HIGH-OLEIC GROUND BEEF, EXERCISE, AND RISK FACTORS FOR CARDIOVASCULAR DISEASE IN MEN AND POSTMENOPAUSAL WOMEN

A Dissertation

by

LINDA ANNE GILMORE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Nutrition

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ABSTRACT

High-Oleic Ground Beef, Exercise, and Risk Factors for Cardiovascular Disease in Men and Postmenopausal Women. (December 2010)

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Chair of Advisory Committee: Dr. Stephen B. Smith

Sixty-six percent of the ground beef consumed in the U.S. contains 16-30% fat by weight, and at the retail level, ground beef fat varies widely with regards to saturated, monounsaturated and *trans*-fatty acid content. Through two independent studies the effect of fatty acid composition of ground beef on selected cardiovascular disease risk indicators was evaluated.

In the first study, 27 free-living normocholesterolemic men completed a three-way crossover dietary intervention. Subjects consumed five, 114-g ground beef patties per week for 5 wk with intervening 4-wk washout periods. Patties contained 24% total fat with monounsaturated fatty acid:saturated fatty acid (MUFA:SFA) of either 0.71 (low-MUFA, pasture-fed), 0.83 (mid-MUFA, short-term corn-fed), or 1.10 (high-MUFA, long-term corn-fed). Blood was collected from each subject before and at the end of each diet period. Overall, the ground beef interventions decreased plasma insulin, HDL_2, and HDL_3 particle diameter and α-linolenic acid (18:2 (n-3)), and increased plasma arachidonic (20:4(n-6)). The greatest increase in HDL cholesterol from baseline (0.07 mmol/L) was after the high-MUFA ground beef intervention. An increase from baseline in LDL particle diameter (0.5 nm) occurred after the mid- and high-MUFA interventions.

We concluded that low-MUFA ground beef from pasture/hay-fed cattle was no more "heart healthy" than high-MUFA ground beef from corn-fed cattle as judged by common clinical criteria.

In the second study, 19 of 29 post menopausal women completed a two-way crossover design. Subjects consumed five, 114-g ground beef patties per week for 6 wk periods separated by a 4 wk washout period. The low-MUFA patties contained 19.4% fat with MUFA:SFA of 0.9. The high-MUFA patties contained 22.5% fat with a MUFA:SFA ratio of 1.3. In addition to patty consumption, the subjects completed a bout of exercise during the last week of each phase. Blood was taken before, each diet phase (24 hr before exercise) and 24 hr post exercise. Total cholesterol was increased by the high-MUFA patties with the most significant increase seen in HDL cholesterol, mainly HDL_{2b} subfraction. Lipid-rich lipoprotein fractions were increased with the low-MUFA diet, but not by the high-MUFA diet. Very long chain fatty acids were depressed by low MUFA patty consumption. When unadjusted for plasma volume shifts (raw), exercise decreased triglycerides in all three phases. Raw VLDL cholesterol was reduced after exercise during the intervention phases. Raw RLP and IDL cholesterol were reduced after exercise during the high-MUFA intervention. HDL_{2b} was reduced after exercise during the high-MUFA phase. LDL mean size increased and LDL mean density decreased after exercise during the low-MUFA intervention. HDL mean density increased after exercise during both ground beef interventions. The data indicate that high-oleic ground beef can reduce some cardiovascular disease risk factors and can be a part of a healthful diet. Exercise can have a beneficial impact on cardiovascular disease risk factors independent and in conjunction with ground beef consumption.

DEDICATION

This work is dedicated to my family. Thank you all for your unconditional love and support at this time and always.

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Thank you also to my colleagues and the department faculty and staff for making my time at Texas A&M University a great experience. I also want to extend my gratitude to the National Cattlemen's Beef Association, which provided funding for the grass- versus corn-fed study, to HeartBrand Beef, which provided Waygu patties and discretionary funding, the J.L. Huffines Institute, which provided the exercise equipment testing facilities, and funding, and to all the men and women who were willing to participate in the study, without whom this study would not have been possible.

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CHAPTER I

INTRODUCTION

Introduction

Cardiovascular disease (CVD), a multifaceted disorder, is the leading cause of death in the United States. Atherosclerosis is the major contributor to CVD and has been plaguing the human race for centuries with early cases being seen in Egyptian mummies (1). Galkina and Ley (2) defined atherosclerosis as a chronic inflammatory process characterized by plaque formation within the vessel wall of arteries and extensive necrosis and fibrosis of surrounding tissues. Being chronic, this disease process progresses over time. The progression of CVD begins with an imbalance of endothelial function, lipid metabolism, and lipid retention. Ross (3) summarized the theory that attributed the initiation of endothelial dysfunction to a response to injury whether it be mechanical injury, toxins, or oxygen radicals. The first visible lesions of atherosclerosis are foam cells, which with lymphocytes form fatty streaks that consist of lipid-rich macrophages and T cells within a thin layer of lipid on the arterial wall. Fatty streaks are formed as a response to the disruption of normal endothelial cell function. The plaque formation and immune response are self-propagating. As the fatty streaks are formed, macrophages are recruited, surround the lipid, and become activated foam cells.

This dissertation follows the style of Journal of Nutrition.

Many inflammatory molecules, including cytokines, are released by the foam cells which in turn attract more lymphocytes, intensifying the immune response. This perpetuation results in a plaque with a fatty core covered by a stabilizing fibrous matrix (4).

Inflammation and CVD

Inflammation plays an integral role in the initiation and progression of CVD (5). Inflammation is also seen with conditions such as obesity, insulin resistance, hypertension, metabolic syndrome, type 2 diabetes, hypertriglyceridemia, low HDL cholesterol and smoking, all of which are correlated with increased CVD risk (6). For this reason, inflammatory proteins such as high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and serum amyloid A (SAA), when elevated chronically, are considered markers of CVD (7, 8). hs-CRP and SAA are acute-phase proteins which become elevated as an innate immune response during periods of infection, inflammation, and tissue damage. After an innate response is initiated, the adaptive immune system is activated with B- and T-cell responses such as production of IL-6 (9). Unlike hs-CRP which travels freely in the plasma, SAA is an apolipoprotein primarily associated with HDL particles. SAA levels also have been correlated to the incidence of CVD (6). Inflammation markers are important predictors of cardiovascular events in women (8).

Homocysteine is not an inflammatory protein, but it is regarded as a biomarker strongly associated with CVD. A single mechanism by which homocysteine contributes to CVD is not established. Homocysteine may cause endothelial damage and dysfunction by affecting nitric oxide production and reactivity (10). With all the biomarkers, a

multimarker approach that may be predictive of cardiovascular events should be utilized (11).

Lipoprotein Metabolism

The particles responsible for the packaging and transport of lipid-soluble constituents in an aqueous environment are lipoproteins. The main goal of lipoprotein metabolism is to package and transport cholesterol and lipids from the liver to extrahepatic tissues and then back to the liver. Lipoproteins are classified into five main groups based on particle density: high-density lipoproteins (HDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL), very low density lipoproteins (VLDL), and chylomicrons. Lipoproteins also can be grouped according to surface charge as "alpha" or "beta." Each lipoprotein class is responsible for specific activities related to lipoprotein metabolism.

High Density Lipoproteins

High density lipoproteins, as a class of lipoproteins, are a heterogeneous mix of HDL subfractions. These subfractions can be characterized and sorted by density, size, shape, apolipoprotein composition and surface charge. When separated by size and density, HDL are classified as, $HDL_{2b,}$ HDL_{2a}, HDL_{3a}, HDL_{3b} and HDL_{3c}. Based on apolipoprotein composition, HDL can be divided into subpopulations A-I HDL which contain apoA-I but no apoA-II, and A-I/A-II HDL, which contain both apoA-I and apoA-II. Agarose gel electrophoresis separates HDL based on surface charge. HDL are then classified as alpha, pre-alpha, pre-beta or gamma based on migration relative to the

plasma proteins. The surface charge then influences particle shape. Alpha HDL contain both apoA-I and apoA-II and are spherical lipoproteins whereas pre-beta HDLs are lipid-poor apoA-1 discoidal particles. HDL particles are not limited to one characteristic, but can bear characteristics of each category (12).

HDL cholesterol is well known as the "good' cholesterol. This is due in part to its role in reverse cholesterol transport, which is the means in which cholesterol is removed from the peripheral tissues, including the macrophages in vessel walls. Reverse cholesterol transport is one of the major defense mechanisms against the development of atherosclerosis. Many enzymes are involved in reverse cholesterol transport, including lecithin cholesterol acyltransferase (LCAT) which transesterifies the sn-2 or sn-1 fatty acid of a phosphatidyl-choline molecule to the 3-β-hydroxyl group of cholesterol, resulting in a cholesterol ester and lysophosphatidyl-choline. LCAT is primarily associated with HDL and plays a major role in HDL metabolism. HDL formation begins with an apoA-1 and nascent HDL aggregate. The LCAT reaction is used to fill the nascent HDL with cholesterol ester, forming HDL_3 and then the larger HDL_2. HDL_2 cholesterol then can be taken up by the liver via scavenger receptor class B member 1 (SR-B1). If LCAT activity is decreased, mature HDL production also is decreased. Like many mechanisms in the body, cholesterol efflux depends in part on a gradient. By esterifying fatty acids to free cholesterol and creating more cholesterol ester, which is then packaged into lipoproteins, LCAT helps maintain the cholesterol gradient favorable for the diffusion of free cholesterol from the peripheral tissues to HDL.

Other enzymes important in reverse cholesterol transport include cholesteryl ester transfer protein (CETP) and hepatic lipase. CETP facilitates the exchange of cholesterol

ester from HDL for triglyceride in apoB_{100}-containg lipoproteins such as LDL. Cholesterol esters removed in this way can be taken up by the liver by the LDL receptor. A decrease in CETP activity may increase HDL cholesterol concentrations and decrease VLDL and LDL cholesterol concentrations. However, the relationship of CETP to CVD (atherogenic or antiatherogenic) is still controversial (13). Hepatic lipase is responsible for hydrolyzing triglycerides and phospholipids in lipoproteins.

