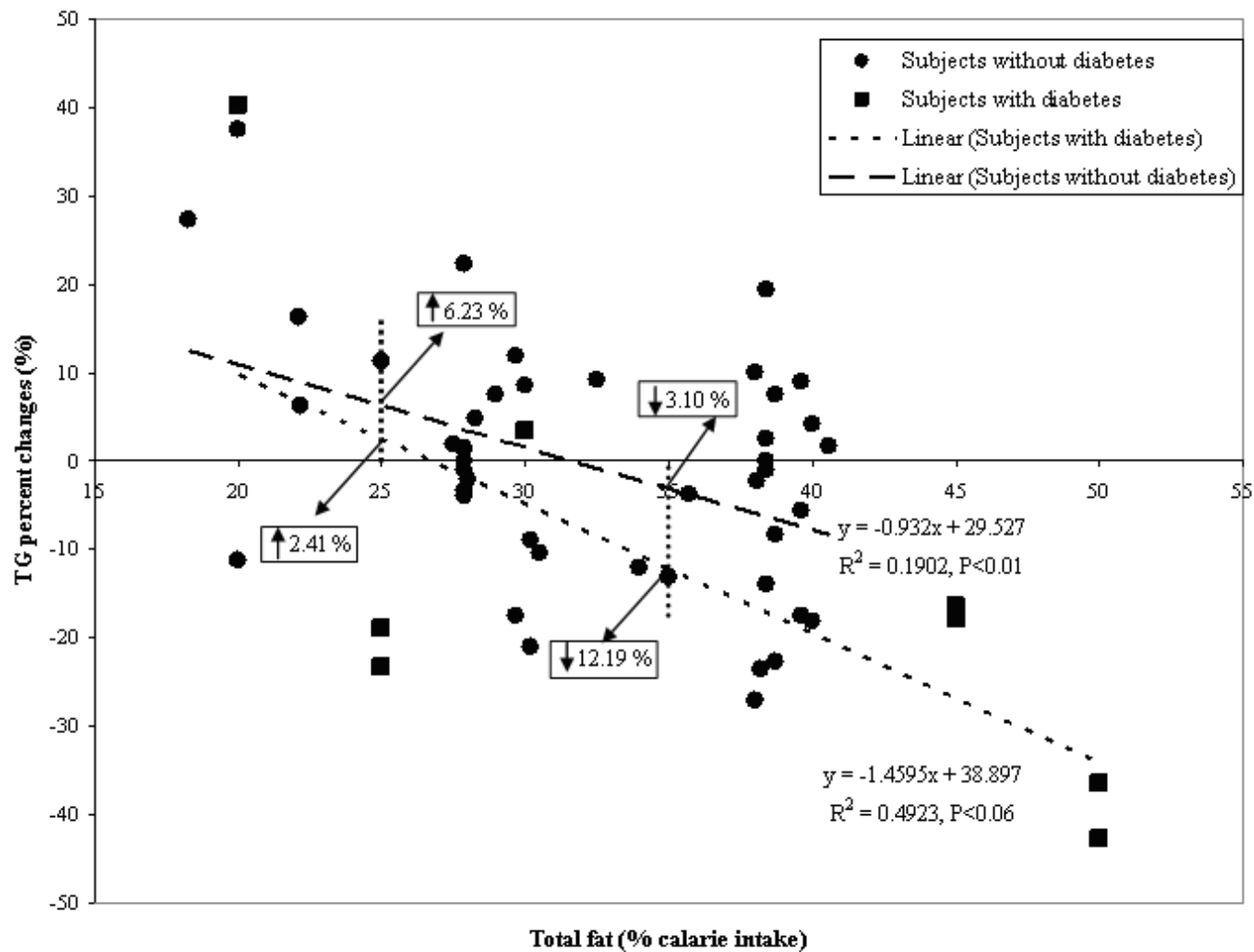


Figure 3-9 The correlation between HDL-C percent changes from baseline and total fat

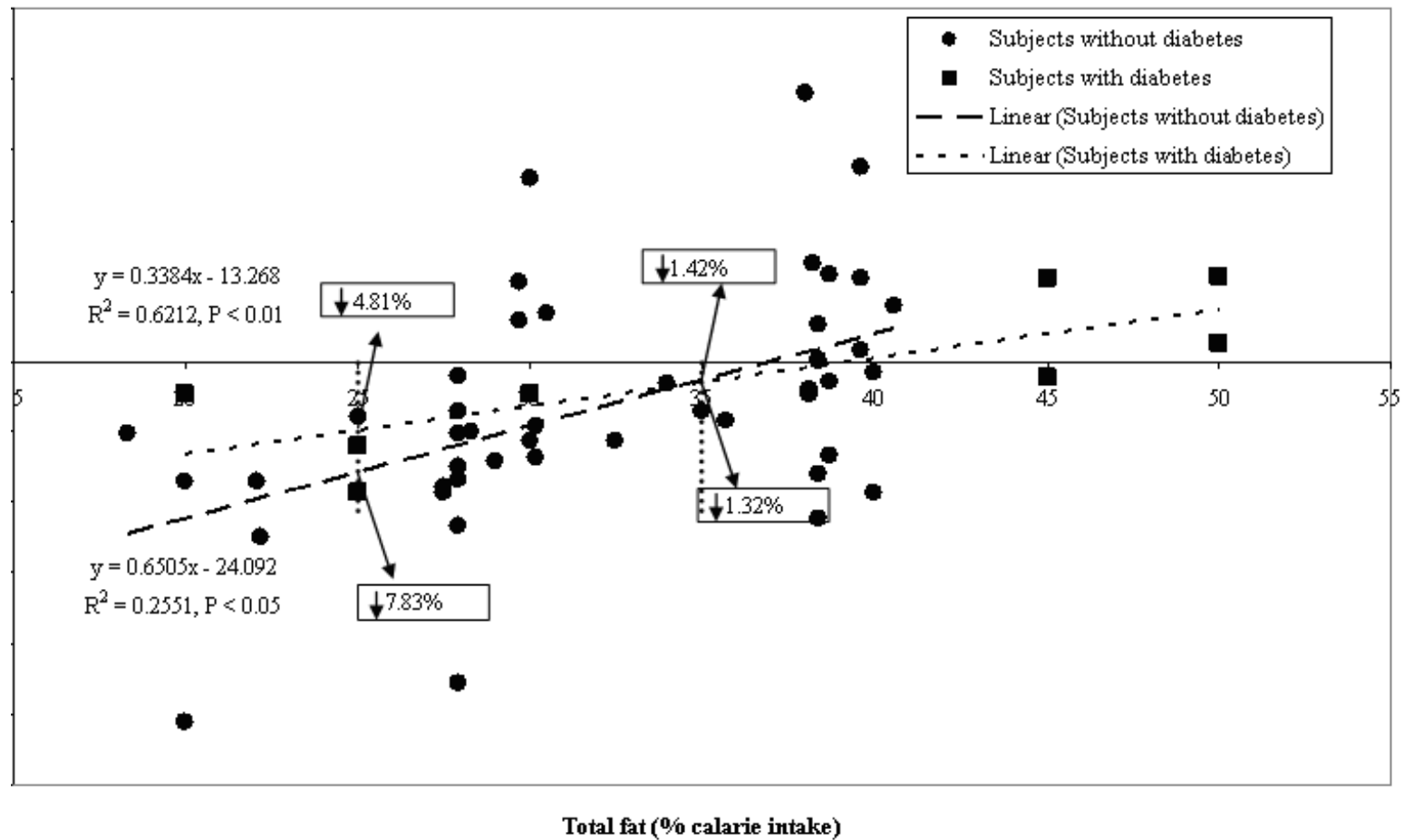
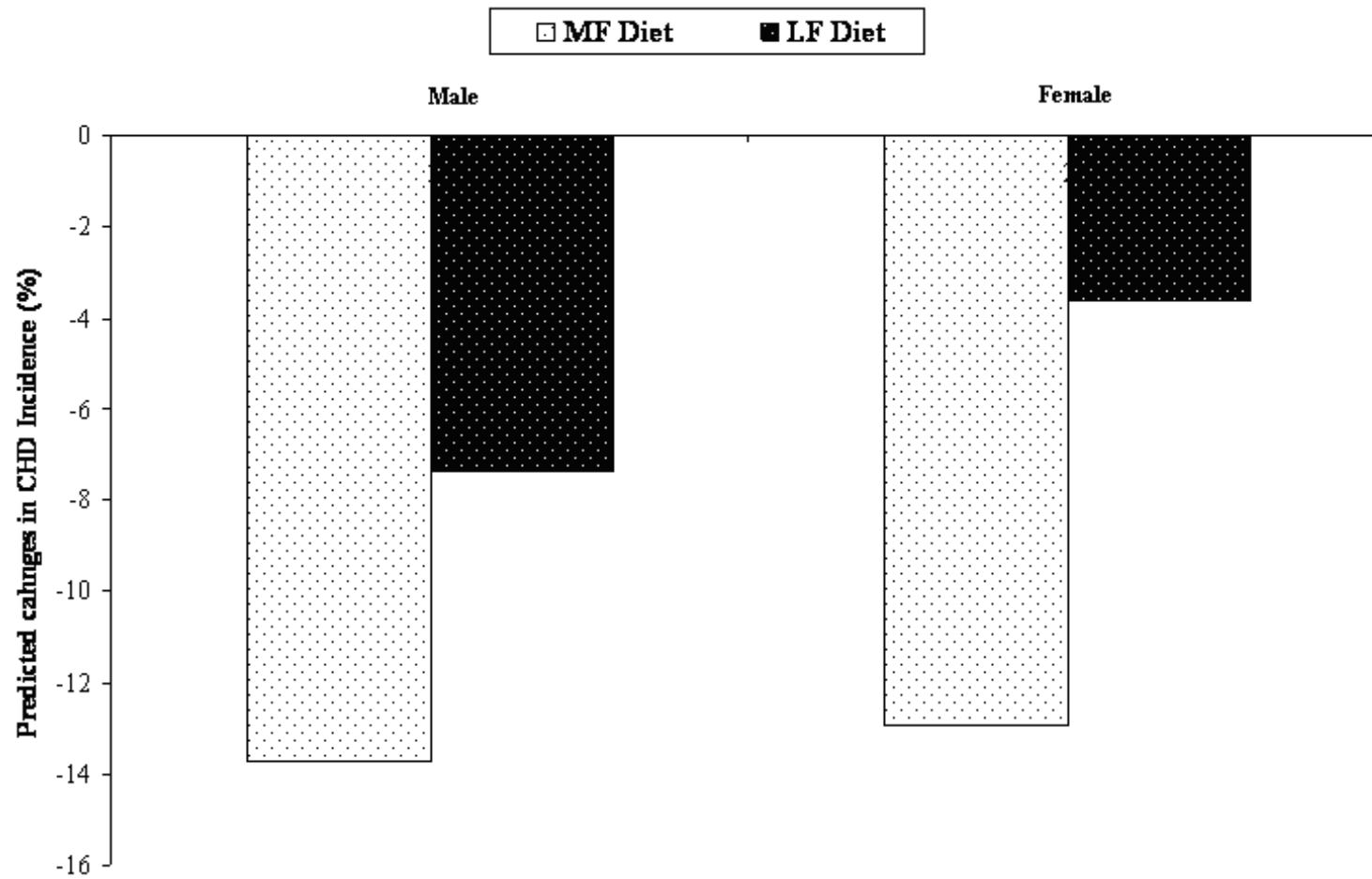


Figure 3-10 Predicted changes in CHD incidence in males and females in response to MF and LF diets



Chapter 4

LIPIDS AND OXIDATIVE STRESS RESPONSES TO A DIET RICH IN MACADAMIA NUTS ARE MODIFIED BY BASELINE PLASMA C-REACTIVE PROTEIN

4.1 ABSTRACT

C-reactive protein (CRP), a marker of inflammation, is a predictor of cardiovascular disease (CVD). Inflammation contributes to increased risk by altering cholesterol and triglyceride metabolism. We evaluated the effect of baseline CRP on lipid, lipoprotein, and oxidative stress responses to a cholesterol-lowering diet rich in macadamia nuts (MAC) versus an average American diet (AAD). Twenty-five mildly hypercholesterolemic men (n = 15) and women (n = 10) participated in a 2 - period randomized, crossover, controlled feeding study. Subjects were classified according to baseline CRP $<$ or \geq 1 mg/L. The MAC diet (33 % total fat, 7 % SFA, 18 % MUFA, 5 % PUFA) was compared with an AAD (33 % total fat, 13 % SFA, 12 % MUFA, 5 % PUFA). In individuals with baseline CRP levels $<$ 1 mg/L only, TC, LDL-C, and non-HDL-C were reduced following the consumption of MAC diet versus the consumption of AAD (TC: -24.7 mg/dL (-11.0 %), $P < 0.0001$; LDL-C: -16.2 mg/dL (-10.6 %), $P < 0.001$; non-HDL-C: -20.7 mg/dL (-11.3 %), $P < 0.0001$). In contrast, these risk factors were not decreased in individuals with baseline CRP $>$ 1 mg/L. Lipid hydroperoxide (LOOH) decreased after the consumption of AAD ($-0.6 \pm 0.2 \mu\text{M}$; $P < 0.01$) and MAC diets ($-0.4 \pm 0.2 \mu\text{M}$; $P < 0.06$) vs. baseline in the CRP $<$ 1 mg/L group, and was unchanged in the CRP \geq 1 mg/L group. In summary, our results demonstrate that inflammation prevents the beneficial effects of a heart healthy diet on CVD lipid risk factors, as well as oxidative stress.

4.2 INTRODUCTION

Inflammation plays an important role in the pathogenesis of atherosclerosis and cardiovascular disease (CVD) (1). Elevated serum C-reactive protein (CRP), a marker of systemic inflammation, is an independent risk factor for CVD (2). Inflammation may contribute to an increase in CVD risk by altering cholesterol and triacylglycerol (TG) metabolism (3). This is characterized by elevated TG levels [due to an increase in very low-density lipoprotein (VLDL)] low levels of high-density lipoprotein cholesterol (HDL-C) (4, 5), and an increase in small, dense LDL particles, which, collectively, are associated with an increase in CVD risk (6, 7). In addition, many individuals with an elevated low-density lipoprotein cholesterol (LDL-C) also present with high CRP levels (2).

Several studies (8-10) have shown that inflammation may affect the lipid and lipoprotein responses to cholesterol-lowering diets. In the Dietary Approaches to Stop Hypertension (DASH)-Sodium trial, individuals who had baseline CRP levels below median (low CRP; CRP < 2.37 mg/L) had a significant reduction in TC (- 9.8 %; P < 0.0001) and LDL-C (- 11.8 %; P < 0.0001) in response to the blood cholesterol lowering DASH diet (9). In contrast, both TC and LDL-C did not change in individuals with baseline CRP levels above median (high CRP; CRP > 2.37 mg/L). TG was increased significantly in individuals with high CRP (19.8 %, P < 0.0001) while it remained unchanged in those with low CRP. Similarly, Hilpert et al. showed that soy protein or milk protein included in a Step I diet both decreased LDL-C (-3.5 %) and LDL: HDL-C ratio (-4.8 %) in individuals with low baseline CRP (below median; CRP < 3.5 mg/L) (10). In contrast, LDL-C (4.8 %) and LDL: HDL-C ratio increased significantly in

individuals with high baseline CRP (above median). More recently, it has been reported that TG and VLDL-TG were decreased significantly in individuals with low baseline CRP (< 1 mg/L) but increased in individuals with high baseline CRP (≥ 1 mg/L) after consumption of a low-fat diet (8). Furthermore, TG, VLDL-TG, and VLDL-C were reduced in individuals with low baseline CRP only ($P < 0.0001$). Collectively, these studies indicate that inflammation blunts lipid/lipoprotein responses to dietary intervention. In the current study, we hypothesized that inflammation status at baseline (mainly CRP status) could modify the lipid and lipoprotein responses to a blood cholesterol-lowering diet high in unsaturated fatty acids provided by macadamia nuts (MAC diet).

It is well established that oxidation plays an important role in the early development of atherosclerotic plaques (11). Susceptibility to oxidation of plasma LDL is reduced after consumption of a high MUFA diet (12). Moreover, a diet high in MUFA provided by almonds (which also are a source of many antioxidants) has been shown to reduce oxidized LDL (13). Therefore, we hypothesized that oxidative stress [measured by lipid hydroperoxide (LOOH) level (14)] would be reduced by the consumption of MAC diet. Moreover, we hypothesized that CRP status would modify oxidative stress response to diet.

4.3 MATERIALS AND METHODS

4.3.1 Participants

Twenty-six men and women (10M and 15F) were recruited through advertisements in local newspapers. One male did not complete the study (he completed the first five-week period but elected to not continue participating in the second five-

week period); his data were not included in the analysis. Subjects were eligible if they were: (1) 25 - 65 years old; (2) BMI (body mass index) 22 - 35; (3) non-smokers; (4) HDL-C levels between 10 - 90th percentile (33.98 - 69.11 mg/dL), LDL-C levels between 25 - 90th percentile (101.93 - 174.90 mg/dL) according to NHANES (National Health and Nutrition Examination Survey) (15). Subjects were excluded if: (1) they had CVD, diabetes, cancer, or any other severe medical conditions; (2) they were taking lipid lowering medications or other medications that would affect inflammation; (3) they had nut allergies; (4) women were lactating or pregnant. The study protocol was approved by the Pennsylvania State University Institutional Review Board and all participants gave written informed consent.

4.3.2 Experimental Design

In this randomized, two-period crossover, controlled feeding study, subjects were randomly assigned to either the Average American Diet (AAD) or a blood cholesterol-lowering diet rich in macadamia nuts (MAC) for five weeks. After a two-week compliance break, subjects switched to the other diet and consumed it for another five weeks. Subjects were instructed to maintain their usual physical activity and discontinue vitamin supplements during the study. Consumption of alcoholic beverages was limited to two drinks per week. Body weights were measured daily at the Metabolic Diet Study Center when subjects arrived for their meal. Fasting blood samples were collected for two consecutive days at the beginning of the study and at the end of each of diet period at the General Clinical Research Center (GCRC) on the University Park campus. All samples were then stored at - 80°C and analyzed together at the end of the study. Further details about the experimental design have been reported previously (16).

To assess the effect of inflammatory status on diet effects on lipids, lipoproteins and LOOH, participants were assigned to two groups based on their baseline CRP levels. According to the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA), low, average, and high relative risk categories were recognized as CRP < 1.0, 1.0 - 3.0, and > 3.0 mg/L, respectively (17). The participants in the current study were relatively healthy. Therefore, the CRP groups were stratified by CRP level ≥ 1 or < 1 mg/L.

4.3.3 Experimental Diets

A 6-day menu cycle for each diet was developed by the dietitian (AMC) using Food Processor SQL software. All meals were prepared daily in the Metabolic Diet Study Center and subjects consumed at least one meal per day on weekdays. Other meals and snacks for weekdays and the weekend were prepared and packed in coolers for subjects to take out and eat at a time and place of convenience. Macadamia nuts were incorporated into entrees or consumed as snacks, along with other popular snacks, some of which were provided on the AAD. Snacks on the AAD included pretzel twists, cheddar cheese cubes, peaches, pears, granola bars and chocolate candy. The composition of the experimental diets has been reported previously (16). The two diets were matched in carbohydrate, protein and total fat. Briefly, saturated fat, monounsaturated fat, cholesterol, and dietary fiber of the two test diets reflected the average intake of these nutrients in a typical American diet (AAD), and the recommended nutrient profile of a blood cholesterol-lowering diet (MAC diet). Both diets had the same amount of polyunsaturated fat (5 % energy). AAD had 13 % energy from saturated fat and 12 % energy from monounsaturated fat while MAC diet had 7 % and 18 % energy,

respectively. AAD had 21 g/d of fiber and 290 mg/d of cholesterol while MAC diet had 23 g/d of fiber and 280 mg/d of cholesterol. In addition, the MAC diet provided 1.5 oz/d of macadamia nuts.

4.3.4 Biochemical Assays

Blood samples were collected from subjects (12-h fast; 48-h alcohol absentia) before and after each diet intervention. Blood samples were centrifuged and then stored at - 80 °C. CRP, TNF- α , IL-1, and IL-6 were measured using high sensitive Enzyme-Linked ImmunoSorbant Assay (ELISA) developed by the Cytokine Core Laboratory of the Pennsylvania State University GCRC. Serum TC, HDL-C, and TG were determined by standard enzymatic assays with commercially available kits. HDL-C was determined after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium. LDL-C was then calculated using Friedewald equation (18) and non-HDL-C was calculated as HDL-C subtracted from TC. LOOH, a measure of total lipid peroxidation, (19-21) was measured by spectrophotometric techniques using a commercial assay (Cayman Chemical Co., Ann Arbor, MI)(22). All of the endpoints were measured at two consecutive days except for LOOH. The values of means of the two days were used for the analyses.