Cholesterol can be removed from the macrophages found in vessel walls through many independent pathways involving ATP-binding membrane cassette transport protein AI (ABCA1), SR-B1, caveolins, sterol 27-hydroxylase, and passive diffusion (14). ABCA1-mediated cholesterol efflux correlates positively with pre-β-1 HDL levels, whereas SR-B1 selectively removes cholesterol from HDLα-1 particles and correlates positively with HDLα-1 and α-2 particle levels (15, 16). Whereas ABCA1 mediates cholesterol efflux to preβ-HDL (17), ABCG1 stimulates efflux to larger HDL particles, especially HDL_2 (18).

HDL cholesterol is also beneficial in preventing CVD due to its antioxidant properties through the enzyme paraoxonase. Paraoxonase, though not directly involved in reverse cholesterol transport, is an HDL-bound enzyme that hydrolyzes oxidized lipids in LDL and HDL particles. If not hydrolyzed, these oxidized lipids may impair endothelial function (19). Platelet-activating factor acetyl hydrolase and LCAT are also HDL-associated enzymes that are able to remove oxidized phospholipids (20). Apo A-I acts as an antioxidant as well by removing oxidized lipids from LDL and possibly undergoing oxidative modification in return (21).

Bérard et al. (22) has shown that through regulation of the many enzymes involved in reverse cholesterol transport, fatty acids are able to influence HDL cholesterol concentration and functionality. HDL level alone may not dictate the level of CVD risk (22). Demonstrating HDL subfractions and functionality has become more important than HDL cholesterol. The HDL subpopulation profile of CVD patients with low total HDL cholesterol levels has significantly less large cholesterol-rich HDL and more small lipid depleted particle levels than patients with no cardiovascular event (23).

The functionality of each HDL subclass still is equivocal. Low proportions of large HDL_{2b} particles and high proportions of small HDL particles has been associated with other CVD risk factors such as increased body mass index (BMI), hyperinsulinemia, hypertriglyceridemia, increased visceral adipose tissue, increased homeostatic model assessment of insulin resistance (HOMA-IR) scores, and increased carotid intima-media thickness (IMT) (24-28). Although the correlation between high proportions of small, dense HDL and CVD is strong, some argue that small, dense HDL particles are more functional and protective against CVD. In an *in-vitro* system, at similar physiological concentration and circulating levels, HDL antioxidant activity increased in the order $HDL_{2b}<HDL_{2a}<HDL_{3c}<HDL_{3b}<HDL_{3a}$. When HDL particles were studied in equal number (not representative of physiological conditions) antioxidant activity increased in the order $HDL_{2b}<HDL_{2a}<HDL_{3a}<HDL_{3b}<HDL_{3c,}$ with the antioxidant activity of HDL_{3c} most effective at the late stages of oxidation (29).

Together these findings indicate that small dense HDL particles protect LDL against oxidative stress and HDL antioxidative activity is dependent, in part, on the concentration of the particle (29). Along with high antioxidative activity, small HDL are

better cholesterol acceptors and have a high capacity to inhibit expression of adhesion molecules in patients with low total HDL concentrations (30, 31). However, the antioxidative activity of small, dense HDL, especially HDL_{3b} and HDL_{3c}, is impaired in patients with metabolic syndrome and established coronary artery disease (32). Oxidative damage can impair the ability of apoA1 to remove cholesterol from macrophages (33). During an inflammatory state, HDL has been shown to be proatherogenic, being depleted of specific proteins such as apo-A1, apo-AII, and paraoxonase while becoming enriched in SAA. SAA incorporation into HDL may inhibit its ability to deliver free cholesterol to the liver for clearance. Though the HDL cholesterol will rise, the antioxidant function of HDL will decrease (34).

Very Low Density Lipoproteins

VLDL are thought to be the first lipoprotein synthesized by the liver in the sequence of lipoprotein metabolism. When secreted, VLDL are large triglyceride-rich particles and contain apoB100. Once in circulation, VLDL acquire apoE and apoC (I,II, and II). VLDL are delipidated by lipoprotein lipase, which is found on the surface of extrahepatic tissues. As VLDL triglycerides are hydrolyzed, the cholesterol, phospholipids, and proteins are transferred to higher density lipoproteins such as HDL and LDL, leaving a small, dense remnant lipoprotein (RLP). RLP are further metabolized to smaller more dense lipoproteins called IDL, which are remodeled to form the endproduct LDL. Due to the retention of apoB-100, both IDL and LDL can be cleared from the plasma by the LDL receptor, which has a high affinity for apoB-100 and apoE (35, 36). The clearance of lipoprotein particles by the LDL receptor is down-regulated

when the need for cholesterol by the peripheral cells is met, which suggests accumulation of LDL, IDL, RLP by arterial foam cells may not be mediated by the LDL receptor (37). This idea has been supported with the characterization of the scavenger receptor, which is found on the surface of macrophages and endothelial cells and facilitates the uptake of lipoproteins such as modified LDL, IDL, and RLP particles, but not native LDL particles (38). Hyperinsulinemia and increased free fatty acids secondary to insulin resistance lead to an increase in plasma triglycerides and VLDL concentrations. Although VLDL and triglycerides correlate strongly to LDL density and decreasing LDL size, these characteristics are inversely related to HDL cholesterol, especially the HDL_2 subfraction (39, 40).

Low Density Lipoproteins

The National Cholesterol Education Program defines the desirable LDL cholesterol concentration as <3.62 mmol/L (41). Like HDL, LDL are heterogeneous and can be defined on the basis of particle density, size, charge and chemical composition. LDL particles are commonly separated by density ultracentrifugation, resulting in LDL I through IV, with LDL IV being the most dense (42). LDL concentration, particle size and density are influenced by age, gender, hormone replacement therapy or contraceptive use, abdominal adiposity, diet and exercise. Small, dense LDL particles with increased triglycerides and low HDL cholesterol are often associated with metabolic syndrome and diabetes mellitus secondary to insulin resistance (43). Though there is much colinearity between the CVD risk factors, it is still believed that small, dense LDL particles are a contributing factor to atherosclerosis based on evidence that smaller LDL are taken up

less readily by LDL receptors (44), are more apt to penetrate arterial tissue (45) and are oxidized at a greater rate than larger LDL particles (46). Some argue that it is not particle size or amount of cholesterol carried alone that matters, but the particle number as well (47, 48). LDL cholesterol concentration can be the same among individuals, but particle number can vary greatly. Cromwell and Otvos (47) suggest LDL particle numbers be used instead of LDL cholesterol concentrations to better assess risk for CVD.

As stated previously, macrophages and endothelial cells specialize in the uptake of modified LDL particles, which leads to the progression of atherogenesis. LDL particles may be modified in a number of different ways, including acetylation, malondialdehyde addition, acetoecetylation, carbamylation, LDL-dextran sulfate complex formation, oxidation, glycation and desialylation, but not all modifications lead to increased uptake by the scavenger receptor. Oxidation of LDL particles may be the most studied mechanism in which LDL particles are modified and lead to increased uptake by the scavenger receptor. Oxidation of the LDL can occur through phospholipid oxidation (mainly phosphatidycholine), which then propagates into the core of the LDL particle, with cholesterol and cholesterol ester oxidation and fragmentation of Apo B. It is the fragmentation and change in amino acid sequence of the Apo B that is thought to be responsible in a shift from particle recognition from the LDL receptor to the scavenger receptor (49). Therefore quantifying LDL cholesterol levels alone can be misleading for assessing CVD risk (50).

Lipoprotein(a) (Lp(a))

Lp(a) is similar to LDL particles, but also contains apolipoprotein(a). Because of the apo(a), Lp(a) is not taken up by the LDL receptor and in return Lp(a) levels are dependent on genetically regulated synthesis and not particle clearance. Lp(a) is a strong risk factor of CVD; because Lp(a) particles accumulate in atherosclerotic lesions, Lp(a) levels correlate positively with CVD associated events, Lp(a) is both proatherogenic and prothrombotic and Lp(a) is thought to be the main scavenger of oxidized phospholipids (51-53).

Plasma Fatty Acids and CVD

Plasma fatty acids can be obtained directly from the diet (exogenously) or from fatty acids synthesized or modified by metabolic pathways in the body (endogenously). Fatty acids can impact metabolism by up- or down- regulating enzymes. Though it is difficult with nonessential fatty acids to distinguish endogenous from exogenous fatty acids, their origin may dictate their impact on metabolism. For example, dietary oleic acid has been shown to have positive health benefits (54), but for oleic acid synthesized by hepatic stearoyl-CoA desaturase (SCD1), negative health effects are seen such as increased plasma triglycerides, decreased HDL, increased VLDL, and increased LDL (55). SCD1 is a delta-9-desaturase responsible for placing a double bond at carbon 9 of a fatty acid chain. The most common substrates for SCD1 are stearic acid and palmitic acid. Desaturation by SCD1 results in oleic (18:1(n-9)) and palmitoleic acid (16:1(n-7)), respectively (56). SCD1-deficient mice are protected from insulin resistance, diet-induced obesity, increase triglycerides, and increased VLDL (57). Many of the effects of

SCD1 deficiency are dependent on diet composition and other genetic factors (58). Though it seems SCD1 deficiency is antiatherogenic, SCD1 deficiency also promotes inflammation and atherosclerosis in mice (59). When increased SCD1 activity is seen in macrophages, palmitoleic acid is increased and relief from lipid-induced endoplasmic reticulum (ER) stress is provided. Erbay et al. (60) demonstrated endogenous fatty acid synthesis and desaturation of fatty acids can be highly beneficial for defending ER function when macrophages are exposed to toxic lipids, which may lead to an antiatherogenic effect of SCD1 activity.

Menopausal Status and CVD

Menopause brings on many changes, one of which is increased risk for CVD. This increased risk is due in part to reduced circulating estrogen and an overall reduction in physical activity. Although women have higher HDL and total cholesterol and lower LDL cholesterol and triglycerides than men (61), HDL cholesterol levels decrease in women after menopause. The decrease in HDL is accompanied by an increase in LDL, total cholesterol, VLDL, triglycerides, glucose and BMI after menopause, contributing to an increased risk for CVD (62-64). HDL changes in postmenopausal women are accompanied by more dense, smaller HDL particles (64). Though postmenopausal women have different lipid profiles than premenopausal women, the changes in their profile as a response to exercise is the same (63). When estrogen is replaced in postmenopausal women in a combination with exercise, HDL lipid peroxidation is reduced (65).

Dietary Influence on CVD

It is well known that CVD can be influenced favorably by diet and exercise, but optimal dietary content continues to be debated. The National Cholesterol Education Program/American Heart Association Step I or Step II previously recommended a diet low in total fat, especially saturated fat (8-10% and < 7% of energy, respectively), and cholesterol was kept below 300 or 200 mg/d, respectively. At that time, carbohydrate was used to replace saturated fat. Though low in total fat, the high-carbohydrate diet increased plasma triglyceride concentrations and decreased HDL cholesterol concentrations, although beneficial effects were seen for total and LDL cholesterol (41, 66). Though a reduction was seen in total LDL concentration, which is thought to be beneficial, Campos et al. (67) reported an associated reduction of LDL particle size with reduced animal fat intake and increased consumption of carbohydrates. LDL:HDL ratios are reduced when carbohydrate is replaced with fat, even with saturated fat (68). When isocaloric substitutions of carbohydrates for monounsaturated fat or polyunsaturated fat were made in the diets of women, their risk of CVD increased 20 and 60%, respectively (69). Changing the quality of the fat in the diet has been shown to be more beneficial than reducing the total amount of fat in the diet (68, 69). High-carbohydrate diets also increase Lp(a) concentrations (70). In the year 2000, the Nutrition Committee of the American Heart Association moved away from its former insistence on low-fat diets and concluded that diets that provided up to 40% of dietary energy in the form of unsaturated fat were as heart-healthy as low-fat diets. An outcome of this official opinion has been the reevaluation of the nutritional properties of a number of higher fat foods such as dairy,

nuts, and dietary oils such as olive oil rich in the monounsaturated fatty acid, oleic acid (71).

Fatty acids are not only sources of energy, but are powerful modulators of cell function and gene transcription. By this, fatty acids modulate metabolic and inflammatory response by the body (72). Differences in fatty acid chain length and saturation/unsaturation account for the differences in biological effects and physical properties. Kris-Etherton et al. (73) performed a meta-analysis which showed the varying effect of individual fatty acids on total cholesterol, LDL, and HDL cholesterol. It was clear that individual fatty acids have different effects on serum cholesterol fraction even if they belong to the same class of lipid, saturated, unsaturated, or *trans*-fats (73). Though types of fats and higher fat foods have been reevaluated and are now considered "heart healthy," the American Heart Association discourages the consumption of red meat due to its saturated fat content.

Due to the current recommendations, beef and beef fat and their effects on CVD have been examined closely. Though beef fat and coconut oil are both considered to be high in saturated fat, they do not have the same effects on cholesterol levels. When compared to coconut oil, which consist of more medium chain saturated fatty acids (lauric (12:0) and myristic acid (14:0)), beef fat reduced total, LDL, and HDL cholesterol (74). When red meat was tested against fish and poultry, there were no significant difference in plasma cholesterol concentrations between the diets consisting of red meat, fish or poultry (75).

Numerous clinical and epidemiological studies have shown saturated fats have adverse effects on cholesterol metabolism and thus increase the risk of CVD (76). Using

regression analysis, Keys et al. (77) and Hegsted et al. (78) evaluated the effect of individual fatty acid classes on cholesterol concentration in humans. Their equations estimated that saturated fat is twice as potent in raising total cholesterol as polyunsaturated fats are in reducing it. Mazaffarian et al. (79) found while eating a low fat diet (25% energy), a greater saturated fat intake (10-16% energy) was associated with less progression of coronary atherosclerosis in postmenopausal women than a lower intake of saturated fat (3-7% energy). There could be many reasons why this study does not support the general trend of saturated fat effects, including participant characteristics, other dietary components including carbohydrates, and individual saturated fatty acids consumed.

Saturated Fats

Stearic acid (18:0) is the second most abundant saturated fatty acid in beef and accounts for 13-20% of total fatty acids (80). Although saturated fats have been positively correlated with CVD, stearic acid has been shown to have neutral effect on lipids and lipoproteins. This has been shown by predictive equations and through replacing carbohydrate with stearic acid in the diet (81, 82). The regression analysis of Muller et al. (81) demonstrated that stearic acid had no effect on total cholesterol, LDL cholesterol or HDL cholesterol concentrations compared to myristic, lauric or palmitic acid (16:0). When stearic acid was substituted for lauric, myristic and palmitic acid, it lowered total, LDL and HDL cholesterol (68). Watts et al. (83) questioned the influence of stearic acid on CVD through a prothrombotic effect independent of plasma cholesterol concentration. A prothrombic effect was seen in hypercholesterolemic men with a

previous occurrence of angina pectoris or myocardial infarction when the usual diet was consumed, which was higher in stearic acid than the test diet. Although a prothrombic effect was observed, the usual diet was higher in other fatty acids, cholesterol, energy, total fat, saturated fat and monounsaturated fat and *trans*-fat than the test diet. The data provided by the angiogram could not specifically be attributed to stearic acid. Stearic acid also has less of an influence on postprandial lipemia and thus the activation of thrombotic factors than oleic, elaidic (18:1*trans*-9), and palmitic acids (84).

Whereas stearic acid is neutral in its effects on cholesterol metabolism, palmitic acid has a negative effect. Palmitic acid is the main saturated fatty acid found in beef (24-25% of all fatty acids) (80). Evidence from metabolic ward studies and epidemiological studies have shown that palmitic acid, when compared to unsaturated fatty acids and carbohydrates, raises total cholesterol, with the greatest increase in LDL cholesterol, and a slight increase in HDL cholesterol and VLDL cholesterol (85). The slight increase in HDL cholesterol associated with the intake of palmitic and myristic fatty acids may be due to an impairment in reverse cholesterol transport and the residence time of HDL particles. HDL may be less capable to return cholesterol to the liver due to a decrease in SR-B1 and 7α-hydroxylase activity (86). Nicholls et al. (87) examined the effect that saturated fats have on the anti-inflammatory potential of HDL. After consumption of a meal high in saturated fat, HDL was less functional than HDL isolated from fasting plasma. The saturated fat diet reduced the ability of HDL to inhibit the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), possibly by reducing HDL anti-inflammatory properties (88).

Monunsaturated Fats

When monounsaturated fats were used to replace carbohydrate in an average American diet, total cholesterol was reduced by approximately 10% and LDL cholesterol by 14% (68). The high-monounsaturated fatty acid diet did not lower HDL cholesterol whereas a high-carbohydrate diet (59% of energy) lowered HDL by 4% compared to the average American diet (70). Mente et al. (89) used the Bradford Hill Guidelines to evaluate the scientific evidence of a causal relationship between each dietary exposure and CVD. Through the analysis of cohort data of 101,521 patients, Mente et al. (89) found higher consumption of monounsaturated fatty acids was associated with significantly lower risk of CVD. Outside of lipid profiles, monounsaturated fatty acids are antiatherogenic by decreasing the susceptibility of LDL to oxidation, improving endothelial function and reducing inflammation marker levels and platelet aggregation. Monounsaturated fatty acids are antihypertensive, and could improve insulin sensitivity (90). These findings may be due to, or are enhanced by, minor compounds found in olive oil, the common source of monounsaturated fatty acids in study protocols (91).

Oleic acid is the main monounsaturated fatty acid found in beef (80), but it typically is associated with olive oil and the Mediterranean diet. There are polyphenolic compounds in olive oil that also have an influence on lipid metabolism, in that they reduce HMG-CoA reductase activity (92). Though Pérez-Jiménez et al. (91) points out the beneficial effects of the polyphenols found in olive oil, he does not disregard the positive effects of the fatty acids in olive oil, mainly oleic acid, on cholesterol metabolism. The effect of olive oil on cholesterol metabolism was confirmed by Ruíz-Gutiérrez et al. (54). They observed an increase in HDL cholesterol in hypertensive

women who consumed a diet enriched with high-oleic sunflower oil or olive oil. Additionally, a significant decrease in plasma HDL_2 and an increase in plasma HDL_3 cholesterol concentrations were seen with the oleic and interventions. In addition to an increase in HDL cholesterol, oleic acid reduces the post-secretory oxidation of LDL particles, making them less atherogenic (54).

Polyunsaturated Fats

The American Heart Association has moved away from low fat/high carbohydrate diets as discussed previously, but what is to replace the carbohydrate? Jakobsen et al. (93) suggested substituting polyunsaturated fatty acids for carbohydrate. Less fatal coronary heart disease events were seen when polyunsaturated fats, especially linoleic acid (18:2(n-6)), were substituted for carbohydrate as compared to saturated fats and even monounsaturated fats. A review by Kris-Etherton (94), indicated that intervention studies with n-6 polyunsaturated fatty acids obtained a 13-16% reduction in total cholesterol. Though a reduction in total cholesterol can be considered beneficial when it comes to cardiovascular health, n-6 polyunsaturated fatty acids are known to be proinflammatory, increasing the production of cytokines, hs-CRP and SAA levels, and stimulating endothelial activation (90).

When n-3 polyunsaturated fatty acids are increased in beef, the increase is seen in α-linolenic acid (18:3(n-3), ALA) not docosahexaenoic acid (22:6(n-3), DHA), or eicosapentaenoic acid (20:5(n-3), EPA). Intervention studies have shown that increased intake of EPA and DHA lowers the risk of CVD (95, 96). For example, supplementation of 3 g DHA/d for 45 and 90 d significantly decreased remnant lipoprotein concentrations

through a reduction in postprandial triglycerides in hypertriglyceridemic men (97). Though humans can convert ALA to EPA and some DHA, the conversion is too limited to replace the consumption of DHA and EPA directly from cold water fish. When ALA is compared to EPA and DHA in regards to CVD risk factors, Goyens (98) saw that EPA and DHA increased LDL cholesterol levels and tissue factor pathway inhibitor activity (an inhibitor of blood coagulation).