4.3.5 Statistical Analyses

All analyses were done using SAS (version 9.1, SAS Institute). Values were presented as mean \pm SEM (standard error of mean) unless otherwise noted. Variables that were not normally distributed were transformed using the natural log.

The coefficient of variance (CV) for serum lipids and lipoproteins on two days was calculated. The potential outliers were detected by using the PROC UNIVARIATE

statement generating inter-quartile range (IQR) in SAS based on the CV between the two days. Observations that were outside of $Q_1 - (1.5 \cdot \text{IQR})$ and $Q_3 + (1.5 \cdot \text{IQR})$ were detected as potential outliers and excluded from the analysis. Therefore, the final analysis included the following number of data points for the lipids and lipoproteins: TC (19), LDL-C (20), HDL-C (21), and TG (23). To be consistent with our previous analyses, the current analyses used the dataset that excluded outliers (16). We had similar results when the analyses were done using all of the subjects (data not shown). The same method was used to detect potential outliers for the CRP values but none was found. One of the CRP values was greater than 10 mg/L due to the subject having a cold and was excluded (17).

Two-sample *t*-tests were used to test for differences between the CRP groups (measured at baseline). The Wilcoxon rank-sum test was used if the baseline variables were not normally distributed. The mixed model (diet, order, period, CRP group, and their interactions) was used to compare the absolute lipids/lipoproteins values at the end of each dietary period based on initial CRP concentrations. Percentage changes from baseline of the two diets on lipids profile were also evaluated. Age, BMI, waist circumferences, baseline lipid and lipoproteins and changes in body weight during the course of the study were included as covariates in the model. The association between CRP levels and changes in lipids and lipoproteins were calculated by Spearman's correlation coefficients. Treatment differences were determined using the Tukey-Kramer test; $P \leq 0.05$ were considered statistically different and $P \leq 0.10$ indicated a trend for significance.

4.4 RESULTS

Subjects in the CRP ≥ 1 mg/L group were older, had a higher BMI and waist circumference, and higher TC, TG, and fasting glucose levels at baseline (**Table 4-1**). Other baseline characteristics (weight, systolic blood pressure, diastolic blood pressure, LDL-C, HDL-C, Total/HDL cholesterol ratio, and LOOH) did not differ between the two groups. There were 14 subjects (7M and 7F) in the CRP < 1 mg/L group and 10 subjects (2M and 8F) in the CRP ≥ 1 mg/L group. Subjects maintained their body weight throughout the study.

4.4.1 Effect of Diet on Inflammatory Markers

Baseline CRP was 1.12 ± 0.18 mg/L. After the consumption of AAD and MAC diet it was 0.92 mg/L and 0.98 mg/L, respectively (diet effect, $P > 0.10$). In a further subgroup analysis, individuals in the CRP ≥ 1 mg/L group experienced a reduction in CRP after consumption of AAD (1.30 ± 0.29 mg/L, $P < 0.01$) and MAC diet (1.49 ± 0.29 mg/L, $P < 0.05$) compared to baseline (2.00 ± 0.29 mg/L) (**Figure 4-1**). In addition, in subjects with baseline CRP < 1 mg/L, CRP significantly increased after consumption of AAD (0.67 ± 0.08 mg/L) compared to baseline (0.48 ± 0.08 mg/L, $P < 0.05$) and MAC diet (0.51 ± 0.08 mg/L, $P < 0.05$) (**Figure 4-1**). In contrast, CRP did not differ from baseline after consumption of MAC diet in subjects with low baseline CRP levels.

The IL-1, IL-6, and TNF-alpha levels were 10.74 (7.83 - 454.31) pg/ml, 28.34 (11.26 - 537.34) pg/ml, and 19.59 (9.97 - 251.29) pg/ml, respectively, after five weeks consumption of the AAD. Following the 5-week consumption of MAC diet, the IL-1, IL-6, and TNF-alpha levels were 0.62 (0.08 - 6.90) mg/L, 9.03 (7.83 - 438.01) pg/ml, 26.27

(11.26 - 566.80) pg/ml, and 15.09 (9.97 - 338.46) pg/ml, respectively. These values did not differ from baseline or following the consumption of AAD.

4.4.2 Lipid Hydroperoxide (LPO) Response to Diets

LOOH decreased significantly after consumption of AAD and MAC diets compared with baseline (**Figure 4-2**); the reductions did not differ between the two diets. We further examined whether the inflammatory status affected LOOH responses to diets. In the CRP < 1 mg/L group, LOOH decreased after the consumption of AAD (-0.6 ± 0.2 μ M; $P < 0.01$) compared to baseline (**Figure 4-2**). There also was a trend for lower LOOH after consumption of the MAC diet (-0.4 ± 0.2 μ M; $P < 0.06$). In contrast, LOOH did not differ in the CRP ≥ 1 mg/L group after the two diets.

4.4.3 Effect of Diets on Lipids and Lipoproteins as a Function of Baseline CRP

Status

As reported previously by Griel et al (16), the consumption of MAC diet resulted in significant reductions in serum TC, LDL-C, HDL-C, and non-HDL-C compared to both baseline and AAD, whereas TG did not change significantly after both diets (**Figure 4-3**). HDL-C was decreased after the consumption of MAC diet compared to AAD.

In subjects with CRP < 1 mg/L, there were significant reductions in TC (13.9 %, $P < 0.0001$), LDL-C (16.7 %, $P = 0.005$), HDL-C (10.6 %, $P < 0.001$), and non-HDL-C (14.6 %, $P = 0.003$) after consumption of MAC diet compared to baseline (**Figure 4-4**). Furthermore, differences in lipid responses to the consumption of MAC diet were noticeable although those did not reach a significance level. In contrast, the reductions were less in subjects with baseline CRP ≥ 1 mg/L (**Figure 4-4**). In addition, the lipid and lipoprotein changes from baseline after consumption of AAD were not significant.

TC, LDL-C, HDL-C, non-HDL-C, TG, and TC: HDL-C decreased significantly in the CRP < 1 mg/L group after the consumption of MAC diet compared to AAD (**Table 4-2**). TC and LDL-C were reduced significantly by 24.7 mg/dL (11.0 %) and 16.2 mg/dL (10.6 %), respectively, after the consumption of MAC diet compared to AAD in subjects with low baseline CRP. In contrast, in subjects with high baseline CRP, the reductions were not significant [TC, 7.0 mg/dL (3.6 %) and LDL-C, 1.5 mg/dL (2.0 %), respectively]. Similarly, TG decreased significantly by 28.2 mg/dL (14.6 %) ($P < 0.05$) in the CRP < 1 mg/L group but not in the CRP \geq 1 mg/L group ($P = 0.82$) after the consumption of MAC diet compared to AAD. There were similar reductions in HDL-C when subjects consumed the MAC diet versus AAD in low and CRP \geq 1 mg/L groups [4.1 mg/dL (7.3 %) and 2.9 mg/dL (5.2 %), respectively]. The decrease in non-HDL-C was greater in the CRP < 1 mg/L group [20.7 mg/dL (11.3 %)] compared to the CRP \geq 1 mg/L group [4.2 mg/dL (2.7 %)] after the consumption of MAC diet versus AAD ($P < 0.01$). Furthermore, TC: HDL-C was significantly reduced while LDL-C: HDL-C was marginally decreased after the MAC diet in the CRP < 1 mg/L group only (0.4, $P < 0.05$ and 0.2, $P = 0.08$, respectively).

Tests for an interaction between diet and CRP group were significant for TC, LDL-C and non-HDL-C. Moreover, after adjustment for age, BMI, waist circumference, baseline lipid and lipoproteins and changes in body weight during the course of the study, the interactions remained significant.

CRP concentrations were significantly correlated with changes in TC, LDL-C, and LDL-C: HDL-C ratio in the MAC diet group only. Changes in non-HDL-C after the

consumption of MAC diet and changes in TC after the consumption of AAD also were negatively correlated with CRP levels ($P < 0.1$).

4.5 DISCUSSION

The present study demonstrates that inflammatory status influences the lipid and oxidative stress responsiveness to a macadamia-rich, cholesterol-lowering diet. The MAC diet lowered TC, LDL-C, non-HDL-C in subjects with low baseline CRP levels only. Subjects in the $CRP \geq 1$ mg/L group had a higher BMI, waist circumference, fasting glucose, TC and TG at baseline compared to $CRP < 1$ mg/L group. LOOH only decreased in the $CRP < 1$ mg/L group after consumption of MAC diet and AAD compared to baseline but not in the $CRP \geq 1$ mg/L group. The significance of these findings relates to the growing overweight and obesity problem in the United States and worldwide. Obesity is accompanied by inflammation and insulin resistance and both have been shown to diminish diet response (9, 10, 23). Overweight/obese individuals are at an increased risk for coronary heart disease (CHD) because they have higher LDL-C, high blood pressure, and frequently, metabolic syndrome (24). Our results and those of others demonstrated a blunted diet response: overweight/obese people do not achieve the full benefits of a blood cholesterol lowering diet. An explanation for this is that the synthesis of total body cholesterol in obese individuals is higher than that for non-obese individuals, resulting in an increase in circulating cholesterol (25, 26), principally LDL-C. This would have the consequent effect of suppressing the LDL receptor in the liver further increasing LDL-C (26). This, again, reinforces the importance of weight loss for overweight/obese individuals to achieve maximal benefits of a blood cholesterol lowering diet.

The mechanism by which inflammation alters lipids and lipoproteins is not clear. There is some evidence that CRP binds to TG-rich and apoB-containing particles (27, 28) which may induce changes in lipid metabolism. Animal studies have shown that hepatic cholesterol synthesis was increased whereas LDL clearance, hepatic cholesterol catabolism and excretion were decreased (29) by infection and inflammation. TNF and IL-1 increase TG by stimulating hepatic VLDL secretion (30-32), and IL-6, a potent CRP stimulator, has been shown to reduce lipoprotein lipase activity in adipose tissue (33) and induce hepatic TG secretion resulting in an increase in serum TG (34). While there is growing evidence that inflammation affects lipid and lipoprotein metabolism in multiple ways, the mechanisms that account for this are not clear.

Six subjects in our study had metabolic syndrome; five were in the CRP \geq 1 mg/L group and only one was in the CRP < 1 mg/L group. A large-scale cohort study of 14,719 healthy women designed to examine the potential interrelationships between CRP, metabolic syndrome, and the incidence of cardiovascular events showed that CRP added independent prognostic information at all levels of metabolic syndrome with regard to cardiovascular risk (2). Furthermore, Ridker et al. reported that acute coronary syndrome patients who had achieved LDL-C less than 70 mg/dL and CRP levels less than 1 mg/dL after statin therapy had the lowest rate of recurrent events (1.9 per 100 person-years, $P < 0.001$) compared with the other patients that had higher LDL-C or CRP levels (35). It was concluded that monitoring both CRP levels and LDL-C should be part of the medical management of patients on statin therapy (35). Therefore, it is reasonable to speculate that CRP lowering would be synergistic with recommended dietary interventions that decrease CVD risk.

There are a number of strategies for lowering CRP that include statin drugs, weight loss, exercise, and a healthy diet. Cross-sectional studies report an inverse association between CRP, fitness, and physical activity. In addition, CRP decreases with weight loss in obese people (36-38). However, it is still controversial whether CRP can be reduced by exercise training without corresponding weight loss (39-42). Furthermore, most of the studies were conducted with overweight or obese individuals. Consequently, the effects of exercise training (and physical activity) in normal weight individuals with elevated CRP remain unclear. A recent study was conducted in 47 overweight and obese adults to investigate the effects of exercise training and lifestyle modification on CRP (43). The CRP levels were reduced significantly (-25%, $P < 0.01$) in the lifestyle modification group along with weight loss (-9%, $P < 0.01$) (43). Population studies have shown that a high fiber diet as well as a diet with low glycemic index were associated with lower CRP (44, 45). Specifically, there was an inverse relation between highest quartile of total dietary fiber intake and CRP > 3 mg/L (OR: 0.37; 95% CI: 0.16, 0.87; P for trend = 0.01), when compared with the lowest quartile of dietary fiber intake. In addition, the median CRP level for the lowest quintile of dietary glycemic load was 1.9 mg/L and for the highest quintile was 3.7 mg/L, P for trend < 0.01 . In a randomized crossover intervention study, mean CRP level decreased by 13.7% ($P = 0.046$) after the high-fiber (30 g/d) Dietary Approaches to Stop Hypertension (DASH) diet and by 18.1% ($P = 0.02$) after the fiber (psyllium) - supplemented diet (30 g/d) without weight loss (46). The Portfolio Diet [high in plant stanols (1.0 g/kcal), soy protein (21.4 g/1000 kcal), viscous fibers (9.8 g/1000 kcal), and almonds (14 g/1000 kcal)] as well as a diet high in α -linolenic acid provided by walnuts and flaxseed oil have been shown to reduce CRP by

23.8% ($P = 0.001$) (47) and 75% ($P < 0.01$) (48), respectively, without weight loss. Changes in CRP levels after consumption of a Mediterranean diet or a diet high in monounsaturated fat are inconsistent (49-51). In the current study, plasma CRP concentrations were decreased significantly after the consumption of AAD and MAC diets compared to baseline in individuals with $CRP \geq 1$ mg/L but not in those with $CRP < 1$ mg/L. One explanation is that our AAD (which has fruits and vegetables and whole grains) might be a healthier diet compared to what our subjects typically consume. Therefore, both treatment diets decreased CRP levels in the $CRP \geq 1$ mg/L group. In subjects with low baseline CRP, it might not be possible to lower CRP further. The MAC diet was a healthier diet compared to the AAD, and CRP levels did not increase as they did after the consumption of AAD compared to baseline in subjects with $CRP < 1$ mg/L levels. Specifically, the MAC diet was lower in SFA and higher in MUFA which could explain the different CRP diet responses in subjects with low baseline CRP levels.

LOOH, generated from polyunsaturated fatty acids, has been shown to be elevated in association with myocardial ischemia (52). Moreover, elevated LOOH levels were suggested to be predictive of cardiovascular events in patients with stable coronary artery disease (53), independent of traditional risk factors and inflammatory markers (54). In the present study, LOOH was reduced in response to the consumption of MAC diet ($P < 0.06$) and consumption of AAD ($P < 0.01$) in the $CRP < 1$ mg/L group only. The AAD provided fruits, vegetables and whole grains which might explain the reduction in LOOH. It has been suggested that at the earliest stages of CHD development, oxidative stress might be a determinant of CRP concentrations, promoting pro-atherosclerotic inflammatory processes (55). Although the precise association between CRP and

oxidative stress markers is not well established, urinary F2 isoprostanes (correlation coefficient, 0.67; $P < 0.001$) (56) and oxidized LDL (correlation coefficient, 0.13; $P=0.011$) (57) have been shown to positively associate with CRP in individuals without CHD. Furthermore, CRP is positively correlated with LOOH in patients with chronic kidney disease (58), and is associated with oxidative stress markers in healthy young people (59). We did not observe this relationship using Spearman correlation analysis (data not shown), which may reflect our small sample size. Nonetheless, our results provide important additional information with regard to the interaction between inflammatory markers and oxidative stress markers.

A limitation of the present study is that only one measure of oxidative stress and inflammation are included. Further studies are needed to confirm our findings and extend them to other biomarkers of oxidative stress and inflammation. Based on the current literature, however, it is evident that individuals who present with inflammation and insulin resistance have a blunted lipid and lipoprotein response to a blood cholesterol-lowering diet.

In conclusion, our results demonstrate that CRP level not only modulates the effects of a cholesterol-lowering diet rich in macadamia nuts on lipid and lipoproteins, but also modifies the LOOH response to diet. This indicates that individuals with low inflammatory status benefit the most from cholesterol-lowering dietary interventions in terms of lipid/lipoprotein and LOOH responses. Furthermore, our results show that individuals with higher inflammatory status do not benefit from a healthy diet as much as individuals who are not inflamed (as measured by CRP). Further studies are needed to understand the mechanisms that explain how CRP modulates lipids, lipoproteins and

LOOH. It is evident that healthy dietary interventions will have the greatest benefits on multiple CVD risk factors if inflammation can be quelled.

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Table 4-1 Characteristics of the study participants at baseline¹

	CRP < 1 mg/L group (n=14)	CRP ≥ 1 mg/L group (n=10)
Age (y)	48.0 ± 9.6	53.1 ± 6.3 ²
Weight (lbs)	166.8 ± 23.3	166.2 ± 33.2
BMI (kg/m ²)	25.1 ± 2.9	27.6 ± 3.3 ²
Systolic BP (mmHg)	118.9 ± 13.7	124.1 ± 15.2
Diastolic BP (mmHg)	77.7 ± 7.0	81.0 ± 8.4
Waist circumference (cm)	91.2 ± 7.4	98.8 ± 8.0 ²
TC (mg/dL)	210.2 ± 23.7	235.7 ± 30.1 ²
LDL-C (mg/dL)	139.1 ± 21.5	148.2 ± 27.7
HDL-C (mg/dL)	46.8 ± 11.9	54.4 ± 12.9
TG (mg/dL)	121.1 ± 45.4	165.8 ± 78.8 ²
TC/HDL-C ratio	4.7 ± 1.1	4.6 ± 1.3
Fasting glucose (mg/dL)	86.6 ± 7.0	96.4 ± 10.0 ²
CRP (mg/L)	0.5 ± 0.1	2.0 ± 0.3 ²
LOOH (μM)	0.8 ± 1.0	0.9 ± 0.9

¹ All values are mean ± SD. BMI: body mass index; BP, blood pressure; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triacylglycerol; CRP, C-reactive protein; LOOH, lipid hydroperoxide.

² Significantly different between groups, $P < 0.05$.