Trans-Fats

Over the last two decades, *trans*-fats and their relation to CVD increasingly have been an area of interest. *trans*-Fats are not metabolically equivalent to the *cis*-isomers and have adverse effects on serum lipid profiles (99). *trans*-Fats raise LDL cholesterol, lower HDL concentrations, increase triglycerides and increase Lp(a) when substituted for saturated fat (100). In addition to the reduction of HDL-cholesterol, *trans*-fats have been shown to reduce serum paraoxonase activity (19).

Little has been studied concerning individual *trans*-fatty acids, but several studies have been done dividing *trans*-fats into those from industrial sources, or partially hydrogenated oils, and those from ruminant animals as a result of biohydrogenation of fatty acids by rumen bacteria. Epidemiologic studies have shown a positive association between CVD risk and the intake of *trans*-fat from industrial sources, but not between CVD risk and ruminal *trans*-fats (101). These epidemiological data have lead to multiple intervention studies which have supported past data. Motard-Bélanger et al. (102) showed high intakes (10.2 g/2,500 kcal) of both industrial and ruminal *trans*-fats, significantly increased LDL cholesterol concentrations and reduced HDL cholesterol. Whereas high

intake of ruminal *trans*-fat leads to deleterious changes in cholesterol homeostasis, moderate intake (4.2 g/2,500 kcal) had no effects. Compared to industrial *trans*-fats, ruminal *trans*-fats significantly increased large HDL and LDL concentrations (103).

Mozaffarian (104) reported that consuming *trans*-fats not only influences lipoprotein concentrations, but also is associated with higher circulating markers of systemic inflammation, including TNF-α, IL-6 and hs-CRP. When mononuclear cells from hypercholesterolemic subjects were cultured, increased production of IL-6 and TNF-α was seen with a soybean margarine diet (6.7% energy from *trans*-fats), compared with a soybean oil diet (0.6% energy from *trans*-fats) (105). When using cow feeding strategies to increase the vaccenic acid (18:1(trans-11)) concentration in milk fat, Tholstrupt (106) did not see a significant difference between the butter made with high-vaccenic milk and a control diet in their effects on hs-CRP concentrations, urinary excretion of $PGF_{2\alpha}$, or other hemostatic risk and oxidative stress markers. There also was a reduction in total and HDL cholesterol levels. This may have been due in part to the method in which the high vaccenic butter was made. Through vaccenic acid was increased by the feeding method, so was conjugated linoleic acid, oleic acid and stearic acid, with a decrease in palmitic and myristic acid. This profile may reflect the actual consumption of products containing ruminant produced *trans*-fatty acids by most people.

Conjugated linoleic acid (CLA) has many isomers. The main isomers, *trans*-10, *cis*-12 CLA and *cis*-9,*trans*-11 CLA, are commonly found in ruminant animal fat, dairy products, and partially hydrogenated vegetable oils (107). When given encapsulated individual CLA isomers, an increase in plasma triglycerides, total cholesterol, and LDL cholesterol was seen with *trans*-10, *cis*-12 CLA, but not with *cis*-9,*trans*-11 CLA

supplementation (108). The *trans*-10, *cis*-12 and CLA *cis*-9,*trans*-11 CLA isomers were studied *in vitro* at high concentrations. Both CLA treatments resulted in lower concentrations of esterified cholesterol and stimulated HDL-dependent cholesterol efflux in mouse macrophage-derived foam cells (109).

Dietary Cholesterol

As reviewed by Grundy and Denke (110), the relationship between dietary cholesterol and serum circulating cholesterol levels was first observed when cholesterol was fed to rabbits and other species. From these studies it was assumed dietary cholesterol increased serum cholesterol and thus led to an increased risk for CVD. However, humans respond less to dietary cholesterol than most species (110, 111). The direct influence of dietary cholesterol and serum cholesterol levels is interrupted by the intense regulation of cholesterol biosynthesis by the liver. Cholesterol synthesis is regulated, in part, by a negative feedback mechanism. When dietary cholesterol is high, biosynthesis is down regulated (112). Dietary cholesterol reduces endogonous cholesterol synthesis by suppressing 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) and up-regulates acyl CoA:cholesterol acyltransferase (ACAT) activity. ACAT is responsible for esterifying a free fatty acid, likely unsaturated, to free cholesterol making cholesterol ester. When ACAT activity is increased, the free cholesterol pool is decreased, and LDL receptor activity, and thus LDL catabolism is increased. Oleic acid, the primary fatty acid substrate for ACAT, (along with arachidonic acid and eicosapentanoic acid) stimulates ACAT activity (113, 114).

Exercise and Lipid and Lipoprotein Metabolism

Inactivity is a major risk factor for CVD due, in part, to its effects on individual lipid and lipoprotein profiles. The American College of Sports Medicine and the American Heart Association now recommend that older adults participate in moderate aerobic exercise for an accumulation of 30 min/d, with each bout lasting a minimum of 10 min at least 5 d/wk, or vigorous activity for 20 min/d for at least 3 d/wk. Moderate muscle strengthening activities should be done for 30 min at least 2 d/wk or vigorous muscle strengthening activity for 20 min/d at least 2 d/wk (115). High levels of exercise are associated with reduced prevalence of hypertension, elevated cholesterol, and diabetes mellitus. Even with low- to moderate- intensity activities (walking), women who expend 600-1499 kcal/wk are less likely to develop CVD with no additional reduction of risk seen at higher energy expenditures (116).

Endurance exercise may be beneficial to blood lipid profiles due to its influence on key lipoprotein metabolism enzymes. Such effects include increased lipoprotein lipase activity, decreased hepatic lipase activity (117), increased LCAT activity and reductions in CETP activity. By influencing these enzyme activities, changes in blood lipids are seen in postmenopausal women and in men including decreases in total cholesterol, LDL cholesterol and triglycerides with elevations in HDL cholesterol including HDL_3 and HDL_2 (62, 117). Through meta-analysis, Kelley et al. (118) reported that chronic exercise significantly lowered total cholesterol, LDL and triglyceride concentrations while increasing HDL cholesterol concentrations in women (118) and men (119). Greater reductions in total cholesterol were associated with reductions in body fat percentage (118, 119). HDL cholesterol was increased in White and African American males and

females, while triglycerides were decreased only in White male and females after 3 y of increased regular exercise (61).

An acute bout of exercise can be used to isolate the acute effects of exercise on blood lipids. These effects may mirror that of endurance exercise training such as acute reductions in triglycerides, increases in HDL cholesterol, and improved glucose control (120, 121). After an acute bout of exercise in which moderately trained females expended 800 kcal at 75% VO_{2peak}, an increase in total HDL cholesterol was seen at 48 h after exercise and an increase in HDL_3 was seen immediately after exercise, but returned to baseline 24 and 48 h after the exercise bout (122). As long as caloric expenditure is held constant, the changes in serum lipids after an acute bout of exercise are not influenced by intensity (121).

Present Study

The objectives of this study were:

1. To establish the relationship between the monounsaturated:saturated fatty acid (MUFA:SFA) ratio in beef and plasma lipoprotein cholesterol and inflammatory markers of CVD in normocholesterolemic men and postmenopausal women.
2. To establish the relationship between the MUFA:SFA ratio of ground beef and the metabolic and inflammatory responses to a single bout of aerobic exercise in postmenopausal women.

Our primary hypothesis was differing fatty acid profiles in ground beef would differentially affect CVD risk with the high-MUFA ground beef having a more beneficial impact on CVD risk than low-MUFA ground beef in normocholesterolemic men and postmenopausal women. Also, exercise would have a beneficial additive effect on CVD risk factors when performed during ground beef patty consumption.

CHAPTER II

GROUND BEEF FORMULATED FROM CORN-FED CATTLE INCREASES HDL CHOLESTEROL AND LDL DIAMETER AND DECREASES PLASMA INSULIN IN NORMOCHOLESTEROLEMIC MEN

Introduction

Researchers previously concluded that dietary saturated fatty acids (SFA) elevate serum cholesterol concentrations, whereas polyunsaturated fatty acids (PUFA) reduce serum cholesterol concentrations, and monounsaturated fatty acids (MUFA) have little or no effect (77, 78). While a recent WHO/FAO expert consultation generally confirmed these concepts (123), specific reports noted the importance of dietary context for the effects of SFA (124) as reductions in its intake is associated with reductions in HDL cholesterol (HDL-C) as well as LDL cholesterol (LDL-C) The major MUFA in beef, oleic acid, has been studied in more detail and found to lower LDL-C without affecting the HDL-C (70, 89, 125, 126). Monounsaturated fatty acids typically constitute 35 to 45% of the total fatty acids in beef produced in the U.S. (80, 127). Some studies concluded that beef consumption resulted in decreased serum cholesterol in human subjects (128), while others conclude that it had no effect (75, 129, 130). The wide variation of oleic acid and *trans*-fatty acid content (TFA) in market beef (131, 132) may have been responsible for inconsistent study results.

Carcasses from pasture-fed cattle are lower in total fat and typically attain USDA quality grade Select, whereas carcasses from corn-fed cattle are higher in total fat, typically grading to USDA quality grade Choice (133, 134). Select beef contains 3 – 4%

total extractable lipid while Choice beef contains 5 – 7% total extractable lipid (135). Additionally, because grasses contain a greater proportion of α-linolenic acid (18:3(n-3), ALA) than corn or other grains, beef from grass-fed beef cattle contains more ALA than beef from grain-fed cattle (131, 133), albeit the total amount remains very low. For example, a 114 g (4 oz) strip steak from corn-fed beef contains approximately 6 mg of ALA, whereas the same size strip steak from grass-fed cattle would contain about 25 mg of ALA (values calculated from 17). The current DRI for macronutrients recommends daily intakes of 1,100 mg and 1,600 mg ALA for adult women and men, respectively. Thus, a 114-g steak from grass fed cattle could provide 1.6 – 2.3% of the daily recommendation of ALA and about 4 g of total fat.

At least 40% of average 67 pounds of per capita beef consumption in the U.S. is consumed as ground beef, and low-income households consume more ground beef per capita than high-income households (136). Unlike intact cuts of beef, ground beef is fabricated to a specific fat level, which can be as low as 3% total fat to an upper legal limit of 30% total fat (137). Statistics from July 2009 to July 2010 show that 31.4% of ground beef consumed in the US contains 22-30% fat, while the next 34.8%, contains 16-22% fat. In contrast, ground beef containing 10% or less fat comprises about 18.8% ground beef consumption (138). Beef fat can be a significant component of the diet, and nutritional optimization of beef fat composition could have important health impacts (89).

Previous work from our laboratories has shown that beef fat composition varies by depot, animal age and method of feeding, with corn feeding being an effective means to reduce SFA and TFA (131, 132, 139). However, these improvements came at the expense of ALA content. In an earlier study, hypercholesterolemic individuals showed

evidence of reduced CVD risk following consumption of 35% fat hamburger patties from corn-fed cattle. It became of interest to determine whether the intake of ground beef of a common total fat content (24% total fat) from pasture- and corn-fed cattle would differentially affect risk factors for CVD in normocholesterolemic individuals.

Methods

Approval. The study was conducted according to the Declaration of Helsinki guidelines. All procedures involving human subjects were approved by the Texas A&M University Institutional Review Board for use of human subjects in research (Protocol number 2005-0435). Written consent was obtained from all subjects.

Participants and study design. Healthy, non-smoking males between the ages of 23 and 60 y were screened for eligibility. The 30 subjects selected were not on restrictive diets or medications. Family histories were obtained as part of a complete physical examination that included a treadmill exercise test with an electrocardiogram. Baseline blood chemistries were analyzed by a local laboratory (St. Joseph's Hospital, Bryan, TX) and all blood chemistries were within normal ranges as defined by the testing laboratory. All participants were free-living and were instructed to maintain routine activities and body weight (± 2.2 kg of entry weight). Exercise and physical activities were not restricted, but participants were requested not to change their habitual level of physical activity. Twenty-seven of the initial 30 participants completed the study. Of the three non-completers, one had a reoccurrence of a previous illness, another relocated, and data from the third was omitted following three baseline samples that showed high TAG concentrations (> 5 mmol/L).

A three-period randomized, cross-over design was used. Each participant completed three, 5-wk diet interventions in a randomly assigned order with a 4-wk washout period between the test diet interventions. The men consumed 5 ground beef patties per week for 5 wk during each dietary intervention. The three interventions were low-MUFA ground beef, mid-MUFA ground beef and high-MUFA ground beef. The three diet interventions and two washout periods required a total of 23 wk to complete.

To facilitate product distribution and blood sampling, participants were assigned to one of two groups comprised of 3 blocks of 5 men each, balanced with regard to age, body weight and total cholesterol concentration at the initial screening. Group 1 began the study 2 wk before group 2. Both groups rotated through all three test diets, but the pattern in which they crossed over differed between groups. Therefore, crossovers included all possible rotation sequences. Body weights were recorded weekly during the test phases, and body composition was measured by dual energy X-ray absorptiometry (DEXA) at the initial screening and at the completion of the study.

Diet records. Diet records were obtained to establish baseline observations and encourage compliance to guidelines to consume one patty daily, five times each week for 5 wk. Between each diet phase and once during each phase, participants completed a 3-d record. Records included one weekend day. Daily intake of major nutrients and dietary exchanges was analyzed by a registered dietitian using Nutrient Calc version 1.1 (University of Minnesota, St. Paul, MN).

Source of raw materials. Cattle were fed pasture or grain-based diets specifically with aim to produce ground beef with MUFA:SFA of 0.70, 0.85 and 1.20, representing grass-fed, typical chubpack (regular), and intensively corn fed (premium)

ground beef (15, 16). Eighteen Angus steers were purchased as calves at 8 mo of age, transported to the Texas AgriLife Research Center in McGregor, TX, and fed Coastal burmudagrass hay (9.5% crude protein) free choice for 8 d. Twelve steers were fed a high-energy, corn-based diet containing (as-fed basis) 48% ground corn, 20% ground sorghum, 15% cottonseed hulls, 6.5% molasses, 6% cottonseed meal, 3% limestone, trace mineral salt (NaCl, 98%, Zn, 0.35%, Mn, 0.28%, Fe, 0.175%, Cu, 0.35%, and I, 0.007%), vitamin premix (vitamin A, 2.2 x 10^6 IU/kg; vitamin D, 1.1 x 10^6 IU/kg; and vitamin E, 2.2 x 10^6 IU/kg), and a monensin premix (Elanco Animal Health, Greenfield, IN) to provide 25 mg of monensin/kg of feed (18). The remaining six steers grazed on Coastal burmudagrass pasture and were offered free choice coastal Burmudagrass hay supplemented with non-protein nitrogen.

Six of the 12 corn-fed steers were fed for 117 d (to 12 mo of age), and the remaining 6 corn-fed steers and the 6 pasture/hay-fed steers were fed for 228 d (to 16 mo of age). The 12-mo-old, corn-fed steers were processed at a commercial facility in McGregor, TX, whereas the 16-mo-old corn-fed and pasture/hay-fed steers were transported to the Texas A&M University Rosenthal Meat Science and Technology Research Center, College Station, for processing. At each facility, subcutaneous adipose tissue was sampled over the 12th thoracic rib along the dorsal midline according to commercial grading protocols and adipose tissue collected for immediate fatty acid composition determination to facilitate ground beef patty formulation.

Carcasses from the pasture/hay-fed steers graded USDA low Select and carcasses from the steers fed corn for 228 d graded USDA low Choice (Table 1). Carcass data were not available for steers fed corn for 117 d because they were processed at a commercial

plant. However, based on previous studies (139), the cattle should have produced high Select/low Choice, yield grade 3 carcasses, values intermediate between grass-fed and long-fed cattle.

TABLE 1 Production and carcass characteristics of Angus steers used to produce the test ground beef

	Targeted ground beef MUFA:SFA ratio		
Item	0.80	1.10	1.40
Age at slaughter, mo	20	15	20
Average daily gain, kg/d	0.88	1.53	1.32
Time on pasture or corn, mo	8 mo, pasture	4 mo, corn	8 mo, corn
Carcass weight, kg	284.2	NA[1]	363.9
Adjusted fat thickness, cm	1.09	NA	2.45
Subcutaneous adipose tissue MUFA:SFA	0.8	1.07	1.43
Ribeye area, cm^2	67.2	NA	73.7
Kidney, pelvic & heart fat %	2	NA	2.67
Marbling score[3]	340	NA	500
Quality grade	Select[16]	NA	Choice[08]
Yield grade	3.03	NA	4.82

[1]NA = not available.
[2]MUFA:SFA ratio = (14:1n-5 +16:1n-7 + 18:1n-9 +18:1n-7)/(14:0 + 16:0 + 18:0).
[3]300 = slight marbling, 400 = small marbling, 500 = modest marbling.

Subcutaneous adipose tissue overlying the 12th thoracic rib from the pasture/hay-fed steers had a MUFA:SFA of 0.80 (low-MUFA). In the short-fed, corn-fed steers, the subcutaneous adipose tissue had a MUFA:SFA of 1.07 (mid-MUFA), whereas subcutaneous adipose tissue from corn-fed, long-fed steers had a MUFA:SFA of 1.43 (high-MUFA).

Preparation of ground beef. Carcasses were fabricated at the Texas A&M Rosenthal Meat Science & Technology Center, Texas A&M University. Fat and lean trims from the plate and flank were combined at the appropriate ratios to yield 24% total fat. Notably, the plate and flank regions from the pasture/hay-fed cattle did not contain sufficient fat trim to achieve 30% total fat.

Ground beef patties (114 g; 4 oz) were formed with an automated patty maker, quick-frozen to -20°C, and individually vacuum-packaged. The final MUFA:SFA of the low-MUFA, mid-MUFA and high-MUFA ground beef patties were 0.71, 0.83 and 1.10, respectively. These values were lower than predicted by the subcutaneous fatty acid composition because the plate and flank used to formulate the ground beef are more saturated than the subcutaneous fat overlying the loin (132). Each low-MUFA ground beef patty contained 2.5 g more SFA and 3.4 g less MUFA than the high-MUFA patties (Table 2). Also, each low-MUFA patty contained 37 mg more *trans*-vaccenic acid and 60 mg more ALA than the high-MUFA ground beef patties.

TABLE 2 Fatty acid composition of low MUFA, mid MUFA, and high MUFA ground beef[1]

Fatty acid	Low MUFA	Mid MUFA	High MUFA
	g/114-g ground beef patty		
14:0 (myristic)	0.99	0.97	0.66
14:1 (myristoleic)	0.28	0.26	0.22
16:0 (palmitic)	8.78	8.43	7.89
16:1(n-7) (palmitoleic)	0.85	0.83	0.97
18:0 (stearic)	5.57	4.98	4.31
18:1(*trans*-11) (*trans*-vaccenic)	1.06	0.97	0.69
18:1(n-9) (oleic)	10.07	11.06	13.25
18:1(n-7) (*cis*-vaccenic)	0.29	0.36	0.46
18:2(n-6) (linoleic)	0.55	0.49	0.56
18:3(n-3) (α-linolenic)	0.09	0.04	0.03
18:2(*cis*-9,*trans*-11)	0.18	0.13	0.14
18:2(*trans*-20,*cis*-12)	0.04	0.07	0.09
Total *trans*	1.28	1.17	0.92
MUFA:SFA ratio	0.71	0.83	1.10

[1]Data are means for three batches of ground beef per treatment group.

On the day of the initial blood sampling, each participant received an unlabeled box containing all of the patties for the first 5-wk trial. A new box of patties was provided at the beginning of the subsequent, two intervention periods. No restrictions were placed on how the beef was to be prepared. Participants were instructed to consume all to the beef from a single patty at one meal.