Table 4-2 Lipid and lipoproteins changes after AAD and MAC diets consumption stratified by baseline CRP level¹

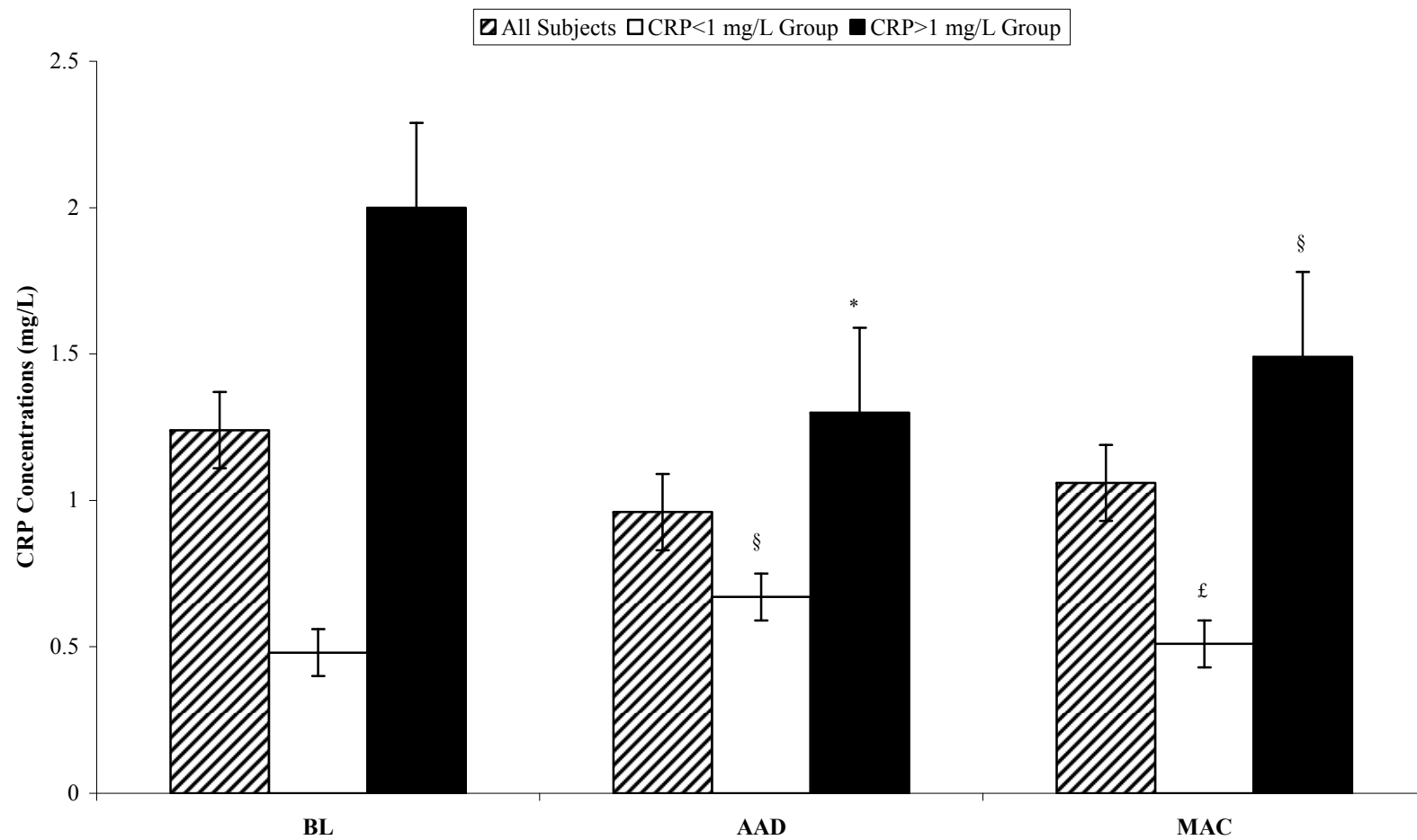
	CRP < 1 mg/L Group (n = 14)		CRP ≥ 1 mg/L Group (n = 10)		Difference between Groups		Adjusted P ³
	Changes ²	P	Changes ²	P	Effect	P	
TC (mg/dL)	-24.7 ± 3.5	< 0.0001	-7.0 ± 4.4	0.12	Group*Diet	< 0.01	< 0.01
LDL-C (mg/dL)	-16.2 ± 3.6	< 0.001	-1.5 ± 4.5	0.75	Group*Diet	< 0.05	< 0.05
HDL-C (mg/dL)	-4.1 ± 0.9	< 0.001	-2.9 ± 1.1	< 0.05	Group Diet	0.41 < 0.0001	0.31 < 0.0001
TG (mg/dL)	-28.2 ± 12.7	< 0.05	-3.6 ± 15.3	0.82	Group Diet	0.12 0.78	0.10 0.60
Non-HDL-C (mg/dL)	-20.7 ± 3.8	<0.0001	-4.2 ± 4.6	0.38	Group*Diet	< 0.05	< 0.01
TC: HDL-C	-0.4 ± 0.1	<0.05	0.004 ± 0.2	0.98	Group Diet	0.13 0.63	0.13 0.81
LDL-C: HDL-C	-0.2 ± 0.1	0.08	-0.04 ± 0.1	0.74	Group Diet	0.17 0.31	0.20 0.50

¹All values are mean ± SEM. AAD, Average American Diet; MAC, Macadamia nut-rich Diet; CRP, C reactive protein TC: total cholesterol; LDL-C: low-density-lipoprotein cholesterol; HDL-C: high-density-lipoprotein cholesterol; TG: triacylglycerol

²Changes are the difference between MAC diets and AAD

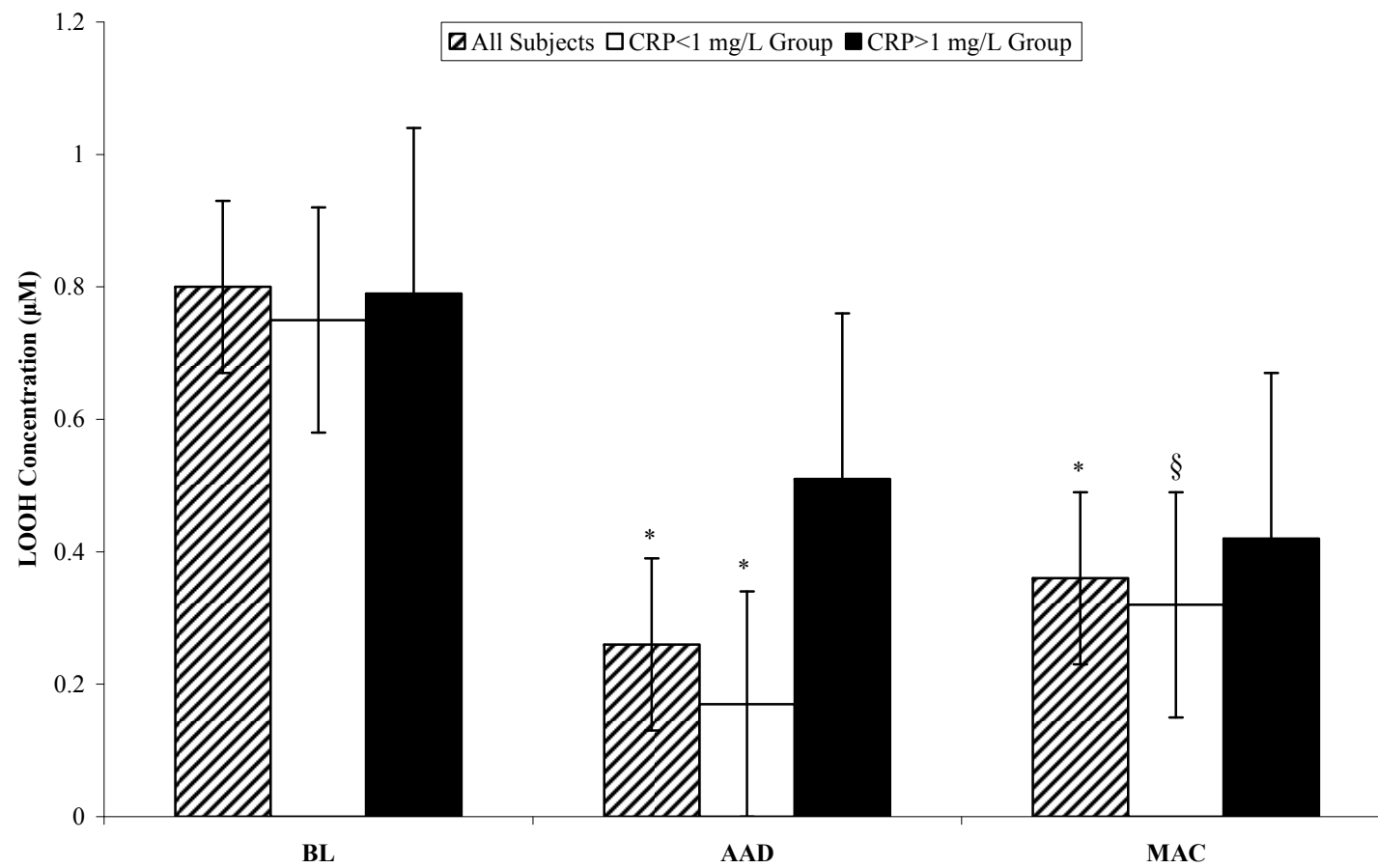
³Adjusted for baseline lipid level, age, BMI, waist circumferences, and weight changes

Figure 4-1 Serum CRP concentration changes after consumption of the AAD and MAC diet



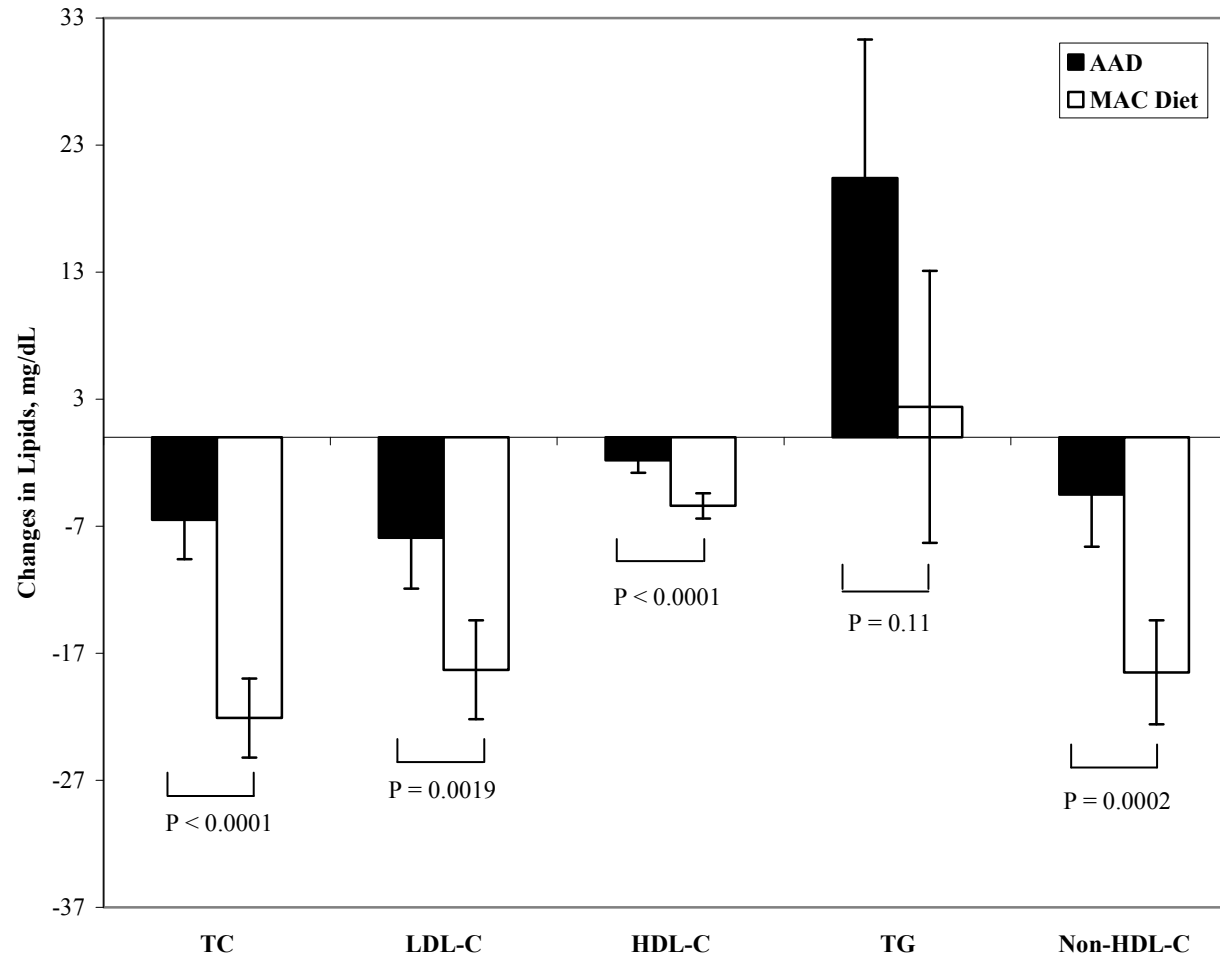
*Significantly different from baseline ($P < 0.01$). §Significantly different from baseline ($P < 0.05$). £Significantly different from AAD ($P < 0.05$).

Figure 4-2 Serum lipid hydroperoxide (LOOH) responses after consumption of the AAD and MAC diet



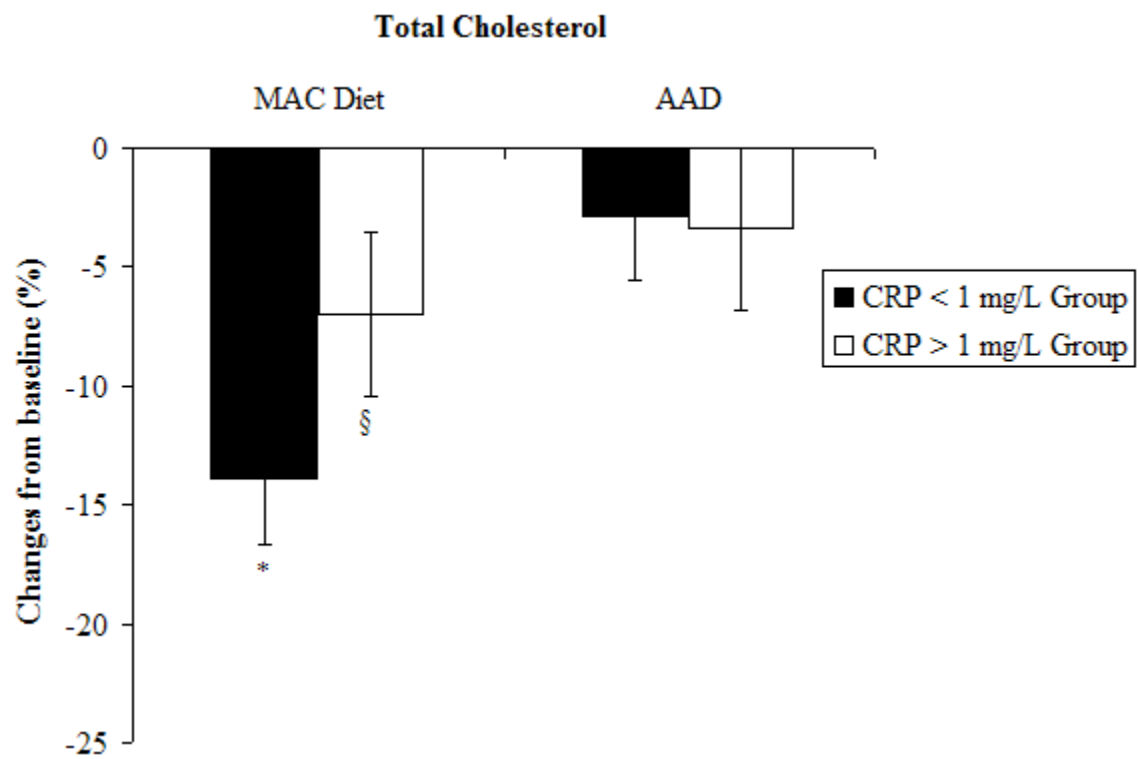
*Significantly different from baseline ($P < 0.01$). §Significantly different from baseline ($P < 0.06$).

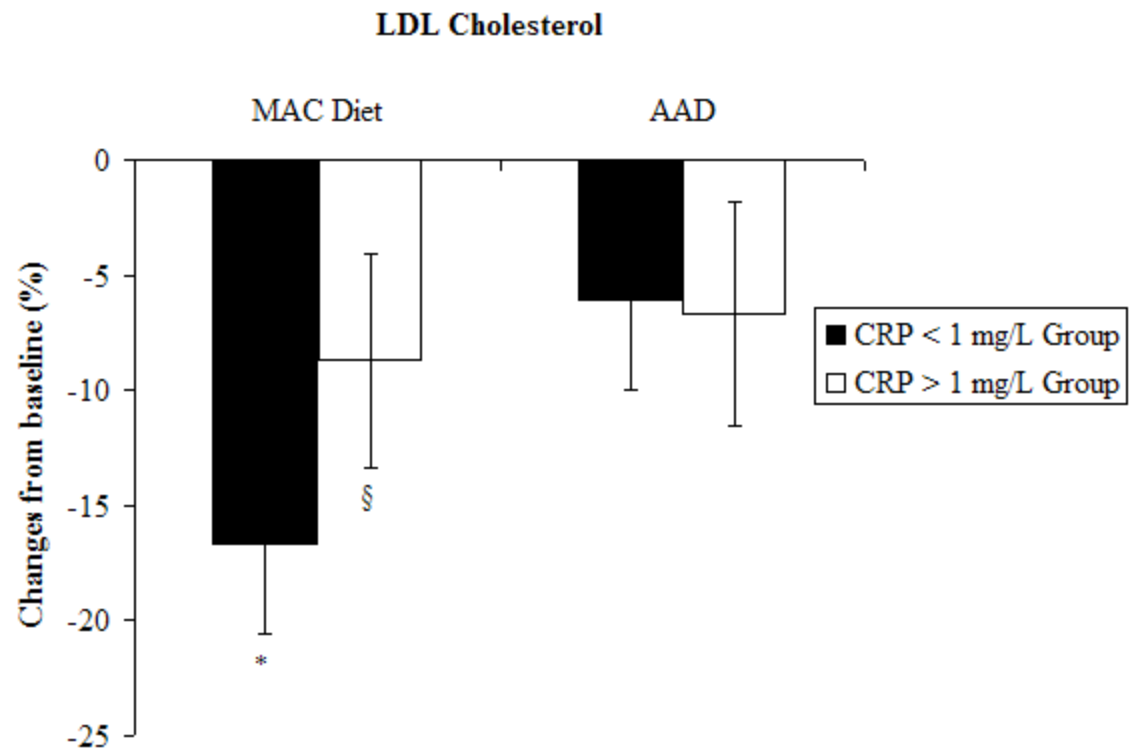
Figure 4-3 Changes from baseline in serum lipid and lipoproteins after consumption of the MAC diet and AAD

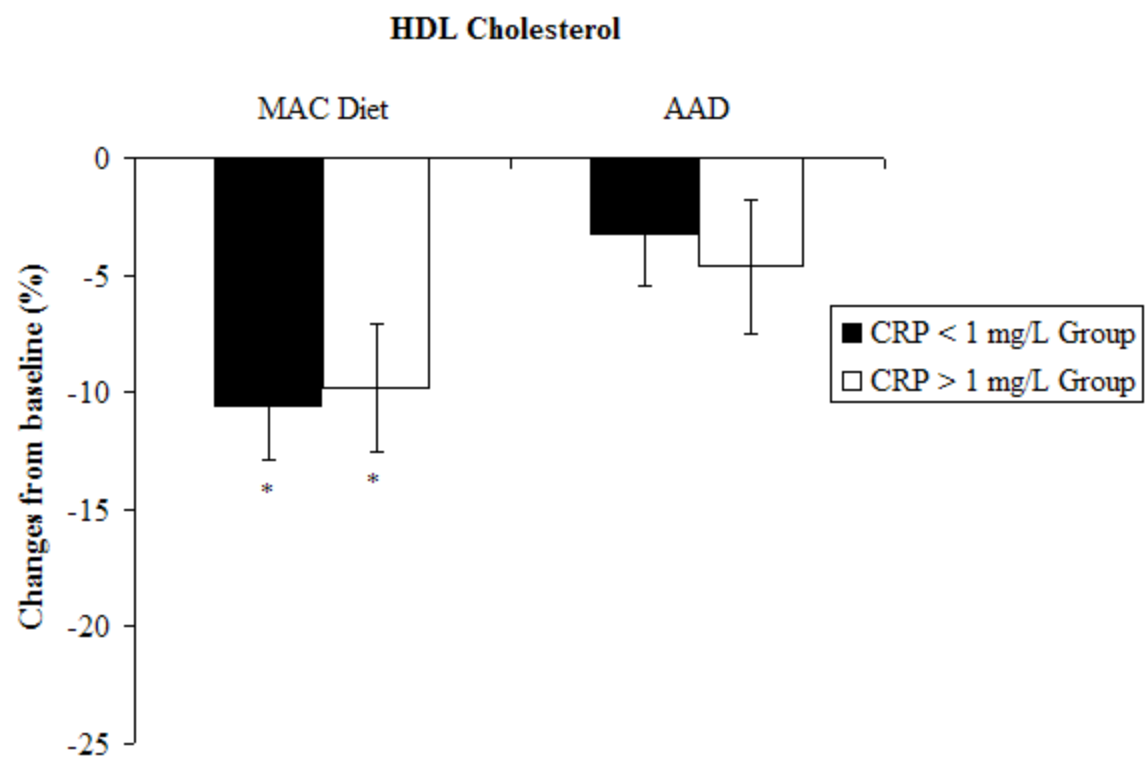


Change is the difference between baseline and end-of-study values. The bars are changes within diets, and the P values are for differences between diets.