Collection and analysis of blood samples. Prior to the initiation of the dietary treatments and at the end of each diet phase, after 5 min of seated rest, blood was collected from the subjects into EDTA vacutainers from a vein in the antecubital fossa using standard phlebotomy procedures. Plasma was harvested from the blood collected with EDTA, and lipoproteins preserved (140). Lipoprotein separation involved density gradient ultracentrifugation employing human density intervals (131). The diameters of LDL and HDL particles isolated from plasma were determined by non-denaturing

gradient gel electrophoresis (141). Particle diameters were determined by comparison with migration distances of standard proteins of known hydrated diameter (High Molecular weight standards, Amersham Pharmacia, Piscataway, NJ) (142).

Plasma total cholesterol, triacylglycerol, insulin and glucose were determined by separate enzymatic assays (Sigma Chemical Co., St. Louis, MO). Serum hs-CRP was determined using an ELISA test kit (Alpha Diagnostic International, Inc., San Antonio, TX).

Fatty acid composition of plasma and test ground beef. Plasma fatty acids and fatty acid composition of every batch of ground beef (n = 3 for each ground beef type) were measured. Total lipid was extracted and methylated (143, 144). Fatty acid methyl esters (FAME) were analyzed with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA) equipped with a fused silica capillary column CP-Sil88 [100 m x 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands) (16). Helium was used as the carrier gas (1.2 mL/min). After 32 min at 180^{o}C, oven temperature was increased at 20^{o}C/min to 225^{o}C and held for 13.75 min. Injector and detector temperatures were at 270 and 300^{o}C, respectively. Individual FAME were identified using genuine standards (Nu-Chek Prep, Inc., Elysian, MN) and expressed as a g/100 g total FAME analyzed or as g/114 g ground beef patty.

Statistical analysis. Nutrient and dietary exchange data were analyzed by analysis of variance (SuperAnova, Abacus Concepts, Inc., Berkeley, CA). Intakes during each test phase were compared to the pooled values for two habitual periods. Final (after test, pooled across treatment; n = 81) plasma glucose, lipids, insulin, hs-CRP, lipoprotein particle sizes and fatty acids were compared to pooled initial values (n = 81) by analysis

of variance. Also, changes from corresponding initial values for each variable were calculated and compared statistically to no change by analysis of variance. Because each participant randomly rotated through all three diets, values reported for changes from baseline are means ± standard error of the mean (SE) for $n = 27$ observations.

Results

Nutrient and dietary exchange intake. Dietary records indicated no significant differences in energy intake or intakes of protein, carbohydrate, cholesterol, linoleic acid (18:2(*n*-6)) or ALA (Table 3).

Saturated fat intake was greatest when participants consumed low-MUFA ground beef, while MUFA and oleic acid intake was greatest when participants consumed the high-MUFA ground beef. Consumption of the mid-MUFA ground beef caused no significant change in the intakes of any major fatty acid. Differences in total TFA intake could not be evaluated due to inadequate food database documentation. However *trans*-fatty acids from beef were greatest while consuming the low-MUFA beef, 1.28 g/d, and lowest while consuming the high-MUFA ground beef, 0.92 g/d. When diets were characterized as dietary exchanges, only the intake of high-fat meat changed between the habitual and test diets (from 0.5 to approximately 1.5 exchanges/d; Table 4). The increase in intake of high-fat meat was offset sufficiently by reduced intake of medium-fat meat so that there was not a significant difference in total meat intake.

TABLE 3 Daily intake of major nutrients for habitual diets and for test diets of men rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), medium in monounsaturated fatty acids (Mid MUFA), or high in monounsaturated fatty acids (High MUFA)[1]

	Treatment group				
Item	Habitual	Low MUFA	Mid MUFA	High MUFA	*P*-values
Total energy, *kjoule/d*	8,632 ± 501	9,337 ± 909	8,968 ± 1,049	9,065 ± 535	0.20
Protein, *g/d*	97 ± 5	97 ± 10	92 ± 10	91 ± 8	0.32
Carbohydrate, *g/d*	211 ± 13	224 ± 26	225 ± 27	222 ± 15	0.23
Cholesterol, *mg/d*	353 ± 45	325 ± 44	347 ± 59	325 ± 44	0.37
Saturated fat, *g/d*	30 ± 2^{b}	39 ± 4^{a}	36 ± 4^{ab}	36 ± 2^{ab}	<0.01
Monounsaturated fat, *g/d*	26 ± 2^{b}	31 ± 4^{ab}	27 ± 3^{ab}	32 ± 3^{a}	0.05
Polyunsaturated fat, *g/d*	10 ± 1	11 ± 1	9 ± 2	12 ± 2	0.15
Oleic acid, 18:1(n-9), *g/d*	22.4 ± 2.0^{b}	27.0 ± 3.3^{ab}	23.8 ± 2.9^{ab}	27.4 ± 2.2^{a}	0.04
Linoleic acid, 18:2(n-6), *g/d*	8.4 ± 0.8	7.6 ± 1.0	6.7 ± 1.3	9.0 ± 1.5	0.35
α-Linolenic acid, 18:3(n-3), *g/d*	1.1 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.2	0.36

[1]Data were derived from 3-d diet records collected during each test period, to include one weekend day. Data for the habitual diets were pooled over the two washout periods. [ab]Means without common superscripts differ ($P < 0.05$).

TABLE 4 **Dietary exchanges for habitual diets and for test diets of men rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), medium in monounsaturated fatty acids (Mid MUFA), or high in monounsaturated fatty acids (High MUFA)[1]**

	Treatment group				
Item	Habitual	Low MUFA	Mid MUFA	High MUFA	*P*-values
Bread/starch	8.1 ± 0.5	9.1 ± 1.2	9.2 ± 1.3	8.6 ± 0.5	0.18
Fat	6.7 ± 0.9	7.0 ± 1.6	7.0 ± 1.8	7.7 ± 1.1	0.26
Fruit	0.9 ± 0.2	1.0 ± 0.3	1.3 ± 0.3	0.7 ± 0.2	0.24
Meat, high-fat	0.5 ± 0.1[b]	1.7 ± 0.4[a]	1.4 ± 0.4[a]	1.4 ± 0.4[a]	<0.01
Meat, medium-fat	6.2 ± 0.5	6.0 ± 0.9	5.7 ± 0.7	5.5 ± 0.4	0.30
Meat, low-fat and lean	2.3 ± 0.4	2.6 ± 0.6	2.2 ± 0.6	2.6 ± 0.6	0.44
Meat, total	8.9 ± 0.8	10.6 ± 1.3	10.4 ± 1.8	9.9 ± 1.2	0.19
Milk, whole, low-fat and skim	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.3	0.7 ± 0.2	0.25
Other carbohydrates	3.3 ± 0.4	3.4 ± 0.6	2.7 ± 0.6	3.4 ± 0.7	0.28
Vegetables	1.3 ± 0.2	1.4 ± 0.4	1.6 ± 0.4	1.1 ± 0.3	0.41

[1]Data were derived from 3-d diet records collected at each interval. Data for the habitual diets were pooled over the two washout periods.
[ab]Means without common superscripts differ ($P < 0.05$).

TABLE 5 Initial and final characteristics of men rotated through ground beef types low in monounsaturated fatty acids, medium in monounsaturated fatty acids, or high in monounsaturated fatty acids (pooled across dietary interventions)[1]

Item	Baseline	Intervention	*P*-values
Age, *y*	35.8 ± 1.3	---	---
Body weight, *kg*	85.6 ± 2.7	86.2 ± 2.7	0.15
Body mass index, *kg/m*^{2}	27.1 ± 0.8	27.3 ± 0.8	0.17
Android fat, *% total fat*	35.3 ± 2.4	35.6 ± 2.4	0.69
Gynoid fat, *% total fat*	32.7 ± 2.5	31.1 ± 1.8	0.29
Total body fat, *%*	27.4 ± 2.0	26.9 ± 1.8	0.31
Lipid values			
Glucose, *mmol/L*	5.10 ± 0.04	5.03 ± 0.04	0.14
Triacylglycerol, *mmol/L*	1.28 ± 0.06	1.21 ± 0.11	0.09
Total cholesterol, *mmol/L*	4.73 ± 0.10	4.72 ± 0.09	0.97
LDL cholesterol, *mmol/L*	3.02 ± 0.09	3.00 ± 0.09	0.55
HDL cholesterol, *mmol/L*	1.17 ± 0.02	1.22 ± 0.04	<0.01
LDL:HDL	2.68 ± 0.10	2.57 ± 0.18	0.04
Insulin, *mU/L*	5.96 ± 0.53	4.52 ± 0.42	<0.01
hs-CRP, *mg/L*	1.97 ± 0.44	1.82 ± 0.29	0.70
LDL particle diameter, *nm*	26.05 ± 0.13	26.38 ± 0.12	0.05
HDL_2 particle diameter, *nm*	11.45 ± 0.08	11.25 ± 0.08	0.04
HDL_3 particle diameter, *nm*	9.29 ± 0.03	9.12 ± 0.04	<0.001

[1]Data are means ± SE for 27 values for age and morphometric measurements and 81 values for lipid measurements, pooled across 27 men and three ground beef interventions.

Main effects of ground beef interventions. Final lipid and lipoprotein parameters did not differ among the ground beef interventions and so the effects of ground beef consumption per se were assessed using pooled data comparisons between baseline (n = 81) and intervention values (n = 81). Body weight, BMI, android fat, gynoid fat and total body fat were unchanged over the 23-wk duration of the study (Table 5).

Ground beef interventions decreased plasma TAG (P = 0.09), and increased HDL-C (P < 0.01) and the plasma LDL:HDL (P = 0.04). Plasma insulin was uniformly decreased by the ground beef interventions (P < 0.01) and aggregate hs-CRP was unaffected. The ground beef interventions increased LDL particle diameter (P = 0.05), but decreased HDL_2 and HDL_3 particle diameters (P = 0.04 and P < 0.001, respectively).