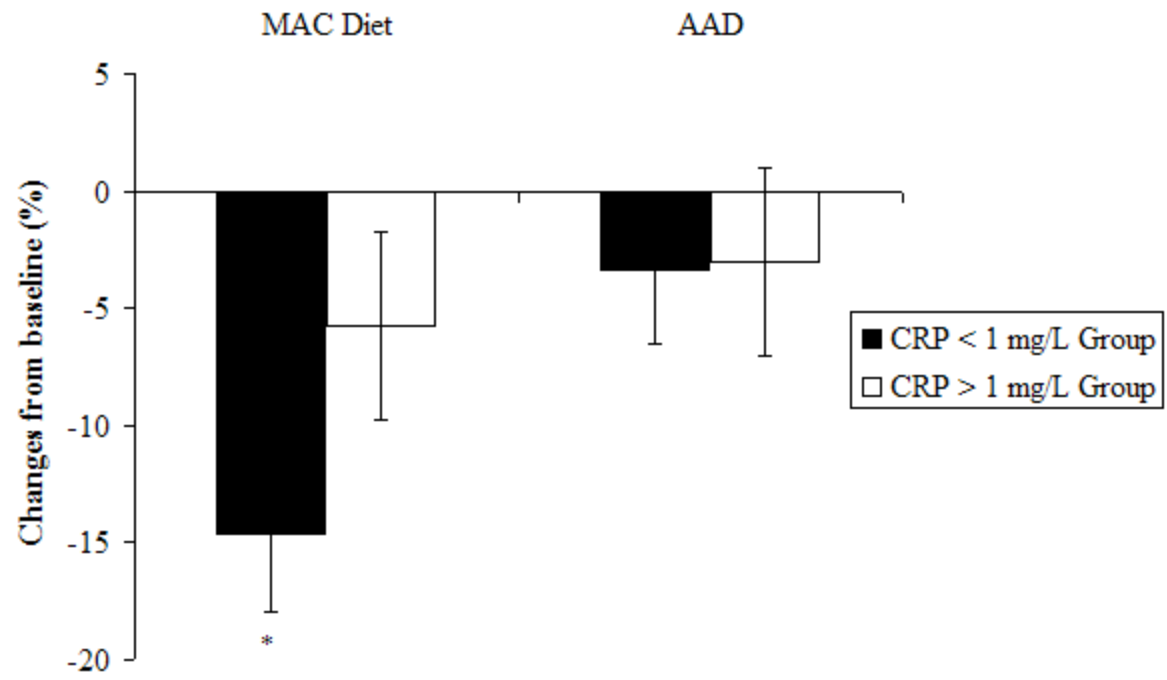
Figure 4-4 Percentage changes from baseline in serum lipid and lipoproteins in the two CRP groups

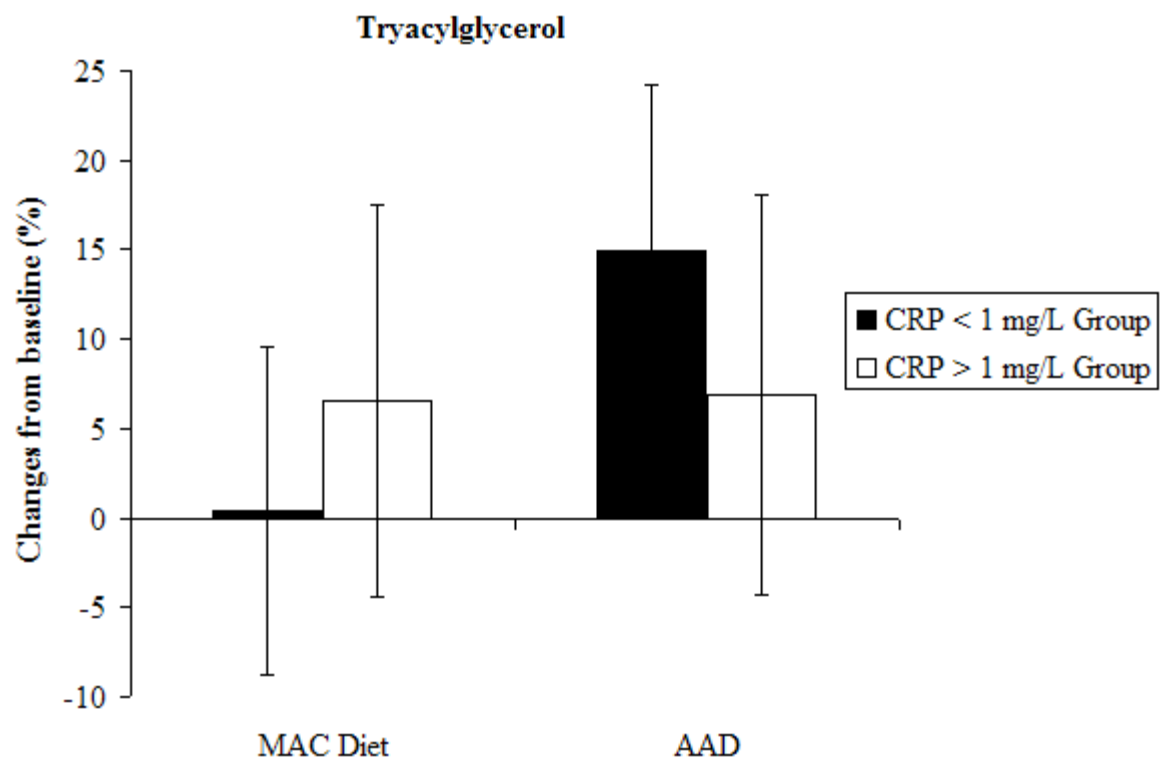






Non-HDL Cholesterol





*P < 0.05; §P < 0.1 versus AAD

Chapter 5

EFFECTS OF ACUTE AND CHRONIC INTAKE OF PROCESSED TOMATOES ON LDL OXIDATION AND PARAOXONASE ACTIVITY

5.1 ABSTRACT

Low density lipoprotein (LDL) oxidation is an initiating event in atherogenesis. Paraoxonase-1 (PON1) hydrolyzes and decreases lipid peroxides in oxidized LDL and macrophages, reduces macrophage-mediated LDL oxidation, and reduces oxidized LDL uptake by the macrophage. We investigated the effects of consuming processed tomatoes on oxidized LDL postprandially in response to a single meal or after six weeks, and on PON1 activity in healthy overweight participants. The PON1-192 genotype also was assessed to determine whether possible treatment effects were associated with PON1-192 polymorphism. Fifty-four men (n = 21) and women (n = 32) aged 21 to 70 years old nonsmokers with a body mass index (BMI) of 25 to 35 kg/m² (inclusive) were recruited in this randomized, treatment-controlled, 2-center, 2-arm, parallel designed study. A subset of 26 participants was recruited for the postprandial studies reported herein. At the run-in period, two postprandial meals were fed in which participants consumed a high fat meal with or without processed tomatoes. After six weeks of low or high tomato consumption, a third postprandial test (a high fat meal without processed tomatoes) was administered. In the run-in period, oxidized LDL increased at 6 H compared to 0 H after the high fat meal without processed tomatoes but did not change after the high fat meal with processed tomatoes. After six weeks of processed tomato consumption, PON1 activity increased at 6 H compared to 3 H ($P < 0.05$) after the high fat meal without processed tomatoes. Furthermore, tomato group participants showed a trend for greater PON1 activity ($P < 0.07$) compared to those in the control group in response to the high fat meal without processed tomatoes (for PON1 QR/RR carriers only). In conclusion, consumption of processed tomato products decreases oxidative stress; when consumed

with a meal, postprandial LDL oxidation is decreased, and daily consumption of tomatoes for six weeks increases PON1 activity which would decrease LDL oxidation.

5.2 INTRODUCTION

The postprandial period is a time that typically is accompanied by a pro-oxidative state, inflammation and endothelial dysfunction, which all contribute to the development and progression of cardiovascular disease (CVD). Excess superoxide production in response to a meal contributes to oxidative stress and endothelial dysfunction (1). Thus, strategies that attenuate postprandial oxidative stress would quell the potential adverse effects associated with consumption of a meal. Beneficial effects of foods rich in antioxidants have been shown to protect against LDL oxidation (2-4). For example, nuts have been shown to lower postprandial oxidative stress (5), and pomegranate juice consumed daily for 18 months decreases LDL susceptibility to oxidation(6). Thus, identifying nutrition strategies that can be implemented chronically, as well as during a meal occasion that would blunt an oxidative response would attenuate the sequelae of events that stimulate inflammation and endothelial dysfunction.

Oxidative modification of LDL is a key step in the development of atherosclerotic lesions; the LDL particle atherogenicity is enhanced as it undergoes oxidative modification (7). LDL oxidation involves formation of lipid hydroperoxides that are converted to smaller molecules (e.g., malondialdehyde, 4-hydroxynonenal, etc) that bind covalently to apo B (8). The modified apo B is recognized by the scavenger receptor resulting in oxidized LDL being taken up by the macrophage, with subsequent formation of foam cells (9). Foam cell production results in the formation of the atherosclerotic lesion (10).

Serum paraoxonase (PON1), a glycoprotein synthesized in the liver, is secreted into the plasma where it binds to high-density lipoprotein (HDL) (11, 12). In the late

1980s, Mackness first proposed that serum PON1 protects against LDL oxidation (13). Avoram and Rosenblat subsequently proposed that PON1 hydrolyzes oxidized lipids in oxidized LDL and macrophages, thereby reducing macrophage-mediated LDL oxidation and uptake (14). Thus, it appears that PON1 protects against the development of atherosclerosis. Some studies have evaluated the association between dietary factors (e.g., alcohol, dietary fat) and PON1 activity. Antioxidants also have been reported to modulate PON1 activity, although the results are inconsistent (15-21).

In humans, the PON1 gene is located on chromosome 7 q21-22 (22). There are two major polymorphisms in the PON1 coding sequence: replacement of glutamine (Q or A) by arginine (R or B) at position 192 and substitution of leucine (L) by methionine (M) at position 55 (23). PON1 genotype and activity might play an important role in the pathogenesis of atherosclerosis (24, 25). It has been demonstrated that PON1 192 Q/R polymorphism is the major determinant of the variation in the rate of hydrolyzing organophosphates (22, 26). Furthermore, it has been suggested that the Q allele is more abundant than the R allele, and the Q allele is responsible for the anti-atherosclerosis effects of PON1 while the R allele is related to an increased risk of CHD (27, 28). To date, little is known about how serum PON1 polymorphisms are associated with diet responsive CVD risk factors (19, 29-32).

Since tomatoes and tomato products contain a variety of antioxidants including the carotenoids, lycopene and beta-carotene, as well as vitamins C and E, and polyphenolics, the present study was conducted to investigate the effect of high versus low intake of processed tomatoes on LDL oxidative susceptibility, PON1 activity in healthy overweight participants. Consequently, postprandial studies were performed to

examine: (1) the effect of a single serving of processed tomatoes eaten concurrently with the high fat test meal to attenuate oxidative stress in response to the meal; (2) the effect of a consuming processed tomatoes for six weeks compared to a low intake of processed tomatoes on attenuation of these meal-induced effects. In addition, the PON1-192 genotypes were determined to see whether any treatment effects at chronic and postprandial states were associated with certain PON1-192 polymorphism.

5.3 METHODS

5.3.1 Participants

Fifty-four men (n = 21) and women (n = 32), ages 21 to 70 years, nonsmokers with a body mass index (BMI) of 25 to 35 kg/m² (inclusive) were recruited for the study through advertisements in the local newspaper. Study participants were healthy, not taking lipid lowering medications, anti-inflammatory drugs or dietary supplements (e.g., fish oil or evening primrose oil) that would compromise the outcome of our study. Potential participants were excluded if they had a known allergy or intolerance to tomatoes, atherosclerotic disease, inflammatory disease, diabetes mellitus, uncontrolled hypertension (> 140/90 mm Hg) or any other disease. Persons with hypertension that was controlled by medication were eligible to participate in the study. The Biomedical Committee of the Institutional Review Board at the Pennsylvania State University approved the protocol and written informed consent was obtained from all participants before enrollment in the study.

5.3.2 Experimental Design

We employed a randomized, treatment-controlled, 2-center (the Pennsylvania State University and University of California, Davis), 2-arm, parallel design to evaluate

the effect of chronic and acute consumption of processed tomatoes on LDL oxidation and PON1 activity (**Figure 5-1**). Fifty-four participants were recruited and randomized to either tomato intake group or a control group after three weeks of low tomato diet consumption (run-in period).

Twenty-six (out of the 54) of the participants voluntarily participated in the postprandial studies in which three postprandial tests were conducted (the data presented herein are from the 26 subjects). The first two postprandial tests were conducted during the run-in period to evaluate the effects of processed tomatoes consumed with a meal on measures of oxidative stress. Two weeks after the start of the run-in period, the first postprandial test was conducted; participants were randomly assigned to high fat meal with or without processed tomatoes. The second postprandial test was conducted later during the run-in period just before randomization. Participants consumed the high fat meal with processed tomatoes if they had the high fat meal without processed tomatoes at the first postprandial visit. Otherwise, they had the high fat meal without processed tomatoes. At the end of the 6-week study, after participants had consumed the processed tomatoes or the control, a third postprandial test was conducted (a high fat meal without processed tomatoes was fed).

5.3.3 Experimental Diets

During the three week run-in period, participants minimized consumption of all processed and fresh tomatoes. Dietitians counseled participants to achieve a low tomato product intake (< 300 g/week). After the run-in, participants were randomized to either the control group (processed tomatoes take up to 40 g/d or < 300 g/week, ~ 15-20 % of total fruit and vegetable intake) or the tomato group (processed tomatoes take up to ~ 200

g/d or 1400 g/week, 75-80 % of total fruit and vegetable intake) for six weeks. Fasting blood was drawn at the end of the run-in period and at the end of the six week study.

Participants were randomly assigned to one of the six tomato product sequences, which included tomato paste (19 ounces per week) and processed tomatoes. The latter were rotated in the diet on a weekly basis. The processed tomatoes used were: tomato juice, tomato soup, spaghetti sauce, ketchup, salsa, diced tomatoes, and tomato sauce. Broth and applesauce were included each day in the control group to match the macronutrient contribution of the tomato products in the tomato group. The dietitians helped participants incorporate tomatoes or the alternative products into their habitual diets and maintain body weight during the study. Food diaries were assigned to the participants to monitor study adherence and promote compliance. Participants came to the diet study centers at the Pennsylvania State University or University of California, Davis to pick up their weekly supply of processed tomatoes or the alternatives.

Two test meals (the high fat meal with processed tomatoes and the high fat meal without processed tomatoes) were developed for the postprandial study. Both test meals provided a plain bagel (35 g) and fat free cream cheese (15 g). The high fat meal with processed tomatoes included a tomato milkshake and the high fat meal without processed tomatoes included a control milkshake. Diet composition of the tomato and control milkshakes and nutrient profiles of the two postprandial test meals are shown in **Tables 5-1 and 5-2**, respectively. Participants in the postprandial study consumed their meal over a 20 minute period, and blood samples were collected at timed intervals after meal consumption. Blood collection occurred at 0 time (fasting) and 30 minutes and then hourly thereafter to 5.5 h.

5.3.4 Biochemical Assays

The main endpoints in the study were PON1 activity, LDL oxidation and inflammatory markers [IL-6 (interleukin-6), TNF-alpha (tumor necrosis factor-alpha), VCAM (vascular cell adhesion molecule-1) and ICAM (intercellular adhesion molecule-1)]. Venous blood samples were collected in the appropriate tubes (with anti-coagulants where necessary) after an overnight fast by antecubital venipuncture. Serum samples were subjected to low-speed centrifugation at 1500 x g for 10 min at 4°C.

PON1 activity was measured by highly sensitive, homogeneous fluorometric assay using a commercial assay kit (EnzChek Paraoxonase Assay Kit, Molecular Probes, Inc). Oxidized-LDL was measured by the Merckodia Oxidized LDL Enzyme-Linked Immunosorbent Assay (ELISA) kit that was based on a direct sandwich technique that was adapted from previous studies (33). IL-6, TNF-alpha, ICAM and VCAM were measured using ELISA kits (R&D Systems)

Genomic DNA was extracted from buccal swab samples using a DNA isolation kit (Boca Scientific Inc.). PON1-192 genotype was determined using polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis by Taqman Allelic Discrimination Assay (Applied Biosystems, Foster City, CA). Briefly, 2.0 µl of genomic DNA at 20 to 40 ngs/ul, 0.15 ul of Taqman Single-nucleotide polymorphism (SNP) Genotyping Assay Kit, 0.35 ul of H₂O and 2.5 ul of Taqman Universal PCR Master Mix were added to the wells of a 384 plate, and PCR was performed as recommended in the SNP Genotyping protocol #4332856C provided with the kit. SNP calls were made after the post-read step and based on segregation of the alleles.

5.3.5 Statistical Analyses

Two-sample t-tests were used to test for demographic differences between tomato and control groups (measured at baseline) as well as the polymorphism groups. The Wilcoxon rank-sum test was used if the baseline variables were not normally distributed.

The mixed models procedure (PROC MIXED) was used to test treatment, time, and the interaction on the levels of oxidized LDL, PON1 activity for the chronic study. Repeated measurements were performed for the postprandial study. Gender and study center were included as fixed factors in the model. Age, BMI, waist circumferences, baseline total cholesterol, high-density-lipoprotein cholesterol, LDL-C, PON1 activity and oxidized LDL and changes in body weight during the course of the study were included as covariates in the model.

Only the Pennsylvania State University site collected buccal swabs from participants for the PON1 genotype analyses. Since the RR-carrier was present in only one participant, the data from the QR and RR carriers were pooled. Polymorphism groups also were included into the model in addition to treatment, time, and the interactions.

Pearson correlations were performed to investigate possible relationships between PON1 activity, oxidized LDL and inflammatory markers. The Shapiro-Wilk test for the residuals was used to test for the normality of each variable. A W statistic > 0.90 indicates that the variable is normally distributed. Tukey-Kramer adjusted P values were used to determine whether the differences in the outcome variables are significant. $P \leq 0.05$ were considered statistically different and $P \leq 0.10$ indicated a trend for significance. All statistical analyses were performed using SAS for WINDOWS, release 9.1 (SAS Institute, Cary, NC).

5.4 RESULTS

Of the fifty-four participants, twenty-six (18 F and 8 M) participated in the postprandial studies. Baseline characteristics of the participants are summarized in **Table 5-3**. Despite randomization, participants in the tomato group had higher total cholesterol and LDL-C compared to those in the control group. In contrast, the systolic blood pressure was higher in the low tomato group. Age, BMI, blood glucose, and other lipids parameters did not differ significantly between the two groups.

5.4.1 Inclusion of Processed Tomatoes Alters Acute Response to a High Fat Meal

A different response was observed when processed tomatoes were fed in a single high fat meal. Specifically, oxidized LDL increased at 6 H compared to 0 H after the high fat meal without processed tomatoes; there was no change after the high fat meal with processed tomatoes (**Figure 5-2**).

5.4.2 Effect of Six Weeks of Daily Consumption of Processed Tomatoes on Oxidative Stress and Postprandial Responses

Oxidized LDL and PON1 activity in fasted subjects did not change after six weeks of tomato consumption compared to the control group. Female participants had a greater PON1 activity in the fasted state after consuming tomato products for six weeks (**Figure 5-3**) as well as postprandial state (data not shown) when compared to male participants. After six weeks of processed tomatoes intake, there was an increase in PON1 activity at 6 hour compared to 3 hour ($P < 0.05$) after the high fat meal without processed tomatoes (**Figure 5-4**). LDL oxidative susceptibility after a high fat meal without processed tomatoes did not differ after six weeks of tomato consumption compared to the control group.

5.4.3 Effects of PON-1 Polymorphism on Response to Processed Tomatoes

Only participants (n = 32) at the Pennsylvania State University had their PON1 genotype determined. Two of the participants' PON1 genotype could not be determined because of insufficient DNA in the buccal swabs. Seventeen participants carried the QQ-genotype (57 %) while thirteen carried the QR/RR-genotype (43 %). The QR/RR carriers showed a higher PON1 activity at 0 week (0.126 vs. 0.112 U/ μ L, $P < 0.10$) and there were no other differences between the two groups with regard to the participants' characteristics (data not shown).

PON1 activity was higher in participants with the QR/RR genotype than those QQ carriers in tomato group after the high fat meal without processed tomatoes (**Figure 5-5**). Furthermore, participants in the tomato group showed a trend for having greater PON1 activity ($P < 0.07$) compared to those in the control group after the high fat meal without processed tomatoes in QR/RR carriers (**Figure 5-5**). There were no differences in oxidized LDL with regard to the PON1 polymorphisms.

5.4.4 Association between PON1 and LDL Oxidation and Inflammatory Markers

After six weeks of high and low processed tomatoes intake, PON1 activity was negatively correlated with oxidized LDL in the tomato group only (**Table 5-4**). In contrast, no associations were found between PON1 activity and inflammatory markers.

PON1 activity was positively associated with C-reactive protein (CRP) after the high fat meal with processed tomatoes but negatively associated with VCAM after the high fat meal with or without processed tomatoes (**Table 5-5**). Meanwhile, PON1 activity was positively correlated with oxidized LDL and TNF-alpha after the high fat meal without processed tomatoes (**Table 5-5**).

5.5 DISCUSSION

Our study investigated the effects of processed tomatoes consumption on oxidized LDL and PON1 activity in overweight healthy participants. During the run-in period, oxidized LDL increased at 6 H compared to 0 H after the high fat meal without processed tomatoes but did not change after the high fat meal with processed tomatoes. After six weeks of high or low processed tomato consumption, the tomato group had an increase in PON1 activity at 6 H compared to 3 H ($P < 0.05$) after the high fat meal without processed tomatoes. Furthermore, the tomato group participants showed a trend for having greater PON1 activity ($P < 0.07$) compared to those in the control group in response to the high fat meal without processed tomatoes in QR/RR carriers only.

Tomato products are widely consumed in Western countries. Tomatoes or processed tomatoes may benefit heart disease and other diseases based on epidemiologic and clinical studies (34-36). Lycopene has been shown to be the predominant carotenoid in tomato products that contributes to the protection against atherosclerosis and CVD (37, 38). A possible mechanism to explain this is that tomato products may modulate radical-mediated oxidative damage which then inhibits the progress of many disease (39).

LDL modification is a key step in the development of atherosclerotic lesions and evidence demonstrates that atherogenicity is enhanced as the LDL undergoes oxidative modification (7). Therefore, oxidized LDL has been identified as an important marker of oxidative stress. Di Mascio et al reported that lycopene is the most efficient biological carotenoid singlet oxygen quencher (40). In vitro and in vivo studies have shown that lycopene and other carotenoids can inhibit oxidation of LDL (41, 42). In the current study, we examined the effects of antioxidants in the processed tomatoes on oxidized

LDL. A single serving of processed tomatoes eaten concurrently with a high fat meal blocked the increase in oxidized LDL. However, six weeks of consuming processed tomatoes did not change LDL oxidative susceptibility. We believe that the bioavailability of antioxidants in processed tomatoes helps to explain the current findings. Lycopene and other carotenoids are highly lipophilic and often found within lipid compounds (43). Therefore, the bioavailability of antioxidants in the processed tomatoes might be increased in a lipophilic environment such as a high fat meal as in the present study.

HDL-C is inversely associated with the risk of developing CHD. However, the mechanism for the protective effect of HDL-C is not totally clear. It has been suggested that reverse cholesterol transport and several enzymes that are related to HDL-C metabolism may be involved in this protective process. PON1 is one of the enzymes that may elicit a beneficial effect. Navab et al. reported that in a subset of patients with coronary atherosclerosis ($n = 5$), the PON1 activity was significantly lower compared to the control participants (48 ± 29 vs. $98 \pm 17 \mu\text{g/ml}$, $P < 0.01$) while the HDL concentrations were not different (50 ± 6.1 vs. $48.3 \pm 5.3 \text{ mg/dL}$) (44). In addition, the HDL with normal PON1 activity protected against LDL-induced monocyte migration efficiently while the low PON1 activity HDL failed (44). Therefore, PON1 may partly explain the inverse relationship between HDL levels and risk of atherosclerotic events. A recent study examined PON1 activity in patients with coronary artery disease (CAD) and healthy individuals (45). CAD patients had significant lower PON1 activity and HDL-C compared to the healthy participants (45).

In the late 1980s, Mackness first proposed that serum PON1 was protective against LDL oxidation (13). Avian HDL lacks PON1 activity and when incubated with human LDL, it failed to protect Cu²⁺-induced accumulation of lipid peroxides in LDL (46). HDL isolated from PON1-knockout mice failed to protect LDL against oxidation in a co-cultured cell model of the artery wall (47). In addition, HDL and LDL from PON1-knockout mice were more susceptible to oxidation in co-cultured cells compared to those from wild-type mice (47). These studies demonstrate that PON1 is the major component of HDL that is responsible for protecting LDL against oxidation. PON1 was also shown to protect HDL from oxidation. HDL peroxide and aldehyde formation were reduced significantly and HDL oxidation lag phase was prolonged when adding PON1 to human HDL (48). Avoram and Rosenblat proposed that PON1 exerts beneficial effects in the development of atherosclerosis (14).

Research has been conducted to investigate the relationships between diet and PON1 activity. The results remain ambiguous. Short-term fruit and vegetable consumption have been shown to reduce fasting PON1 activity modestly (19) or have no effect (49). Keemola et al. (18) reported a negative correlation between PON1 activity and fruit/vegetable intakes. PON1 activity increased by 18 % in 13 healthy, nonsmoking men after 50 ml pomegranate juice per day for two weeks (17). No correlations were found between serum PON1 levels and vitamin C, vitamin E, and beta-carotene intake (20). In contrast, Jarvik et al reported that vitamin C or E intakes were significant positive predictors of PON1 activity (21). Twelve healthy men were randomized to receive a milkshake (46 g test fat) either rich in fat used for deep-frying or rich in unused fat (50). The fat used for deep-frying had higher peroxide values, high carbonyl values,

and a lower proportion of unsaturated fatty acids when compared with the unused fat (i.e., not used for frying) (50). Serum PON1 activity was reduced by 27 % for up to 8 hours and it returned to normal by 12 hours after the used fat meal. In contrast, the unused fat meal led to a significant increase in PON1 activity. Fourteen patients with type 2 diabetes were fed meals rich in thermally stressed safflower or olive oil. Postprandial serum PON1 activity was increased in women mainly after the olive oil-enriched meal. In contrast, ten healthy men consumed a high fat, high carbohydrate meal (45 % fat) and the PON1 activity did not change after three hours in the postprandial state (51). The postprandial state may accelerate atherosclerotic plaque formation (52). A high fat meal, therefore, has adverse effects as the result of generating lipid peroxides and resulting in endothelial damage. Antioxidants in blueberries (53), olive oil (54), red wine (55), and grape seed extract (56) have been shown to increase antioxidant capacity in the postprandial state. In the present study, after six weeks of intake of processed tomatoes intake, there was an increase in PON1 activity at time 6 H compared to time 3 H ($P < 0.05$) in the tomato group after the high fat meal without the processed tomatoes. Our results suggest that a high content of antioxidants, as in the processed tomatoes, is protective against the adverse effects caused by a high fat meal.

Humbert et al (22) suggested that PON1-192 genotype predicts PON1 activity in human serum. Our results showed that PON1 activity was lower in QQ-carriers than in RR/QR carriers which was consistent with that observation. In order to investigate whether the PON1-192 genotype is associated with the changes in PON1 activity and oxidized LDL after processed tomatoes consumption, data were analyzed according to QQ-carriers and RR/QR-carriers. Bub et al. reported that healthy elderly participants had

an increase in LDL oxidation lag time (93 ± 14 vs. 86 ± 14 min $P < 0.05$) after consuming 330 ml/day of tomato juice (47.1 mg lycopene, 1.7 mg beta-carotene) for 8 weeks compared to the control participants (31). Both the control and tomato juice groups had an increase in PON1 activity and there was no difference between these two groups (31). Moreover, lag time for LDL oxidation were increased (93 ± 15 vs. 84 ± 11 min $P < 0.05$) in PON1-192R allele carriers only (31). Similarly, Bub et al demonstrated that in healthy young subjects with the PON1-192R allele, plasma malondialdehyde (MDA) was reduced ($P < 0.05$) compared to the PON1-192QQ subjects after consuming a low-carotenoid diet (by abstaining from fruit and vegetables high in carotenoids) supplemented with tomato juice (330 ml/d: 37.0 mg lycopene, 1.6 mg beta-carotene) for two weeks (32). In the present study, we found a trend for a greater postprandial PON1 activity in the tomato group participants ($P < 0.07$) compared to those in the control group after the high fat meal without processed tomatoes in QR/RR carriers only. However, there were no differences in oxidized LDL with respect to the PON1 genotypes which might be due to the small sample size.

The correlation between PON1 activity and oxidized LDL is somewhat controversial (57-59). In the present study, after six weeks of processed tomatoes consumption, the tomato group showed an inverse correlation between PON1 activity and oxidized LDL. However, no changes were seen in PON1 activity and oxidized LDL after the six weeks of processed tomatoes consumption. To date, little is known the relationship between PON1 activity, oxidized LDL and inflammatory markers. In the present study, PON1 activity was positively correlated with oxidized LDL after a single high fat meal without the tomatoes but not a high fat meal with the tomatoes. While

oxidized LDL increased at 6 H compared to 0 H after the vanilla meal, PON1 activity did not change significantly.

A previous study has shown that there was a significant inverse association between PON1 activity and CRP ($r = -0.29$, $P < 0.05$) in the higher activity PON1 genotype group (the PON1 genotype were determined according to the PON1 activity) (60). No such relationship was shown in the current study (data not shown) which might be due to the small sample size. Van Himbergen et al showed a positive relation between PON1 activity and CRP in familial hypercholesterolaemia (61). In healthy subjects, however, it has been suggested that CRP might be inversely correlated with PON1 activity (62). In the present study, PON1 activity was positively associated with CRP after the high fat meal with processed tomatoes. The reason remains unclear. Nevertheless, the association of PON1 activity with oxidative stress and inflammatory markers in the postprandial state needs to be studied further.

The present study suggests that processed tomatoes consumption are protective against oxidative damage caused by a high fat meal via increasing PON1 activity. Furthermore, in the postprandial state, consumption of the tomato milkshake blocked the increase in oxidative stress after a high fat meal. Processed tomatoes might be protective against atherosclerosis by directly blocking the increase in oxidative stress, and increasing the PON1 activity which then indirectly reduces the oxidative stress. In addition, processed tomatoes marginally increased in PON1 activity in PON1 QR/RR carriers, and might therefore play a useful role in reducing the risk of CVD in this subgroup that is at a higher risk for CHD as compared to the QQ carriers. However, oxidative stress did not decrease after consumption of processed tomatoes in the PON1

QR/RR carriers. Further studies with an increased sample size are necessary to ascertain the significance of the current finding and further investigate the relationship between oxidative stress and antioxidants with regard to the PON1 polymorphisms. Although numerous in vitro studies have shown that PON1 protects LDL from oxidation, studies need to be conducted to further confirm the interactions between PON1 and LDL oxidation.

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Table 5-1 Diet composition of milkshakes for the postprandial studies

	Tomato Milkshake	Control Milkshake
Skim Milk (g)	245.0	245.0
Light Cream (g)	9.0	9.0
Corn Oil (g)	23.2	23.2
Soybean oil (g)	23.2	23.2
Splenda (g)	7.5	7.5
White granulated sugar (g)	0.0	12.0
Table salt (g)	0.0	1.8
Banana extract (g)	0.0	0.5
Benefiber (g)	0.0	4.6
Tomato paste (g)	90.2	0.0
Ice (g)	100.0	100.0

Table 5-2 Nutrient profile of postprandial test meals

	High fat meal with tomatoes (722 kcal)	High fat meal without tomatoes (714 kcal)
Total Fat (g)	49.5	49.1
SFA (g)	7.9	7.9
MUFA (g)	11.8	11.7
PUFA (g)	27.5	27.4
CHO (g)	56.1	56.3
Protein (g)	18.2	14.3
Fiber (g)	4.8	4.7
Cholesterol (mg)	12	12
Sodium (mg)	1098	1083

Table 5-3 Baseline characteristics of participants¹

Parameters	All participants (n=54)	Tomato Group (n=30)	Control Group (n=24)
Age	44.09±13.07	44.97±14.45	43.00±11.33
BMI (kg/m ²)	29.37±3.89	29.85±4.61	28.78±2.72
Systolic BP (mmHg)	118.83±13.99	115.47±13.26 ²	123.04±14.01
Diastolic BP (mmHg)	78.57±8.55	77.27±8.65	80.21±8.30
TC (mg/dL)	194.48±39.49	203.80±39.70 ³	182.83±36.77
LDL-C (mg/dL)	121.22±35.32	129.80±35.71 ²	110.50±32.42
HDL-C (mg/dL)	49.39±15.49	50.10±15.93	48.50±15.21
TG (mg/dL)	119.28±63.40	119.20±64.52	119.38±63.34
TC:HDL-C	4.33±1.61	4.46±1.59	4.17±1.65
Glucose (mg/dL)	90.91±8.77	92.40±9.33	89.04±7.80
CRP (mg/L)	2.26±2.58	1.87±2.16	2.72±2.98

¹Data are given as means ± SD

²P < 0.05 compared to control group

³P < 0.06 compared to control group

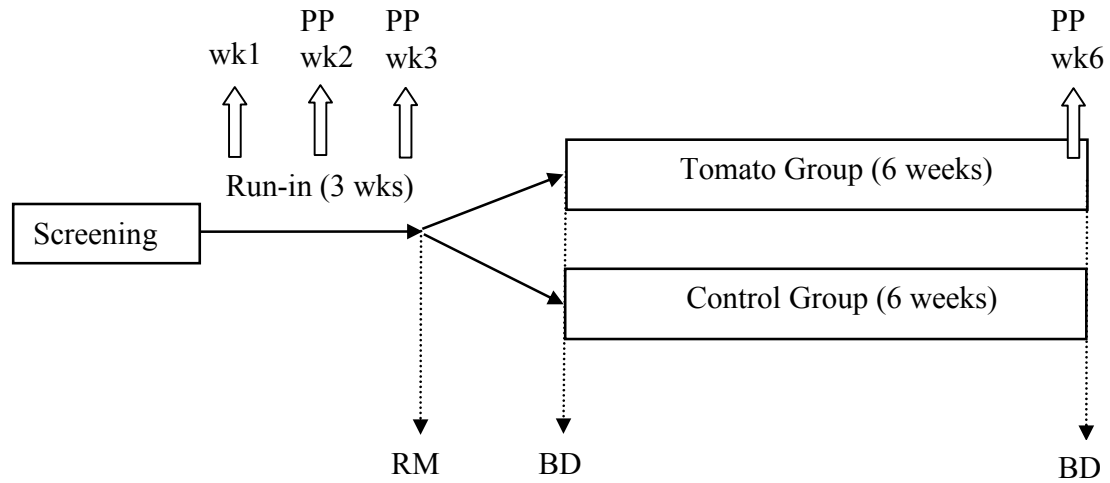
Table 5-4 Correlations of PON1 activity with oxidative stress and inflammatory markers after 6 weeks of high and low tomato consumption

Parameters	Tomato Group		Control Group	
	r	P values	r	P values
Oxidized LDL	-0.39	0.003	-0.01	0.949
CRP	-0.08	0.545	0.03	0.846
IL-6	-0.07	0.600	0.00	0.989
TNF	-0.21	0.110	0.12	0.411
ICAM	0.08	0.582	0.06	0.693
VCAM	-0.02	0.900	-0.19	0.215

Table 5-5 Correlations of PON1 activity with oxidative stress and inflammatory markers after consumption of a single meal with or without tomatoes

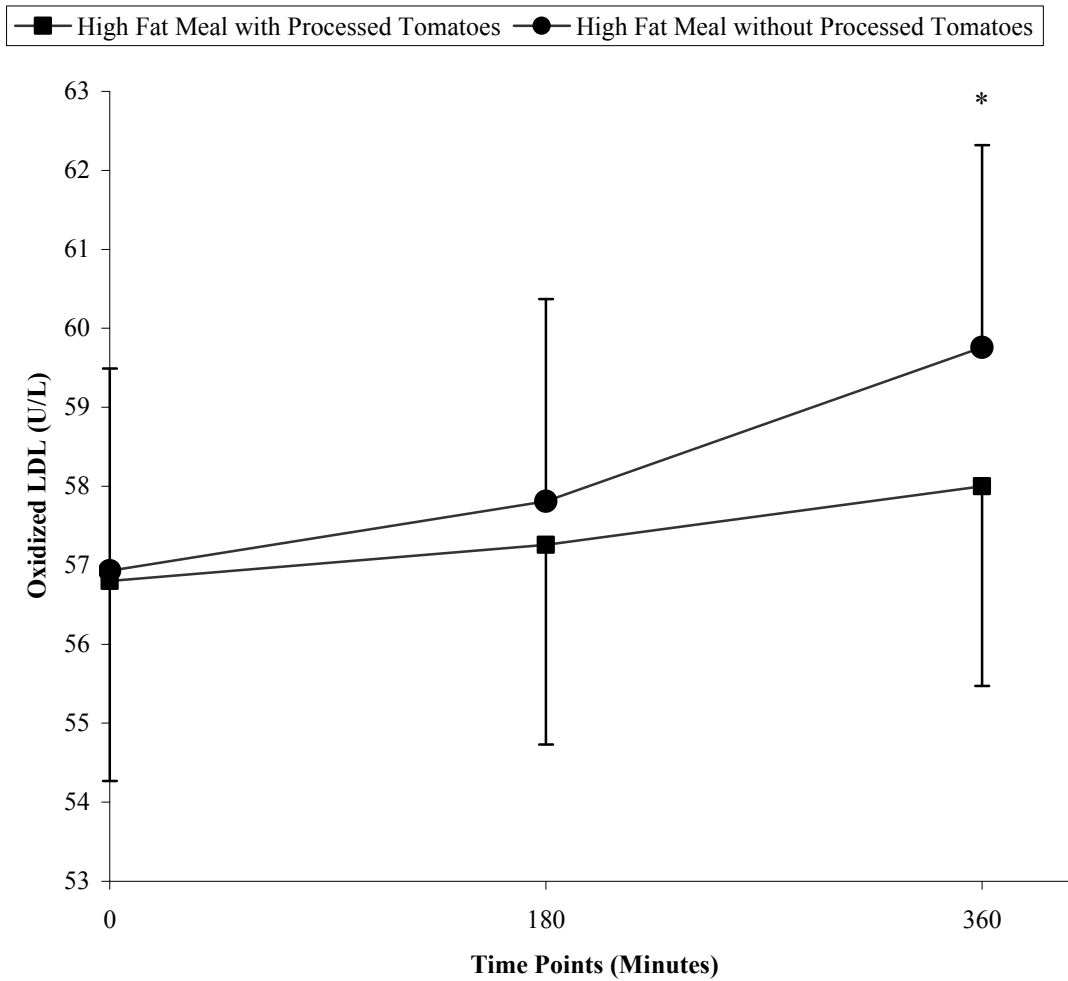
Parameters	High Fat Meal with Processed Tomatoes		High Fat Meal without Processed Tomatoes	
	r	P values	r	P values
Oxidized LDL	0.14	0.230	0.16	0.060
CRP	0.44	<0.0001	0.12	0.152
IL-6	0.01	0.897	-0.05	0.539
TNF	0.02	0.876	0.19	0.023
ICAM	-0.07	0.567	-0.113	0.199
VCAM	-0.49	<0.0001	-0.32	<0.001

Figure 5-1 Experimental design of the tomato study



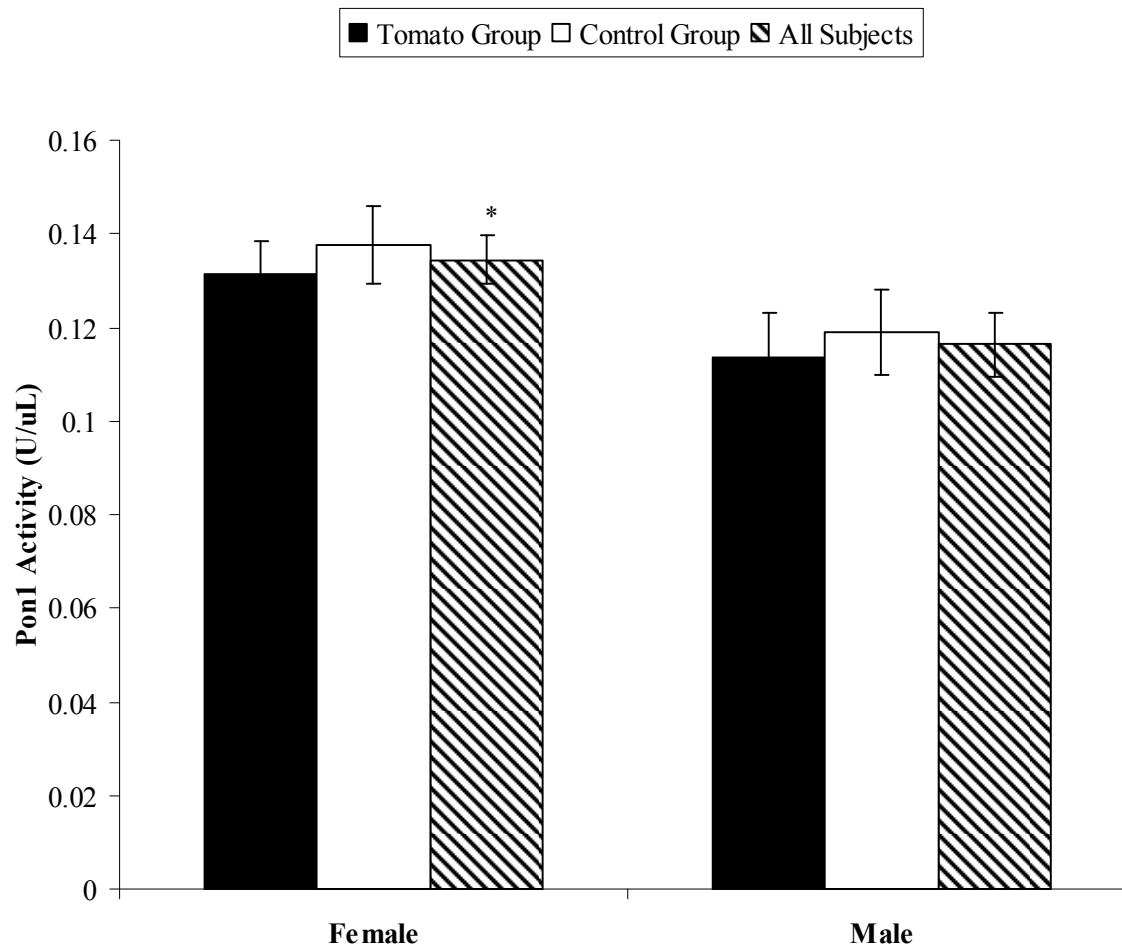
BD: blood draw; RM: randomization; PP: postprandial study