There was a small decrease in plasma palmitoleic acid and a similarly small increase in plasma stearic acid (both P = 0.07) that resulted in a significant (P = 0.04) decrease in the palmitoleic:stearic (from 0.185 to 0.156; Table 6). Plasma *trans*-vaccenic and ALA both were depressed after the ground beef interventions (P = 0.02). Conversely, consumption of the ground beef patties increased plasma arachidonic acid (P < 0.001). The total plasma fatty acid concentration did not change with the ground beef interventions (414 vs 363 μmol/L, respectively; baseline vs intervention P = 0.25).

TABLE 6 Initial and final plasma fatty acids of men rotated through ground beef types low in monounsaturated fatty acids, medium in monounsaturated fatty acids, or high in monounsaturated fatty acids (pooled across dietary interventions)[1]

Fatty acid	Baseline	Intervention	*P*-values
	μmol/100 μmol total fatty acids[2]		
Palmitic, 16:0	23.89 ± 0.29	23.31 ± 0.31	0.36
Palmitoleic, 16:1(n-7)	1.54 ± 0.09	1.34 ± 0.08	0.07
trans-Vaccenic, 18:1(*trans*-11)	0.40 ± 0.07	0.17 ± 0.05	0.02
Stearic, 18:0	8.43 ± 0.12	8.81 ± 0.13	0.07
Oleic, 18:1(n-9)	21.06 ± 0.34	20.85 ± 0.36	0.89
Linoleic, 18:2(n-6)	32.99 ± 0.59	32.85 ± 0.58	0.54
α-Linolenic, 18:3(n-3)	0.33 ± 0.05	0.20 ± 0.04	0.02
Arachidonic, 20:4(n-6)	6.54 ± 0.26	7.92 ± 0.20	<0.001
Eicosapentaenoic, 20:5(n-3)	0.31 ± 0.05	0.34 ± 0.05	0.86
Docosahexaenoic, 22:6(n-3)	0.76 ± 0.11	0.83 ± 0.11	0.31
Palmitoleic:stearic acid ratio	0.185 ± 0.012	0.156 ± 0.010	0.04

[1]Data are means ± SE for 81 values, pooled across 27 men and three ground beef interventions.

[2]Total plasma fatty acid concentrations were 414 μmol/L (baseline) and 363 μmol/L (intervention); concentrations were not different, $P = 0.25$.

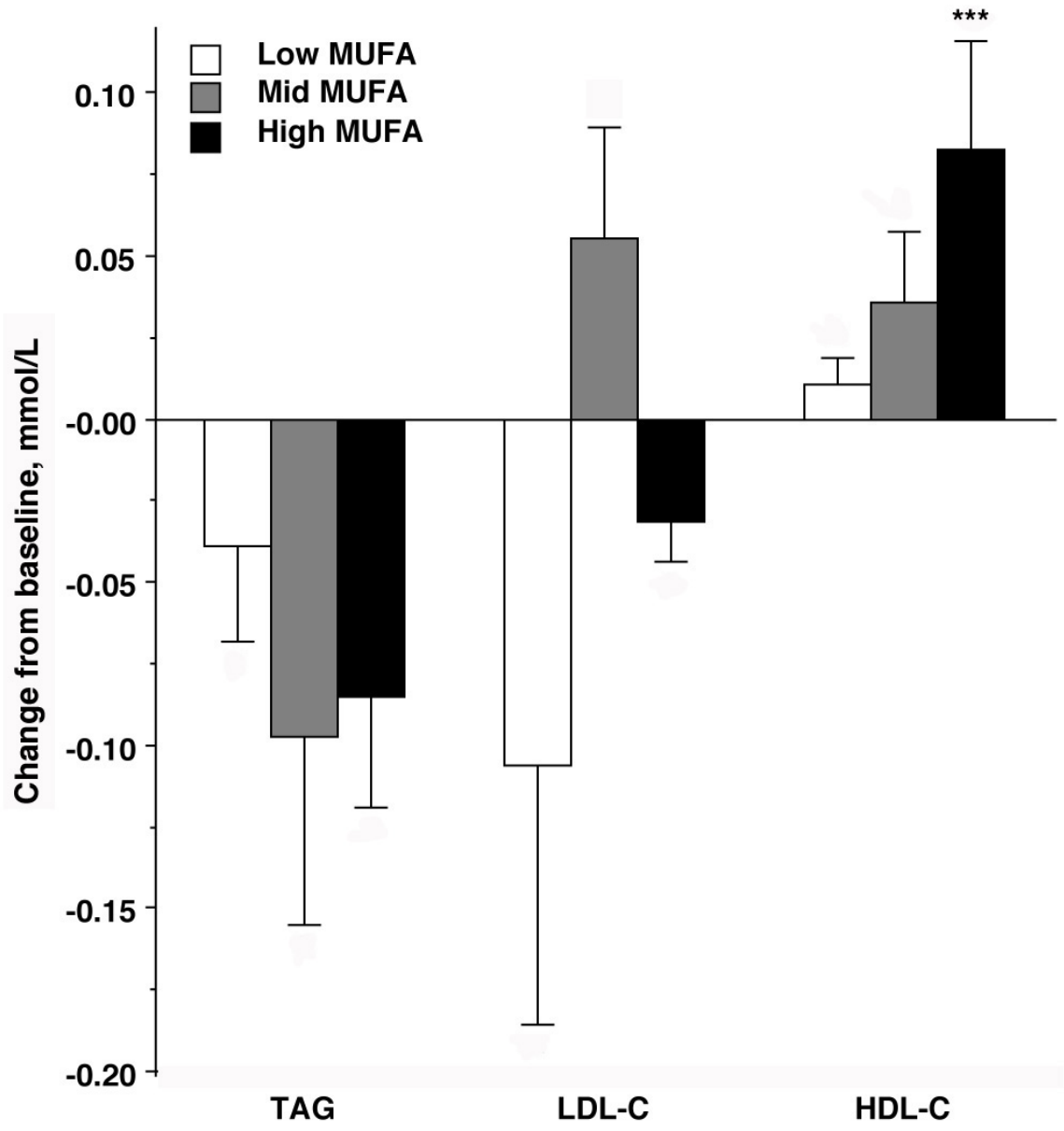

FIGURE 1 Absolute changes from baseline (mmol/L) for triacylglycerol (TAG), LDL-C and HDL-C in men rotated through test group beefs low in monounsaturated fatty acids (Low MUFA), medium in monounsaturated fatty acids (Mid MUFA), or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, $n = 27$. Different from zero change at $^{***}P < 0.001$.

Changes from baseline for each ground beef intervention. Although the low-, mid- and high-MUFA ground beef caused uniform decreases from baseline in plasma TAG (0.04 to 0.10 mmol/L decreases), the change from baseline TAG were not significant ($P > 0.10$). Similarly, there was no overall effect of the dietary interventions on LDL-C (Figure 1**)**. Only the high-MUFA ground beef intervention significantly increased HDL-C from baseline values ($P < 0.001$).

The low-MUFA ground beef had no effect on LDL particle diameter, whereas the mid- and high-MUFA ground beef interventions increased LDL particle diameter from baseline values (0.45 to 0.55 nm increases; $P < 0.05$) (Figure 2). Although the ground beef interventions caused an overall decrease in HDL_2 diameter (Table 5), the changes from baseline for the separate ground beef interventions were not significant ($P > 0.10$). The mid-MUFA ground beef caused the greatest decrease in diameter for HDL_3, but all ground beef interventions significantly reduced HDL_3 diameter.

The mid-MUFA ground beef intervention significantly reduced plasma glucose from baseline ($P < 0.05$) and the high-MUFA ground beef caused a significant depression from baseline for plasma insulin ($P < 0.01$; Figure 3). The low- and mid-MUFA ground beef interventions decreased plasma ALA from baseline ($P < 0.05$).