Figure 5-2 Postprandial oxidized LDL after a high fat meal with or without processed tomatoes



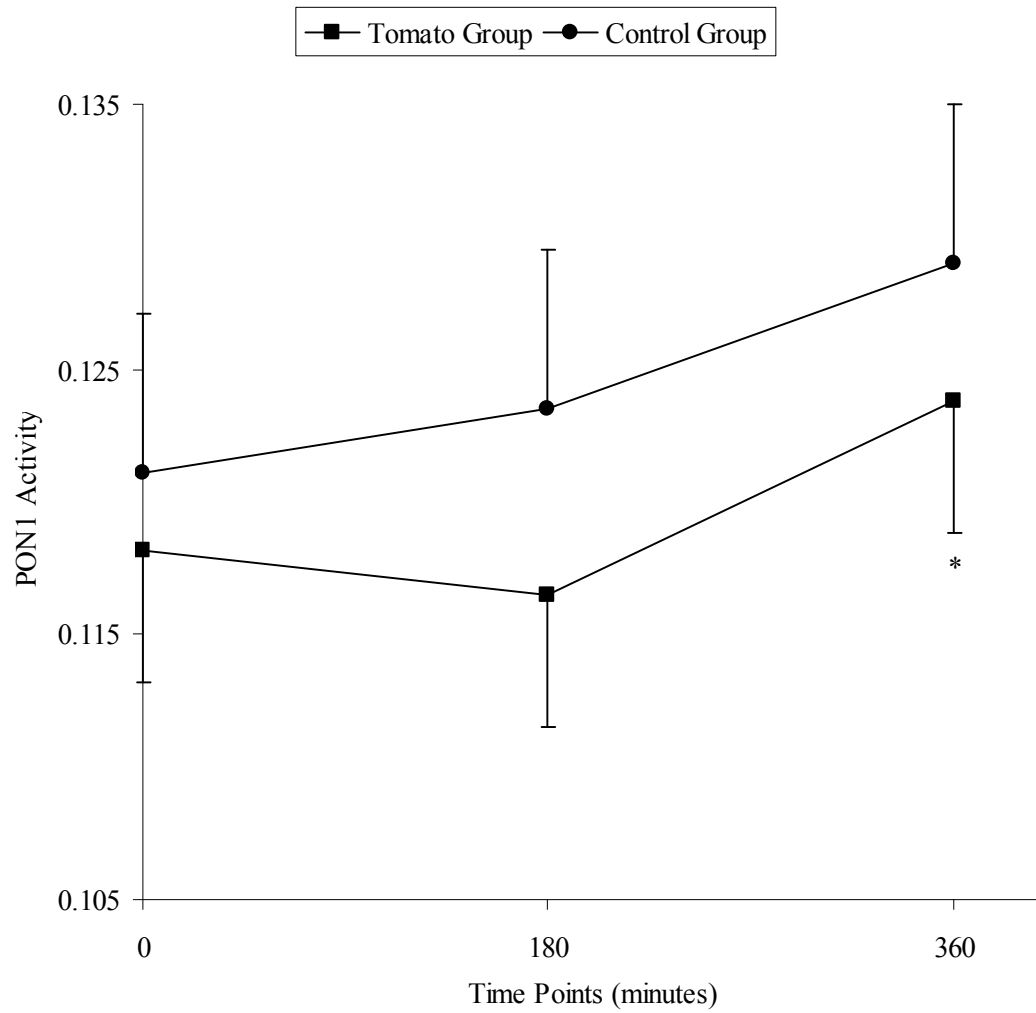
*P < 0.05 at time 0 vs. time 360 for the vanilla meal

Figure 5-3 PON1 activities in males and females after 6 weeks of tomato consumption



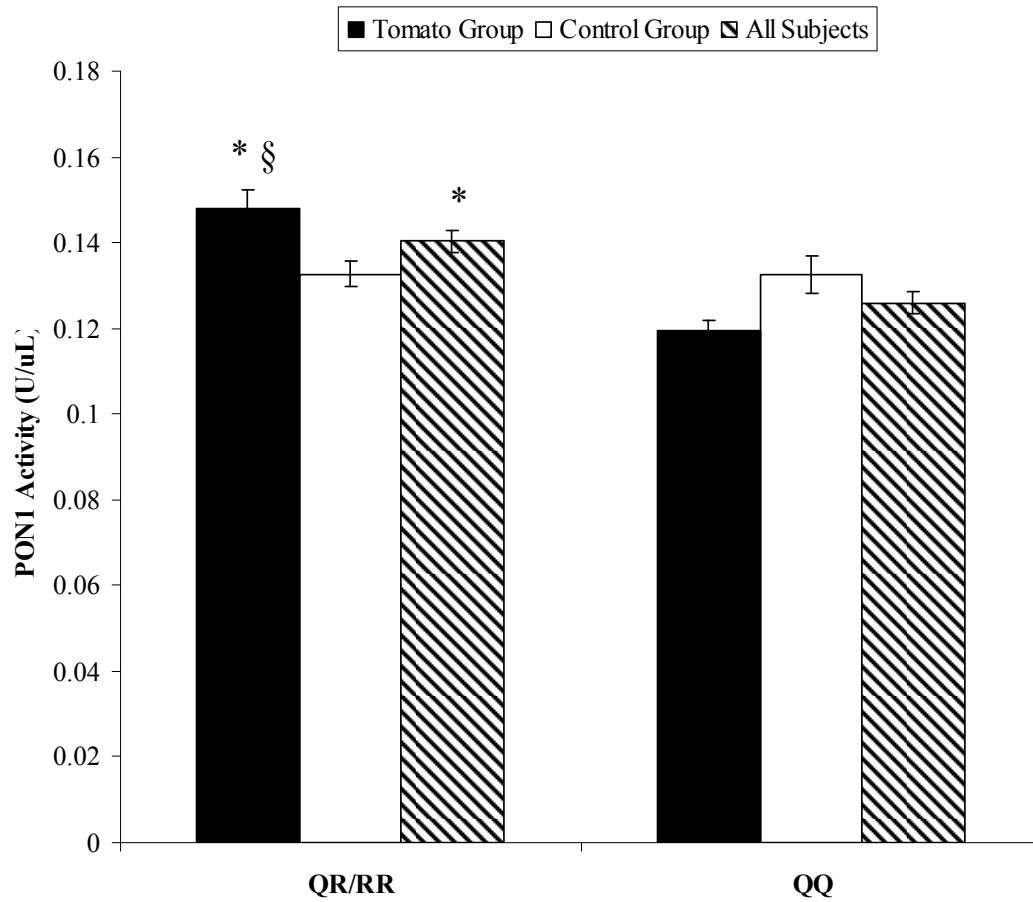
*P < 0.05 vs. Male

Figure 5-4 PON1 activity after a high fat meal without processed tomatoes in the tomato and control groups



*P < 0.05 at Time 360 vs. Time 180 in high tomato group

Figure 5-5 Postprandial PON1 activity (after consumption of tomato products for 6 weeks) after a high fat meal without processed tomatoes in PON1 QR/RR and QQ carriers



*P < 0.01 vs. PON1 genotype-QQ

§P < 0.07 vs. control group

Chapter 6

SUMMARY, STRENGTHS AND LIMITATIONS, AND FUTURE DIRECTIONS

Elevated total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-C), triacylglycerol (TG) and low high-density-lipoprotein cholesterol (HDL-C) are associated with increased risk of coronary artery disease (CAD). Atherosclerosis is an inflammatory disease, and elevated levels of C-reactive protein (CRP), a marker of inflammation, increases risk of cardiovascular disease (CVD). Oxidized LDL, a marker of oxidative stress, is thought to play a key role in the development and progression of atherosclerosis. Paraoxonase (PON1), an antioxidative enzyme found on HDL, has been proposed as one mechanism to explain the protective effect of HDL-C on CVD risk. Therefore, decreased PON1 activity would be expected to increase risk CVD risk. The objective of this thesis was to evaluate the effects of dietary monounsaturated fat and antioxidants on these factors that are related to CVD risk. Three studies were conducted to investigate how different types/amounts of dietary fat and antioxidants affected lipids/lipoproteins, inflammation, oxidative stress, and PON1 activity. Furthermore, how baseline inflammatory status modifies lipids/lipoproteins and an oxidative stress response to diets also was investigated. In addition, the role of PON1 genotype in moderating the dietary effect on PON1 activity and oxidative stress was examined.

In the first study, a meta-analysis of 30 controlled-feeding studies was conducted to quantify the magnitude of lipid and lipoprotein responses to (moderate fat) MF (high in monounsaturated fat) versus (lower-fat) LF cholesterol-lowering, weight maintenance diets in subjects with and without diabetes. MF diets decreased HDL-C less compared to the LF diets. In addition, the MF diets decreased TG whereas LF diets increased TG. In subjects with diabetes, there was a greater reduction in TG after the consumption of MF diets. Based on greater reductions in TG, the TC: HDL-C ratio and non-HDL-C

especially in subjects with diabetes, the CHD risk reduction would be greater for a MF versus a LF weight maintenance, cholesterol-lowering diet. Moreover, both men and women had greater estimated reductions in predicted CHD risk after the MF diets compared to the LF diets. A strength of this study is that in this relatively large meta-analysis, well controlled feeding studies were selected in which body weight was maintained. However, there are some limitations. Some studies did not report all lipid and lipoprotein data at baseline or after diet intervention. Furthermore, the studies analyzed were of relatively short duration (2 to 12 weeks). The analysis therefore does not address effects that may take longer to occur.

The second study evaluated the effect of baseline CRP on lipid, lipoprotein, and oxidative stress responses to a cholesterol-lowering diet rich in macadamia nuts versus an average American diet. In individuals with baseline CRP levels < 1 mg/L only, TC, LDL-C, and non-HDL-C were reduced following the consumption of the macadamia nut-rich diet (MAC diet) versus the consumption of Average American Diet (AAD). In contrast, these risk factors were not decreased in individuals with baseline CRP > 1 mg/L. Lipid hydroperoxide decreased after the consumption of AAD and MAC diets vs. baseline in the CRP < 1 mg/L group, and was unchanged in the CRP ≥ 1 mg/L group. The results demonstrate that inflammation blunts the beneficial effects of a heart healthy diet on CVD lipid risk factors, as well as oxidative stress. A strength of this study is that it was a randomized, two-period crossover, controlled feeding study where body weight was maintained. A limitation of this study is that only one measure of oxidative stress and inflammation were done. Further studies are needed to confirm our findings and extend them to other biomarkers of oxidative stress and inflammation.

The final study used a randomized, treatment-controlled, 2-center, 2-arm, parallel design to investigate the effect of chronic processed tomatoes intake as well as acute intake of processed tomatoes on LDL oxidation and PON1 activity. In the postprandial study that evaluated the effects of tomatoes in single meal, oxidized LDL increased at 6 hour compared to 0 hour after the high fat meal without processed tomatoes but did not change after the high fat meal with processed tomatoes. After the postprandial high fat meal without processed tomatoes, there was an increase in PON1 activity at time 6 hour compared to time 3 hour in subjects who consumed a high tomato diet for 6 weeks. Furthermore, these subjects in the tomato group showed a trend for greater PON1 activity compared to those in the control group after the high fat meal without the processed tomatoes in PON1 QR/RR carriers only. The present data suggest that processed tomatoes are protective against adverse postprandial effects caused by a high fat meal and there is suggestive evidence that this is mediated by increased PON1 activity. Furthermore, in the postprandial state, consumption of the tomato milkshake blocked the increase in oxidative stress after a high fat meal. In addition, processed tomatoes increased PON1 activity in PON1 QR/RR carriers, and therefore may play a beneficial role in reducing the risk of CVD in this subgroup. Thus, processed tomato products might be protective against atherosclerosis by preventing the increase in oxidative stress that occurs postprandially, and by increasing PON1 activity, which would be expected to reduce oxidative stress. In this study, only half of the subjects had the genotype analysis done, which limited power to detect the effect of genotype on PON1 activity and oxidized LDL.

The data presented in this thesis have provided new information about how monounsaturated fat and antioxidants incorporated in the diets influence CVD risk. The lipid-lowering effects of dietary monounsaturated fat in a moderate fat diet were confirmed by the first two studies. The results demonstrate that a high monounsaturated fat diet is superior to a lower-fat diet or Average American diet according to the present data. Further studies are needed to understand the mechanisms that explain how inflammation markers interact with lipids, lipoproteins and oxidative stress. Processed tomato products exert beneficial effects in oxidative stress and PON1 activity (in the postprandial state) which then might reduce CVD risk. PON1 genotype might modify the dietary effects of antioxidants on PON1 activities. However, oxidative stress did not decrease after consumption of processed tomato products in the PON1 QR/RR carriers. Further studies with a larger sample size are needed to ascertain the significance of the current finding and further investigate the relationship between oxidative stress and antioxidants with regards to the PON1 polymorphisms. Although numerous in vitro studies have shown that PON1 protects LDL against oxidation, thereby exerting beneficial effects in the development of atherosclerosis, human studies are needed to confirm the interactions between PON1 and LDL oxidation. The participants in the tomato study were overweight, further studies are needed to confirm the current findings in a normal weight population.

In summary, moderate-fat (high monounsaturated fat) diets decreased TG and increased HDL-C greater than lower-fat diets, especially in subjects with diabetes. Macadamia-nut rich diet improved subjects' lipid profile and oxidative stress compared to baseline and AAD; individuals with low inflammatory status benefited the most from

cholesterol-lowering dietary interventions. Processed tomatoes might be protective against atherosclerosis by directly preventing the increase in oxidative stress and by increasing PON1 activity which would reduce oxidative stress; PON1 QR/RR carriers might benefit more compared to the QQ carrier. The research conducted herein has shown individual responses to diet interventions vary as a function of differences in subjects' clinical profile and genotype. Our research reinforces current dietary recommendations to control major risk factors for CVD so that there is a greater impact of heart healthy diets in the population at large.

Appendix A

MACADAMIA-NUT STUDY INFORMED CONSENT

INFORMED CONSENT FOR CLINICAL RESEARCH STUDY

Title of Project: Effects of a Diet Rich in Macadamia Nuts on Cardiovascular Disease Risk Factors (MAC Study), IRB # 21972

Principal Investigator:

Penny Kris-Etherton, PhD, RD
Department of Nutrition
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Penn State University
University Park, PA 16802
814-863-2923
Email: pmk3@psu.edu

ORP USE ONLY: IRB# 21972 Doc.#1
The Pennsylvania State University
Office for Research Protections
Approval Date: 12/16/05 JKG
Expiration Date: 11/07/06 JKG
Biomedical Institutional Review Board

Study Personnel:

Deborah Maddox Bagshaw, Clinical Coordinator
814-863-8056
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Amy Griel, Study Assistant
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Sarah Gebauer, Study Assistant
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Tricia Psota, Study Assistant
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Amy Cifelli, M.S., Diet Center Manager
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Yumei Cao, Study Assistant
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Jun Zhang, Study Assistant
Email: jzz113@psu.edu

Alison Gerber, Study Assistant
Email: arg5002@psu.edu

This is to certify that you, _____ (**print your name**), have been given the following information regarding your participation as a volunteer in a program of investigation under the supervision of Dr. Kris-Etherton. This consent form may contain words you do not understand. Please ask the study personnel to explain any words or information you do not clearly understand.

PLEASE READ EVERY PAGE CAREFULLY AND INITIAL THE BOTTOM OF EACH PAGE WHEN YOU HAVE HAD ALL OF YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION.

Purpose of the Study

You have been invited to participate in a clinical research study to test the effects of two different diets, one containing macadamia nuts, on the levels of fat in your blood and on the health of your blood vessels. This new study may provide important information about the health effects of macadamia nuts in the diet and how macadamia nuts, which

Initials _____

contain a healthy type of fat, might impact risk factors for developing heart disease. The control diet will contain foods in a typical American diet such as those that are commonly found at a grocery store. The control diet will serve as the basic profile and macadamia nuts will be incorporated into this profile during the macadamia diet. Both diets will have a moderate amount of fat but will have different types of fat. The Macadamia Diet will have more of unsaturated fats from the inclusion of the nuts.

Procedures to be Followed

Screening Tests

If you agree to participate in this study your participation will last for 12 weeks total. There will be two, 5-week diet periods, with a 2-week break between the two diet periods. The two diet periods will be similar except for the addition of macadamia nuts to one of the diets. If you decide to participate in the study and are eligible after the telephone screening, you will be further screened during a visit to the General Clinical Research Center (GCRC) at Penn State to determine eligibility to participate. The visit will consist of filling out forms (Informed consent, medical history, eating attitudes test, personal information); measuring height and weight so your body mass index (BMI) can be calculated; measuring your waist circumference (WC) as well as your blood pressure (BP); and pricking your finger to obtain a drop of blood to measure blood fats (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides). If after these measurements it is determined you are still eligible, a blood sample will be taken from your arm for a blood test for a complete blood count, to check liver and kidney function and for a blood fat panel. (approximately 15 mls of blood or 1 tablespoon will be taken). If you are female, you will be given a urine pregnancy test. You will also be asked to fill out a number of questionnaires dealing with your mood and your attitudes towards food and eating. You will be contacted within 3-5 days with the results of the screening blood sample. A clinician at the GCRC will review all of the screening data and if you are still eligible for the study, you will be told when to report for the beginning of the first feeding period. There will be no charge for the screening blood work or measurements and you will get these results.

Feeding Study

If you agree to participate in the study you will agree to eat only those foods (3 meals and a snack every day) and beverages provided to you (some non-caloric beverages are allowed for free choice) during the feeding periods of the study. You will come to the dining room at the Metabolic Diet Study Center in Henderson Building Monday through Friday for breakfast **or** dinner, where meals will be prepared and provided for you. Your other two meals and a snack will be packed for you to take and eat at a place of convenience. On Friday evenings, you will be given a cooler that contains your Friday dinner and Saturday and Sunday meals and snacks. You will be required to appropriately refrigerate and store all foods provided to you for take-out.

You will be weighed regularly at your mealtime and you will provide the staff dietitian with information about any non-study foods you may have eaten, any study foods not eaten and caffeine (limited to five, caffeine-containing beverages/day) and alcohol

Initials _____

consumption (limited to 2 drinks/week). You are supposed to eat only the foods given to you and nothing else. You must eat all of the food given to you. If for some reason you fail to do this, it is important that you tell the study staff that you did not follow protocol so they can make a note of it in your records. The information you provide to the study coordinators will be collected on two separate forms; one to be completed daily and one to be completed weekly. It should only take about 5 minutes to complete these forms each day. Your calorie intake may be adjusted over the course of the study in order to maintain your screening body weight. This is **not a weight-loss study**. The diets are designed to meet your calorie needs and keep your body weight constant. Calorie intake will be adjusted up or down as necessary to maintain your weight. Also, you must keep your exercise level constant throughout the whole study.

Questionnaires about mood, emotion and diet

You will be asked to complete several questionnaires after the screening visit, and an additional set of questionnaires at the end of each of the two treatment periods. Questions will be asked about your mood and emotions and about sources of stress in your life and about attitudes regarding eating and food. The questionnaires will take about 30 minutes.

Endpoint Testing

Blood sampling:

At the beginning of the first feeding period at the end of each diet period (this will be at least one week after your screening visit), after a twelve hour fast (consumption of no food or drinks except water), a blood sample will be taken from your arm on two consecutive days. This will be done at the General Clinical Research Center on the PSU campus. You cannot drink alcohol during the 48 hours prior to having your blood taken and that you cannot engage in vigorous physical activity 24 hours prior to having your blood taken.

Approximately 80 ml (about 5.5 tablespoons) of blood will be collected (40 mls on two consecutive days). Therefore, over the 12-week study, blood will be taken on 6 days with a total amount of 240 mls. (~ 17 tablespoons) of blood taken. Blood samples will be frozen and analyzed at the end of the study (when all subjects have completed the study). The results of the study will only be available at the end of the entire study (which may take up to 1 year). Your blood will be tested for the following: blood fats (total cholesterol, LDL-cholesterol, HDL-cholesterol), blood sugar (glucose, insulin), inflammation markers (plasma cytokines, and C-reactive protein), and how the blood fat is being metabolized (lipid hydroperoxides). Successful completion of this study depends on the total cooperation of the participants. If during the study, you cannot eat the food provided, you will be asked to leave the study.

Discomforts and Risks

Feeding Study

The diets used in this study are nutritionally adequate, whole-food diets. Foods will be prepared according to accepted standards of sanitation and provisions are made to ensure the safety of foods provided for off-site consumption. However, it is possible that incorrect food handling during shipping, storage or preparation, if not detected, could result in food-borne illness. Every effort will be made to safeguard against this possibility. Feeding studies that require on-site eating of meals and strict adherence to the diets

Initials _____

provided may interfere with social activities centered around eating such as dining in restaurants. While the menus will provide some variety in the diets, the number of food items will be more limited than that available in an average grocery store. The limited variety may become boring over the course of the study.

Blood Sampling

The risks involved with taking blood from you include some local pain and bruising where the blood is taken. Well-trained and experienced phlebotomists will be used to take your blood. Blood sampling can also cause light-headedness and dizziness. If this occurs, the symptoms will be alleviated by having you lie flat with your feet raised. As with any procedure involving taking blood, infection is possible. All precautions will be taken to avoid infection. There is a slight risk of developing a blood clot at the blood draw site.

Questionnaires

You will be asked to fill out questionnaires on 3 occasions throughout the study. You may experience irritation with filling out this amount of paperwork or perhaps feel embarrassed with answering some of the questions.

Benefits to You

You will have a chance to learn the principles of good nutrition practices. You will also receive the results of your screening blood work and information about how your blood cholesterol changed in response to the experimental diets. At the end of the study it will be explained how macadamia nuts could have beneficial affects on your blood cholesterol and inflammation. The final results of the study will not be available until all of the analysis is completed. This may take up to one and a half years. However, no benefit from participation in this study is guaranteed.

Potential Benefits to Society

It is hoped that the information gained from this study will increase our understanding of the effects of macadamia nut intake on cholesterol and heart disease risk factors and may help explain why individuals respond differently to a certain type of diet.

Statement of Confidentiality

Your participation in this research is confidential. All records are coded with a unique ID number and no names are used. Records containing names or other identifying information are kept under lock at the Metabolic Diet Study Center. Only the investigators and their assistants will have access to your identity and to information that can be associated with your identity. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records. Under state law, an exception to confidentiality is required if a person indicates an intention to harm him or her self or another person. In the event of publication of this research, no personal identifying information will be disclosed. Your blood specimens will be coded with your unique ID number and will be maintained until two years after the date from when the study is published, and then destroyed. At the end of the study

Initials _____

(after all subjects have completed the study), you will be given your laboratory results without cost, and informed of the study results, and advised of the implications for your future care.

You should be aware that the Office for Research Protection at Penn State University and the Biomedical Institutional Review Board may review records related to this research.

Right to Ask Questions

You have been given an opportunity to ask any questions you may have, and all such questions have been answered to your satisfaction. Dr. Kris-Etherton is available to answer any questions that you have at the time of your participation in this study or if you have questions in the future. You will be informed of any new information that may affect your willingness to participate. You may call the Office for Research Protections (814-865-1775) if you need further information about your rights as a research participant

Compensation

You will receive all of your food at no cost to you for the two 5-week feeding periods. For your time and participation in the study you will receive monetary compensation of up to \$350. You will receive \$100 for completing the first diet period and \$250 for completing the entire study. If you are an employee of Penn State University, the compensation you receive is treated as taxable income and therefore taxes will be taken from the total compensation amount. If you are not employed by Penn State University, total payments within one calendar year that exceed \$600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income. If you do not complete the study, for whatever reason, your free food and screening blood results will be considered the compensation for your participation up to that point.

Injury Statement

Medical care is available in the event of injury resulting from research but that neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have against the University for injury resulting from negligence of the University or the investigators. For further information about this, you may call the Office of Research Protections at 814-865-1775.

Voluntary Participation

Your participation in this study is voluntary, that you may decline to answer any questions during the screening process or during the study. You are aware that refusing to answer a question may keep you from being able to participate in the study. You may withdraw from this study at any time by notifying the investigators or other study personnel. Your withdrawal from this study or your refusal to participate will in no way affect your care or access to medical services. You may be asked to leave the study at any time if you do not comply with the study protocol.

Initials _____

In the event that abnormal lab test results are obtained during initial screening or subsequently throughout this study, you will be informed as quickly as possible of these results and instructed to contact your private physician for further assessment. The lab test results will be made available to your private physician at your request.

This is to certify that you consent to and give your permission for your participation as a volunteer in the study entitled "Effects of a Diet Rich in Macadamia Nuts on Cardiovascular Disease Risk Factors". You certify that you are 18 years of age or older. You will receive a signed copy of this consent form. You have read this form and understand the contents of this consent form.

Signature of Participant

Date

Printed Name of Participant

I, the undersigned, have defined and explained the study involved to the above volunteer.

Signature of Investigator

Date

Initials _____

Appendix B

MACADAMIA-NUT STUDY MENUS

Average American Diet: 6 Day Cycle Menu

Menu 1

Breakfast

Orange juice
Plain bagel with sliced ham and cheese
Reduced fat (2%) milk

Lunch

Roast beef sandwich on white bread with tomato and light mayonnaise
Apple
M&M's plain chocolate candies

Dinner

Spaghetti with meat sauce
Parmesan cheese
Green beans
Dinner roll with margarine
JELL-O chocolate pudding cup

Snack

Pretzel twists
Cheddar cheese cubes

Menu 2

Breakfast

Apple juice
Cheerios with banana and reduced fat 2% milk
Whole wheat toast with butter

Lunch

Turkey sandwich on whole wheat bread with tomatoes and light mayonnaise
Baby carrots and broccoli florets with fat free ranch dipping sauce

Dinner

Chicken breast with brown rice
Broccoli
Spinach salad with mushrooms and Italian dressing
Dinner roll with butter

Snack

Dannon Fruit on the Bottom lowfat yogurt

Menu 3

Breakfast

Orange juice

Rice Krispies cereal with reduced fat 2% milk

English muffin with butter

Lunch

Ham sandwich on rye bread with Swiss cheese and light mayonnaise

Pineapple chunks

Oatmeal cookie

Dinner

Pork loin with pasta and tomato sauce

Snow peas

Romaine lettuce salad with tomatoes and carrots with French dressing

Dinner roll with butter

Snack

Pear halves

Menu 4

Breakfast

Cranberry juice

Raisin Bran cereal with reduced fat 2% milk

Whole wheat toast with jelly and butter

Lunch

Tuna salad with saltine crackers

Baby carrots

Dinner

Sirloin beef tips with egg noodles and gravy

Corn

Romaine lettuce salad with tomatoes and Ranch dressing

Dinner roll with butter

Snack

Peaches

Menu 5

Breakfast

Orange juice
French toast with syrup and butter
Reduced fat 2% milk

Lunch

Turkey and cheese sandwich on whole wheat bread with lettuce, tomato, and light mayo
Baby carrots
Apple
Fig newtons cookies

Dinner

Chicken cordon bleu entrée
Dinner roll with butter
JELL-O chocolate pudding cup

Snack

Nature Valley Trail Mix granola bar

Menu 6

Breakfast

Dannon Fruit on the Bottom lowfat yogurt
Lowfat granola cereal
Blueberries
Reduced fat 2% milk

Lunch

Ham sandwich on whole wheat bread with light mayonnaise
Pretzel twists
Pear halves
JELL-O gelatin cup

Dinner

Turkey Taco (ground turkey meat with seasoning) Salad
Cheddar cheese, shredded
Romaine lettuce, tomato, corn
Tostitos tortilla chips

Snack

M&M's plain chocolate candies

Macadamia Diet Period: 6 Day Cycle Menu

Menu 1

Breakfast

Orange juice
Plain bagel with sliced ham and cheese
Skim

Lunch

Roast beef sandwich on white bread with tomato and fat free mayonnaise
Apple
Pretzel twists

Dinner

Chicken breast with Vegetable Rice “Mac Pao” (white rice, veggies, unsalted, chopped macadamia nuts in an oriental sauce) ***
Romaine lettuce salad with tomatoes and carrots and Italian dressing
Dinner roll with margarine

Snack

Macadamia nuts, roasted, salted
Dannon Light n’ Fit yogurt with Nature Valley Oats n’ Honey granola bar

Menu 2

Breakfast

Apple juice
Cheerios with banana and skim milk
Whole wheat bagel with light cream cheese

Lunch

Turkey sandwich on whole wheat bread with tomatoes and fat free mayonnaise
Baby carrots and broccoli florets with fat free ranch dipping sauce
Baked Lays potato chips

Dinner

Beef sirloin tips with Spicy Noodles (spaghetti with sesame soy sauce, veggies, and unsalted, chopped macadamia nuts) ***
Romaine lettuce salad with tomatoes and Italian dressing

Snack

Macadamia nuts, roasted, salted
Dannon Light n’ Fit yogurt
Fig newtons cookies

Menu 3

Breakfast

Orange juice
Rice Krispies cereal with skim milk
English muffin with margarine

Lunch

Ham sandwich on rye bread with Swiss cheese and honey mustard
Pineapple chunks
Cranberry Macadamia nut cookie ***

Dinner

Pork loin with pasta and tomato sauce
Snow peas
Romaine lettuce salad with tomatoes and carrots with French dressing
Dinner roll with margarine

Snack

Macadamia nuts, roasted, salted

Menu 4

Breakfast

Cranberry juice
Raisin Bran cereal with skim milk
Whole wheat toast with jelly and margarine

Lunch

Macadamia Mango Chicken Salad with saltine crackers ***
Baby carrots
Baked Lays potato chips

Dinner

Spaghetti with meat sauce
Parmesan cheese
Green beans
Romaine lettuce salad with tomatoes and French dressing
Dinner roll with margarine

Snack

Macadamia nuts, roasted, salted
JELL-O gelatin cup

Menu 5

Breakfast

Orange juice
French toast with syrup and margarine
Skim milk

Lunch

Turkey and cheese sandwich on whole wheat bread with lettuce, tomato, fat free mayo
Baby carrots
Apple
Fig newtons cookies

Dinner

Chicken cordon bleu entrée
Dinner roll with margarine
JELL-O chocolate pudding cup

Snack

Macadamia nuts, roasted, salted
Cinnamon & Sugar Spiced macadamia nuts ***

Menu 6

Breakfast

Orange juice
Dannon Fruit on the Bottom lowfat yogurt
Lowfat granola cereal
Blueberries
Skim milk

Lunch

Mixed greens salad with grilled chicken breast, apples, dried cranberries, unsalted macadamia nuts and California salad dressing ***
Dinner roll with butter
Pineapple chunks

Dinner

Turkey Taco (ground turkey meat with seasoning) Salad
Cheddar cheese (reduced fat), shredded
Romaine lettuce, tomato, corn
Baked Tostitos tortilla chips

Snack

Macadamia nuts, roasted, salted and Fig newtons cookies

Appendix C

TOMATO STUDY INFORMED CONSENT

INFORMED CONSENT FOR CLINICAL RESEARCH STUDY

Title of Project: The effect of processed tomatoes on endothelium and platelet function.

Principal Investigator:

Penny Kris-Etherton, PhD, RD
Department of Nutrition
S126 Henderson
Penn State University
University Park, PA 16802
814-863-2923
Email: pmk3@psu.edu

<p>ORP USE ONLY: IRB#25646 Doc.#1 The Pennsylvania State University Office for Research Protections Approval Date: 02/25/08 T. Kahler Expiration Date: 05/16/08 T. Kahler Biomedical Institutional Review Board</p>

Study Personnel:

Deborah Maddox Bagshaw, Clinical Coordinator
814-863-8056
Email: ddm108@psu.edu

Yumei Cao, Study Assistant
Email: yuc110@psu.edu

Amy Cifelli, M.S., Diet Center Manager
Email: amm392@psu.edu

Pam Davis, Study Assistant
Email: ped4@psu.edu

This is to certify that you, _____ (**print your name**), have been given the following information regarding your participation as a volunteer in a program of investigation under the supervision of Dr. Kris-Etherton. This consent form may contain words you do not understand. Please ask the study personnel to explain any words or information you do not clearly understand.

PLEASE READ EVERY PAGE CAREFULLY AND INITIAL THE BOTTOM OF EACH PAGE WHEN YOU HAVE HAD ALL OF YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION.

Purpose of the Study

You have been invited to participate in a clinical research study to test the effects of the processed tomato products on the health of your blood vessels. The purpose of this study is to test whether certain compounds in tomatoes will help reduce factors in your blood

Initials _____

that are associated with disease risk. This new study may provide important information about the health effects of processed tomato products and how processed tomatoes might impact endothelium and platelet function disease-risk biomarker profiles.

Procedures to be Followed

Screening Tests

If you decide to participate in the study and are eligible after the telephone screening, you will be further screened during a visit to the General Clinical Research Center (GCRC) at Penn State to determine eligibility to participate. The visit will consist of filling out forms (Informed consent, medical history, personal information); measuring height and weight so your body mass index (BMI) can be calculated; measuring blood pressure (BP). If after these measurements it is determined you are still eligible, a blood sample (Approximately 12 ml of blood or ~1 teaspoon) will be taken from your arm for a blood test for a complete blood count, to check liver and kidney function and for a blood fat panel. If you are female, you will be given a urine pregnancy test. You will be contacted within ~1 week with the results of the screening blood sample. A clinician at the GCRC will review all of the screening data and if you are still eligible for the study, you will be told when to report for the beginning of the first feeding period.

Study scheme

You will be in this study for approximately 9 weeks with 2 distinct phases. During the initial 3 weeks, we will ask you to stop or severely limit your tomato product consumption, such as spaghetti sauce, tomato paste, tomato juice, and including fresh tomatoes. This 3-week period is considered a run-in period. Next will be the 6-week experimental or treatment period. You will be randomized to 1 of 2 groups that will follow diets either high or low in tomato products. We will provide you the tomato products or their alternatives (such as applesauce, broth soup) free of charge. The low tomato product intake group will follow a diet similar to the first 3-week run-in period. The high tomato product intake group will consume 4-5 servings of tomato products per day. If you are assigned to the high tomato diet, you will be asked to incorporate products (in the form of tomato paste, tomato juice, tomato soup, salsa, tomato sauce, ketchup, diced tomatoes and spaghetti sauce) into your diet. If you are assigned to the low tomato diet, you will be asked to incorporate different, non-tomato products (such as apple sauce and broth soups) into your diet to provide an equivalent calorie, sugar, and sodium content to the high tomato diet. A dietitian will help you do this. The dietitian will work with you to create a diet consistent with the goals of this study so that you can easily incorporate the study treatments into your daily diet without gaining or losing weight. You will be required to record what you eat and drink up to 3 days per week during two weeks of the study. We will give you the appropriate training and food record logs to do this task. The dietitian or other trained study personnel will see you approximately once per week to review food records, make sure things are going well and answer any questions that you may have. You will be required to pick-up these products weekly at the study site at the Metabolic Diet Study Center in Henderson Building at the Penn State University. You will be weighed at the Diet Center as you come and pick up the products.

Initials _____

This is not a weight-loss study. You will incorporate the tomato products and the alternatives into your daily diet and keep your body weight constant. Also, you must keep your exercise level constant throughout the whole study.

Endpoint Testing

Blood sampling:

At the end of the run-in period and of the six week diet period, after a twelve hour fast (consumption of no food or drinks except water), a blood sample will be taken from your arm. Your weight, waist circumference and blood pressure will also be recorded. This will be done at the General Clinical Research Center on the PSU campus. You cannot drink alcohol during the 48 hours prior to having your blood taken and you cannot engage in vigorous physical activity 2 hours prior to having your blood taken.

Approximately 40 ml of blood will be collected at the end of the run-in period and after the diet period. Therefore, over the 9-week study, blood will be taken on 2 days with a total amount of ~90 ml (10 ml at screening; 40 ml each test day after enrolled in the study or about 6 teaspoons). Blood samples will be frozen and analyzed at the end of the study (when all subjects have completed the study). The results of the study will only be available at the end of the entire study (which may take up to 2 years). Your blood will be tested for the following: blood fats (triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol), blood sugar (glucose, insulin), endothelium functions (cellular adhesion molecules: ICAM and VCAM), inflammation markers (for example: C-reactive protein, interleukin-6, tumor necrosis factor-alpha, PAI-1, and adiponectin), platelet and monocyte function, oxidative stress (for example: oxidized LDL; F2 isoprostane), paraoxonase activity, concentration and function and also target nutrients (lycopene, vitamin C, beta-carotene, quercetin, naringenin, and kaempferol). No personal information will be kept with any sample – only an ID# will be assigned and only the Primary Investigator and the Study Coordinator will have access to the ID# assignments with the study files. If you are female, you will be asked to provide a urine sample at the end of the run-in and at the end of the diet period for a pregnancy test. If you become pregnant during this time, you will be asked to leave the study.

Urine Sampling:

You will be asked to collect a urine sample over a 24 hour period at the end of the run-in period and at the end of the study. You will be provided a sterile canister and instructions for urine collection. The urine will be returned to the study site and frozen for analysis.

Buccal Swab Sampling:

You will be asked to collect a buccal swab sample (by rubbing the inside of your cheek with a small swab) once during the course of the study. DNA (genetic material) will be isolated from this sample, frozen and used to test for whether you have a certain gene that might affect one of the markers we are testing (paroxanase). No part of the genetic profile will be used for anything medically significant nor will you or your physician be given any of the results from this genetic profile. All samples will be coded with your study ID number only.

Initials _____

Successful completion of this study depends on the total cooperation of the participants. If during the study, you cannot eat the food provided or comply with other study procedures, you will be asked to leave the study.

Time Commitment for the Study

You will spend approximately the following amounts of time in study activities –

Screening, visit 1 - 60 min

Filling out forms/picking up food – 15 min per week for 6 weeks – total of 90 min

Meet with dietitian – 45 min at baseline visit; 20 min per week for 6 weeks – total of 165 min.

End-of-run-in/Baseline clinic visit for blood draw – 30 min

End -of-diet period clinic visits – 30 min

Total time for study is approximately 375 minutes or about 6.25 hours.

Discomforts and Risks

Blood Sampling

The risks involved with taking blood from you include some local pain and bruising where the blood is taken. Well-trained and experienced phlebotomists will be used to take your blood. Blood sampling can also cause light-headedness and dizziness. If this occurs, the symptoms will be alleviated by having you lie flat with your feet raised. As with any procedure involving taking blood, infection is possible. All precautions will be taken to avoid infection. There is a slight risk of developing a blood clot at the blood draw site.

Buccal Swab Sampling

There are no known risks of doing a buccal swab sampling.

Benefits to You

You will receive the results of your screening blood work and information about how your blood cholesterol changed in response to the diet treatments. The final results of the study will not be available until all of the analysis is completed. This may take up to two years. However, no benefit from participation in this study is guaranteed.

Potential Benefits to Society

It is hoped that the knowledge gained from this study may increase the understanding of how processed tomato consumption affects acute and chronic indices of disease risk.

Statement of Confidentiality

Your participation in this research is confidential. All records are coded with a unique ID number and no names are used. Records containing names or other identifying information are kept under lock at the Metabolic Diet Study Center. All records associated with your participation in the study will be subject to the usual confidentiality

Initials _____

standards applicable to medical records. An exception to confidentiality is required if a person indicates an intention to harm him or her self or another person. In the event of publication of this research, no personal identifying information will be disclosed. Your blood specimens and other test results will be coded with your unique ID number and will be maintained until three years after the date from when the study is published, and then destroyed unless (see end of document) you give permission for us to keep your blood samples for future research purposes. At the end of the study (after all subjects have completed the study), you will be given your laboratory results without cost, and informed of the study results, and advised of the implications for your future care.

You should be aware that the following may review and copy records related to this research: The Office of Human Research Protections in the U.S. Dept. of Health and Human Services; The Penn State University Biomedical Institutional Review Board; The Penn State University Office for Research Protections.

Right to Ask Questions

You may ask any questions you may have, and all such questions will be answered to your satisfaction. Please contact Dr. Kris-Etherton at 814-863-2923 with questions, complaints, or concerns about the research. You can also call this number if you feel this study has harmed you. Dr. Kris-Etherton is available to answer any questions that you have at the time of your participation in this study or if you have questions in the future. You will be informed of any new information that may affect your willingness to participate. You may call The Penn State University Office for Research Protections (814-865-1775) if you need further information about your rights as a research participant.

If the Primary Investigator or study staff becomes aware of new information or research findings that might impact your willingness to participate in this study, you will be given that information. You will be given the opportunity to ask any questions you might have and to decide if you want to continue to participate in the study.

Compensation

You will receive all of your tomato products and alternatives at no cost to you for the 6-week diet periods. For your time and participation in the study you will receive monetary compensation of \$150.00: \$25.00 for completing the run-in diet period and \$125 for completing intervention period; all payable at the end of a subject's participation. Your screening lab results will be given to you at no cost also.

If you drop out of the study, for whatever reason, but complete the run-in diet period, you will receive \$25.00 compensation.

Total payments within one calendar year that exceed \$600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

Initials _____

Injury Statement

Medical care is available in the event of injury resulting from research but neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have against the University for injury resulting from negligence of the University or investigators.

Voluntary Participation

Your participation in this study is voluntary. You may decline to answer any questions during the screening process or during the study. However, please be aware that refusing to answer a question may keep you from being able to participate in the study. You may withdraw from this study at any time by notifying the investigators or other study personnel. Refusal to take part in or withdrawing from this study will involve no penalty or loss of benefits you would receive otherwise. You may be asked to leave the study at any time if you do not comply with the study protocol.

In the event that abnormal lab test results are obtained during initial screening or subsequently throughout this study, you will be informed as quickly as possible of these results and instructed to contact your private physician for further assessment. The lab test results will be made available to your private physician at your request.

This is to certify that you consent to and give your permission for your participation as a volunteer in the study entitled “The effect of processed tomatoes on endothelium and platelet function”. You certify that you are 18 years of age or older. You will receive a signed copy of this consent form. You have read this form and understand the contents of this consent form.

Signature of Volunteer

Date

Printed Name of Volunteer

I, the undersigned, have defined and explained the study involved to the above volunteer

Signature of Investigator

Date

Initials _____

INFORMED CONSENT FOR CLINICAL RESEARCH STUDY

Title of Project: Secondary study: The postprandial (after meal) effect of processed tomatoes on endothelium and platelet function.

Principal Investigator:

Penny Kris-Etherton, PhD, RD
Department of Nutrition
S126 Henderson
Penn State University
University Park, PA 16802
814-863-2923
Email: pmk3@psu.edu

ORP USE ONLY: IRB#25646 Doc.2
The Pennsylvania State University
Office for Research Protections
Approval Date: 02/25/08 T. Kahler
Expiration Date: 05/16/08 T. Kahler
Biomedical Institutional Review Board

Study Personnel:

Deborah Maddox Bagshaw, Clinical Coordinator
814-863-8056
Email: ddm108@psu.edu

Yumei Cao, Study Assistant
Email: yuc110@psu.edu

Amy Cifelli, M.S., Diet Center Manager
Email: amm392@psu.edu

Pam Davis, Study Assistant
Email: ped4@psu.edu

This is to certify that you, _____ (**print your name**), have been given the following information regarding your participation as a volunteer in a program of investigation under the supervision of Dr. Kris-Etherton. This consent form may contain words you do not understand. Please ask the study personnel to explain any words or information you do not clearly understand.

PLEASE READ EVERY PAGE CAREFULLY AND INITIAL THE BOTTOM OF EACH PAGE WHEN YOU HAVE HAD ALL OF YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION.

Purpose of the Study

You have been invited to participate in a clinical research study to test the effects of the processed tomato products on the health of your blood vessels before and after a meal.

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You are being given the opportunity to participate in this additional testing as part of your participation in the main study (Effects of Processed Tomato Products on Endothelial and Platelet Function). The purpose of this secondary study is to test whether certain compounds in tomatoes will help reduce factors in your blood that are associated with disease risk when you eat them with a meal containing a certain amount of fat. This new study may provide important information about the health effects of processed tomato products and how processed tomatoes might impact endothelium (lining of blood vessels) and platelet (special cells in your blood) function disease-risk biomarker profiles. If you agree to participate in this additional testing, all activities of the main study will remain the same – you will undergo this additional testing (described below) on 3 of the clinic visit days.

Procedures to be Followed

Study scheme

Of the 30 people enrolled at this study, we will have 12 of them (6 men, 6 women) participate in this additional testing. The length of the study is still 9 weeks, but there are 3 study days that you are required to stay with us for up to 7 hours each. We want to see how your body responds to a standard high fat meal with or without tomato products, often referred to as a “postprandial test day”. This will be done after 2 weeks on the run-in phase of the main study and at the end of the run-in before you start your 6 weeks of product consumption and at the end of your 6 weeks test diet period for the main study.

Endpoint Testing

Blood sampling:

To accomplish the additional postprandial study, we will have you come to the GCRC at Penn State in the morning after an overnight fast of at least 12 hours. When you come to the lab you will meet with a nurse or other trained personnel and have your blood pressure, height and weight measured. In addition, a nurse will apply LMX, a numbing cream, to the place on your arm where s/he will be inserting a catheter, a tube about the size and consistency of a piece of spaghetti. After application of the numbing cream you will wait for 30 minutes while the cream takes effect. LMX can be refused if you wish to do so. The catheter will allow for easy blood draws and infusion.

Blood will be collected before eating and for 6 hours after you consume the high fat test meal. You will have 20 minutes to eat the test meal. The meals you eat will be prepared in a metabolic kitchen with strict food safety standards and contain foods appropriate for breakfast along with a milkshake and will contain about 700 calories. The nurse will collect blood from the catheter at 0 (fasting, before test meal – this will also count for your baseline blood draw and end of study blood draw) and then at 30, 60, 90, 120 min (2 h), 3h, 4h, 5h, and 6 h. We will take approximately 20 ml blood out of your arm each time for a total of 180 mls (or ~ 6 oz). We will do this blood collection procedure 9 times during each postprandial test day. Blood will be processed for subsequent laboratory analysis. Your blood will be tested for the following: blood fats (triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol), blood sugar (glucose, insulin),

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endothelial (this refers to your blood vessel health) function (cellular adhesion molecules: ICAM and VCAM; endo-PAT), inflammation markers (such as C-reactive protein, interleukin-6, tumor necrosis factor-alpha, PAI-1, and adiponectin), platelet and monocyte function, oxidative stress (such as oxidized LDL; F2 isoprostane), paraoxonase concentration and function and also target nutrients (lycopene, vitamin C, beta-carotene, quercetin, naringenin, and kaempferol). No personal information will be kept with any sample – only an ID# will be assigned and only the Primary Investigator and the Study Coordinator will have access to the ID# assignments with the study files. At the end of the 6 hour period you will have the catheter removed and briefly evaluated for safety before leaving the study site. You will be offered a small snack before leaving. You will come to lab on 2 additional occasions for this same type of testing for a total of 3 postprandial test days during the 9 week study.

Successful completion of this study depends on the total cooperation of the participants. If during the study, you cannot eat the food provided or comply with other study procedures, you will be asked to leave the study.

Time Commitment for the Study

You will spend approximately the following amounts of time in study activities –

Run-in period clinic visit for postprandial study – 7 hours

Baseline clinic visit for postprandial study – 7 hours

End -of-diet period clinic visits for postprandial study – 7 hours

Total time for study is approximately 21 hours in addition to time for the main study (some of the activities will overlap).

Discomforts and Risks

Blood Sampling

The risks involved with taking blood from you include some local pain and bruising where the blood is taken. Well-trained and experienced phlebotomists will be used to take your blood. Blood sampling can also cause light-headedness and dizziness. If this occurs, the symptoms will be alleviated by having you lie flat with your feet raised. As with any procedure involving taking blood, infection is possible. All precautions will be taken to avoid infection. There is a slight risk of developing a blood clot at the blood draw site. The amount of blood being drawn in this additional testing (~ 180 ml each test day with a total of 540 mls or 18 oz. over 9 weeks) is less than that if you donated blood (16 oz. per unit) every 56 days (8 weeks) as allowed.

Topical Anesthetic Cream: Numbing cream will not be used if you have a sensitivity to lidocaine. Eye contact should be avoided. When used, all sensations within the treated area are blocked. For this reason, unintentional trauma to the treated area, such as scratching, rubbing or exposure to hot or cold temperatures should be avoided until complete sensation has returned. During or immediately after application, mild swelling,

Initials _____

skin redness, or abnormal sensation may develop at the site of treatment. In clinical studies, no serious reactions resulted from the use of the cream. Allergic reactions can occur and can be managed by usual allergic treatment. Body adverse reactions following appropriate use are unlikely due to the small amount of dose absorbed. If effects do occur, they are similar in nature to those seen with other local anesthetic agents and may include light-headedness, nervousness, apprehension, dizziness, drowsiness, twitching, and vomiting. Reactions may be brief or non-existent. Numbing cream can be refused if the subject wishes to do so.

Benefits to You

No additional benefits from participation.

Potential Benefits to Society

It is hoped that the knowledge gained from this study may increase the understanding of how processed tomato consumption affects acute and chronic indices of disease risk with regard to eating these products with meals.

Statement of Confidentiality

Your participation in this research is confidential. All records are coded with a unique ID number and no names are used. Records containing names or other identifying information are kept under lock at the Metabolic Diet Study Center. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records. An exception to confidentiality is required if a person indicates an intention to harm him or her self or another person. In the event of publication of this research, no personal identifying information will be disclosed. Your blood specimens and other test results will be coded with your unique ID number and will be maintained until three years after the date from when the study is published, and then destroyed unless (see end of document) you give permission for us to keep your blood samples for future research purposes. At the end of the study (after all subjects have completed the study), you will be given your laboratory results without cost, and informed of the study results, and advised of the implications for your future care.

You should be aware that the following may review records related to this research: The Office of Human Research Protections in the U.S. Dept. of Health and Human Services; The Penn State University Biomedical Institutional Review Board; The Penn State University Office for Research Protections.

Right to Ask Questions

You may ask any questions you may have, and all such questions will be answered to your satisfaction. Please contact Dr. Kris-Etherton at 814-863-2923 with questions, complaints, or concerns about the research. You can also call this number if you feel this study has harmed you. Dr. Kris-Etherton is available to answer any questions that you have at the time of your participation in this study or if you have questions in the future. You will be informed of any new information that may affect your willingness to participate. You may call The Penn State University Office for Research Protections (814-865-1775) if you need further information about your rights as a research participant

Initials _____

If the Primary Investigator or study staff becomes aware of new information or research findings that might impact your willingness to participate in this study, you will be given that information. You will be given the opportunity to ask any questions you might have and to decide if you want to continue to participate in the study.

Compensation

For each postprandial test day, you will receive an additional \$25 for a total of \$75.

Total payments within one calendar year that exceed \$600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

Injury Statement

Medical care is available in the event of injury resulting from research but neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have against the University for injury resulting from negligence of the University or investigators.

Voluntary Participation

Your participation in this study is voluntary. You may decline to answer any questions during the screening process or during the study. Refusing to answer a question may keep you from being able to participate in the study. You may withdraw from this study at any time by notifying the investigators or other study personnel. Refusal to take part in or withdrawing from this study will involve no penalty or loss of benefits you would receive otherwise. You may be asked to leave the study at any time if you do not comply with the study protocol.

In the event that abnormal lab test results are obtained during initial screening or subsequently throughout this study, you will be informed as quickly as possible of these results and instructed to contact your private physician for further assessment. The lab test results will be made available to your private physician at your request.

This is to certify that you consent to and give your permission for your participation as a volunteer in the study entitled “Secondary Study: The postprandial effects of processed tomatoes on endothelium and platelet function”. You certify that you are 18 years of age or older. You will receive a signed copy of this consent form. You have read this form and understand the contents of this consent form.

Signature of Volunteer

Date

Printed Name of Volunteer

I, the undersigned, have defined and explained the study involved to the above volunteer

Signature of Investigator

Date

Initials _____

Appendix D
TOMATO RECEIPES

Tomato Product Recipes

Sun-Dried Tomato Spread

2 (6 oz) cans tomato paste

2 tbsp chopped fresh basil

¼ cup olive oil

1 tsp crushed garlic

¾ cup sun-dried tomatoes, packed in oil, drained

1 tsp sugar

1 tsp garlic salt

In a blender or food processor, mix tomato paste, basil, olive oil, garlic, sun-dried tomatoes, sugar and garlic salt. Blend to the consistency of a spreadable paste. Chill in the refrigerator until serving. Makes 16 servings, (each serving provides 1.5 Tbsp, approximately ¼ dose of TOMATO PASTE)

Chicken, Spinach and Mango Salad with Warm Tomato Vinaigrette

2 tsp olive oil

4 small boneless skinless chicken breasts cut into thin strips

1 can (14.5 oz) Diced tomatoes

¼ cup French Dressing

1 Tbsp Seasoned Rice Vinegar

1 bag (5-6 oz) Baby Spinach

2 ripe mangoes, peeled, cut into bite size pieces

4 slices Ready Crisp Fully Cooked Bacon, heated, chopped

Heat oil in large skillet over medium-high heat. Add chicken; cook 4 minutes or until lightly browned, stirring occasionally. Add tomatoes with their juice; stir. Bring to a boil.

Reduce heat to low. Cook 5 minutes, or until chicken is no longer pink in the center, stirring frequently. Add dressing and vinegar; cook 2 minutes or until heated through.

Arrange spinach, mangoes and avocados evenly on each of the 4 serving plates. Top with chicken mixture; sprinkle with bacon. Serve warm. Makes 4 servings (each serving

provides 3.6 oz of DICED TOMATOES, or approximately 1 dose)

Little Italy Sausage Sandwiches

1 lb. Sweet Italian Sausage Links, cut in half, lengthwise

1 cup sliced onion

1 can (6 oz) Tomato Paste

2 cups mixed salad greens

Balsamic Vinaigrette Dressing to taste

1 cup water

4 French rolls (about 6 inches long), split lengthwise

Cook sausage and onions over medium high heat in a nonstick skillet about 10 minutes or until sausage is browned and no longer pink; onions are soft. Blend in paste and water.

Bring to a boil reduce heat to low and cook for 10 minutes. Season to taste. Toss salad greens with dressing. Fill each roll with equal amounts of greens and sausage with sauce.

Serve immediately. Makes 4 servings (1.5 oz TOMATO PASTE or

3Tbsp/approximately ½ dose/serving)

Lentil and Kale Stew

1 Tbsp olive oil

4 celery stalks

1 small onion

3 garlic cloves

6 cups cooked lentils

1 28 oz can of diced Tomatoes

24 oz Tomato Vegetable Juice (V8)

1 (half pound) bunch Kale, tough stems removed, and roughly chopped

1 cup shredded reduced-fat cheddar cheese

Pepper to taste

Heat oil in large saucepan over medium high heat. Add celery, onion, and garlic; cook 5 minutes, stirring occasionally (do not brown). Add lentils, tomatoes, juice and kale, stir to combine and bring to a simmer. Reduce heat to low, cover and cook 15-20 minutes.

Season to taste with pepper. Ladle into bowls, top with cheese and serve. Makes 5 (2C) servings, (4.8 oz TOMATO JUICE, or approximately 1.5 doses/serving. 5.6 oz DICED TOMATOES, or approximately 1.5 doses/serving)

Chunky Pizza Dip

4 Tbsp olive oil

5 garlic cloves, minced

2 (14.5 ounce) cans diced tomatoes

2 (6 oz) cans tomato paste

1 Tbsp dried basil

1/2 tsp dried oregano

Heat oil and garlic in large saucepan until garlic starts to sizzle and turn golden. Add tomatoes, tomato paste, basil and oregano; bring to a simmer. Simmer, uncovered, until sauce is thick enough for dipping, 15-20 minutes. Cool and refrigerate, serve with bell pepper strips, celery, breadsticks, and mozzarella sticks. Makes 10 (1/2 C) servings (each serving provides 3 oz, or 3/4 dose of DICED TOMATOES; 2.5Tbsp, approximately 1/2 dose of TOMATO PASTE)

Savory Italian Sausage Stew

1¼ lbs Mild Italian Sausage, cut into 1-inch chunks

1 package (16 oz) frozen Italian style vegetables

2 medium zucchini, sliced

1 can (26 oz) Spaghetti Sauce

1 can (28 oz) Diced Tomatoes, undrained

1 can (6 oz) Tomato Paste

Brown sausage in large skillet until no longer pink and drain. Combine sausage, vegetables, zucchini, sausage, pasta sauce, tomatoes, and paste in Crock Pot (slow cooker). Cook on LOW setting for 8-10 hours or on HIGH for 4-6 hours. Stir before serving. Makes 8 (1C) servings, (3.25 oz SPAGHETTI SAUCE or approximately 1.5 doses/serving, 3.5 oz DICED TOMATOES or approximately ¾ dose/serving, .75 oz TOMATO PASTE or approximately 1.5 Tbsp/¼ dose/serving)

Veggie Lover's Baked Ziti

Non-stick cooking spray

12 oz dry ziti pasta, uncooked (5 C)

2 Tbsp canola oil

3 medium crookneck squash, cut into ¼ inch thick slices

¼ tsp salt

¼ tsp ground black pepper

1 pkg (10 oz) frozen chopped spinach, thawed, squeezed dry

1 can (26 oz) Spaghetti Sauce

1 container (15 oz Alfredo sauce)

3 cups (12 oz) shredded mozzarella cheese, divided

Preheat oven to 425° F. Spray 13 X9- inch baking dish with cooking spray; set aside.

Cook pasta according to package directions. Heat oil in large skillet over medium high heat while pasta is cooking. Add squash, salt and pepper. Cook 5 minutes or until liquid evaporates, stirring frequently. Stir in spaghetti sauce and Alfredo sauce; cook 1 minute or until heated through, stirring occasionally. Drain pasta. Add to sauce along with half the cheese; mix lightly. Spoon into prepared dish; top with remaining cheese. Bake 10 minutes, or until cheese is melted and mixture is heated through. Makes 8 (1¼ C) servings, (3.6 oz SPAGHETTI SAUCE or approximately 1.5 doses/serving)

VITA

Yumei Cao

EDUCATION

- PhD, Nutritional Sciences, The Pennsylvania State University, December 2009
- Master of Applied Statistics, The Pennsylvania State University, December 2007
- Bachelor of Medicine (Equivalent to M.D.), ChengDe College of Medicine, China, July 2000

PUBLICATIONS

- **Yumei Cao**, David T. Mauger, Christine L. Pelkman, Guixiang Zhao, Stacie Townsend, Penny M. Kris-Etherton. Moderate-Fat (MF) versus Lower-Fat (LF) Diets on Lipid and Lipoproteins in Healthy Subjects and Persons with Diabetes: A Meta-Analysis of Randomized Controlled Clinical Trials. *Journal of Clinical Lipidology*. 2009 Feb; 3(1); 19-32
- Amy E. Griel, **Yumei Cao**, Deborah D. Bagshaw, Amy M. Cifelli, Bruce Holub, Penny M. Kris-Etherton. A macadamia nut-rich diet reduces levels of total and LDL-cholesterol in mildly hypercholesterolemic men and women. *J Nutr*. 2008 Apr;138(4):761-7.
- Heather I. Katcher, **Yumei Cao**, Jun Zhang, and Penny M. Kris-Etherton. Which dietary supplements have proven efficacy for impacting serum lipids and cardiovascular outcomes? Are any vitamins harmful to the cardiovascular system? In *Clinical Challenges in Dyslipidemia Management*. 2008
- **Cao, Y**; Zhang, J; Kris-Etherton, P.M. Effects of nutrient supplements and nutraceuticals on risk for cardiovascular disease. In: *Identification and Management of the Patient at High Risk for Cardiovascular Disease*. Edited by A.Gotto and P.Toth. 2007
- Hilpert, K. F.; Griel, A. E.; Psota, T.L.; Gebauer, S.; **Cao, Y.**; Kris-Etherton, P.M. New insights on the role of lipids and lipoproteins in cardiovascular disease: the modulating effects of nutrition. In: *Lipid Metabolism and Health: CRC Handbook*. Edited by R.J. Moffatt and B. Stamford. CRC Press, Boca Raton, FL. 2006

PRESENTATIONS

- **Yumei Cao**, Deborah Bagshaw, Amy Cifelli, Amy E. Griel, Penny M. Kris-Etherton. Mildly Hypercholesterolemic Men and Women with Higher Baseline C-Reactive Protein (CRP) Levels. (San Diego, April, 2008).
- **Yumei Cao**, Penny M. Kris-Etherton, David T. Mauger, and Christine L. Pelkman. Effects of a Moderate Fat (MF) versus Lower-Fat (LF) Diets on Serum Lipid Profile. *Experimental Biology* (Washington D.C., April, 2007).