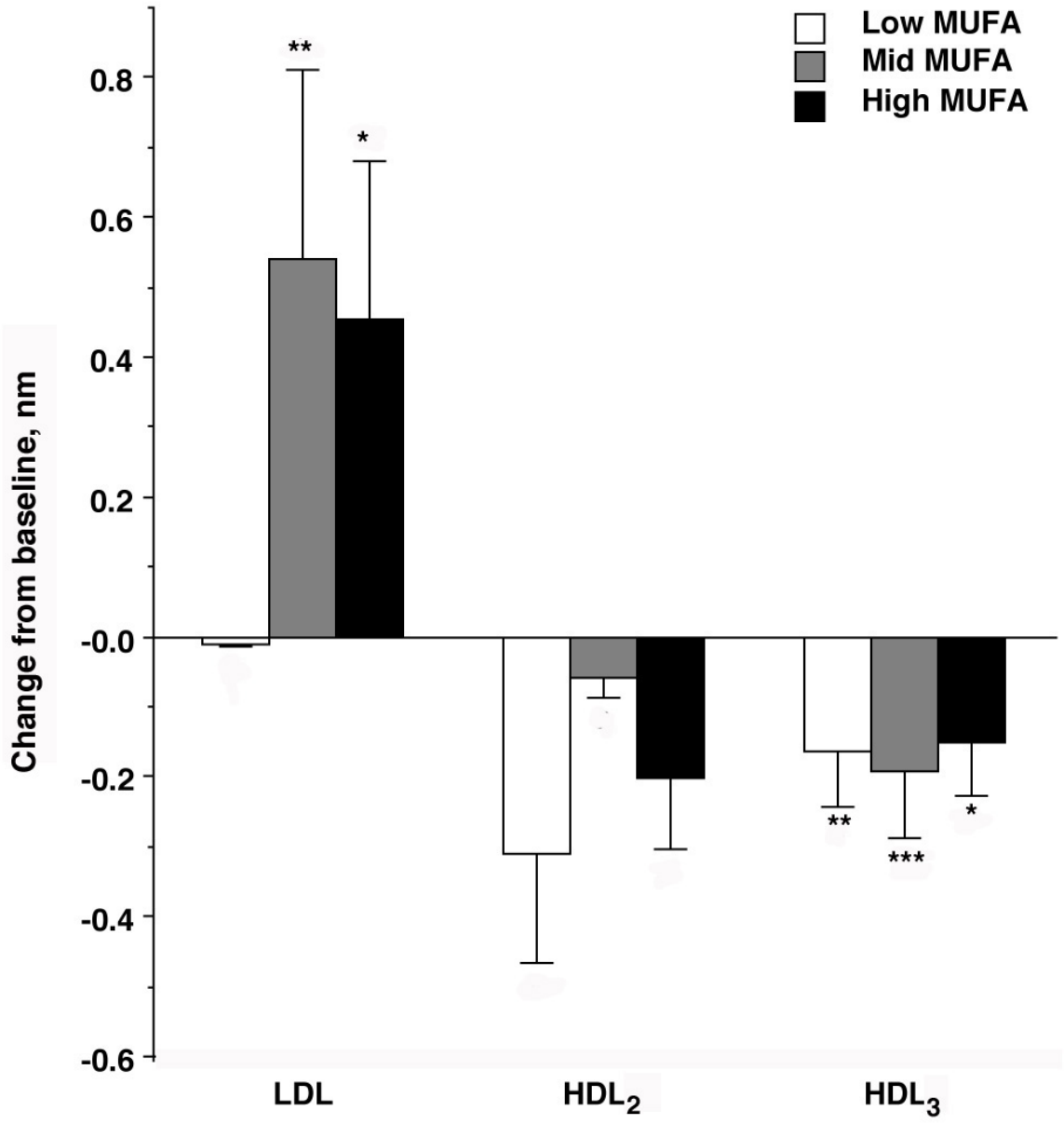

FIGURE 2 Absolute changes from baseline (nm) for LDL, HDL_2 and HDL_3 particle diameter in men rotated through test group beefs low in monounsaturated fatty acids (Low MUFA), medium in monounsaturated fatty acids (Mid MUFA), or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, *n* = 27. Different from zero change at **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

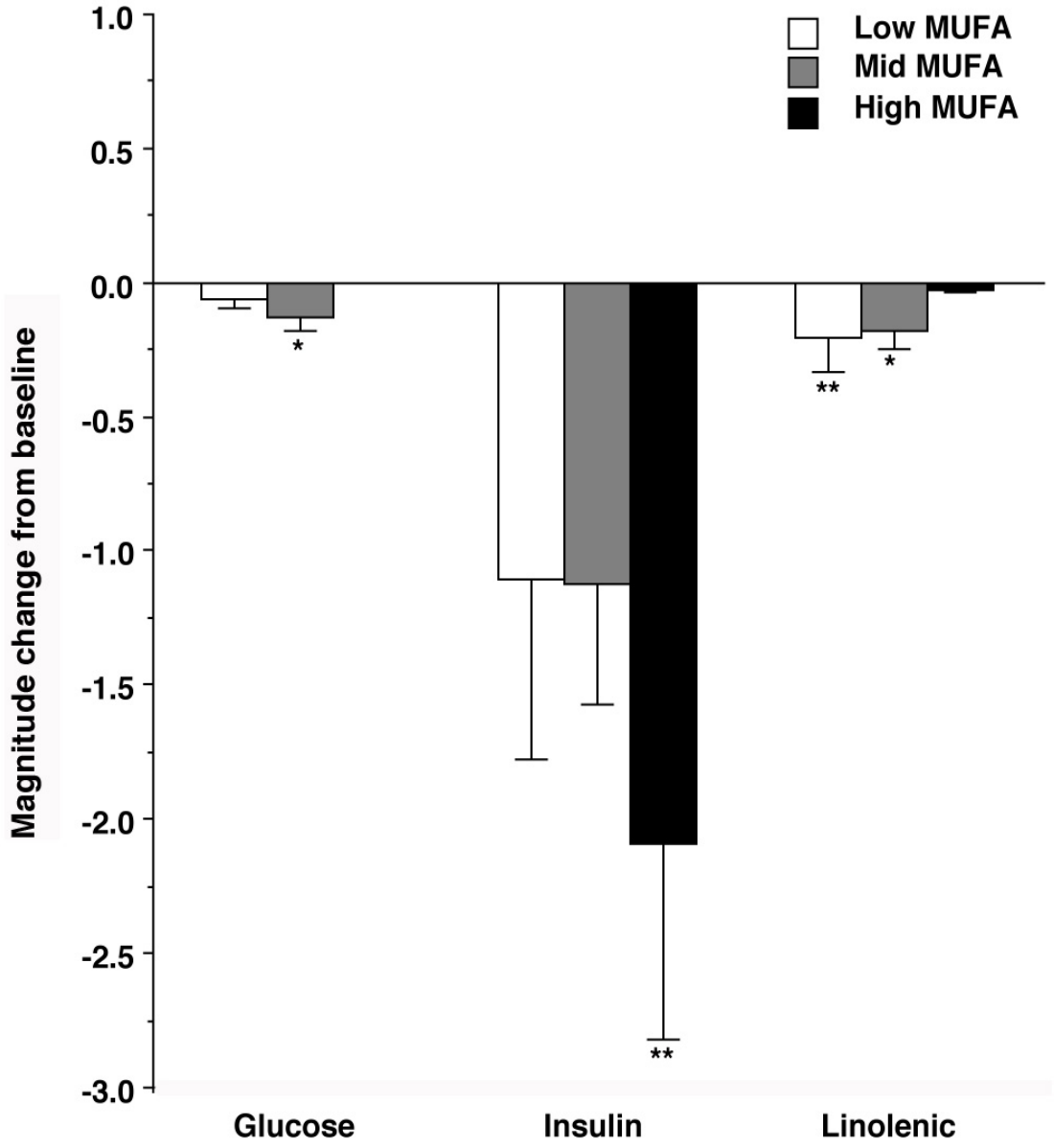

FIGURE 3 Magnitude changes from baseline for glucose (mmol/L), insulin(mU/L) and hs-CRP (mg/L) in men rotated through test group beefs low in monounsaturated fatty acids (Low MUFA), medium in monounsaturated fatty acids (Mid MUFA), or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, *n* = 27. Different from zero change at **P* < 0.05, ***P* <.01.

Discussion

Nutrient and dietary exchange intake. Total energy intake and intakes of protein, carbohydrates, and fat were essentially identical to values reported in two previous studies from our laboratory on the effects of beef on plasma lipoprotein concentrations (130, 131). Although nutrient intake was not strictly controlled, it was carefully monitored. Very few food restrictions were placed on the participants either during the test periods or during the intervening washout periods. We purposely did not strictly control dietary intake of nutrients by participants, but stipulated only that the ground beef patties replaced an equal portion of meat (beef or otherwise) that they normally would have consumed. Dietary records indicated that the participants generally consumed the ground beef patties intact, and usually included them in their noontime meals. This encouraged compliance and allowed us to evaluate the effects of the test ground beef patties in a more likely, i.e., free-living setting.

By design, the high-MUFA ground beef contained nearly 4 g more MUFA per patty than did the low-MUFA ground beef, and the mid-MUFA ground beef was intermediate. Thus, each participant on the high-MUFA diet consumed nearly 20 g more MUFA per week than those consuming the low-MUFA ground beef. The mid-MUFA ground beef intervention was included as it most closely resembled regular chub pack ground beef in total fat and fatty acid composition (131, 132).

Ground beef comprises a large proportion of the beef consumed in the U.S. (145). Also, the ground beef used in this study was representative of the range of ground beef that is readily available to consumers (131, 132), and the amounts of beef consumed

in the study would result in an annual consumption of 65 pounds per year, near to the national average in the US. The MUFA:SFA of the ground beef from pasture-fed cattle (low-MUFA; 0.71) was similar to the MUFA:SFA for a specialty branded ground beef product (0.73) (131). Low MUFA:SFA products typically are produced from cattle that are fed little or no grain, or which are processed at a young age (139). The high-MUFA ground beef (1.10) was similar to that for a branded Angus product (1.12) (130, 131). We demonstrated previously that MUFA in beef, and especially oleic acid, increase with the amount of time that cattle are fed a grain-based diet (139).

The most profound differences in documented fat intake were for saturated and total fat. Individuals in the low-MUFA ground beef group consumed nearly 10 g/d more saturated fat and 15 g/d more total fat than in their habitual diets. This reflected the primary contribution of animal products to saturated and total fat intake. In spite of this, none of the test ground beef patties increased LDL-C, possibly because most of the increase in saturated fat was in the form of stearic acid (146).

Plasma TAG, insulin and glucose. We previously demonstrated that low-MUFA ground beef increased, and high-MUFA ground beef decreased plasma TAG (131). In the current study, the ground beef interventions caused an overall depression in plasma TAG and plasma palmitoleic acid and an increase in plasma stearic acid. There was a highly significant correlation between plasma palmitoleic acid and TAG ($r = 0.38$, $P < 0.0001$), consistent with previous studies (131, 147). Notably, in both this and the previous study (131) the high-MUFA patties increased plasma stearic acid with approximately a 20% decrease in palmitoleic:stearic acid ratio, suggesting that high-

MUFA ground beef may depress hepatic stearoyl-CoA desaturase-1 (SCD1) activity. Hepatic SCD1 activity supports TAG synthesis (148), and SCD1 gene expression and activity are activated by dietary stearic acid and depressed by oleic acid in mice (149). This argues strongly that SCD1 activity in part at least regulates plasma TAG in humans. Similar evidence for plasma palmitoleic as a predictor of cardiovascular mortality was provided by Warensjo et al. (150). The reduction in plasma insulin and the small reductions in TAG and glucose are consistent with this interpretation and effects seen in men consuming additional amounts of dairy fat (151), albeit specific mechanisms underlying the effect may differ.

Plasma lipoprotein cholesterol fractions. We recently reported the effects of two high-fat, hamburger preparations on cholesterol metabolism in a group of mildly hypercholesterolemic men (131). By definition, ground beef contains a maximum of 30% total fat (137). In that study, 10 men were first fed 35% fat hamburger high in SFA and TFA (MUFA:SFA = 0.95, TFA = 1.72 g/patty), and then were rotated to 35% hamburger high in MUFA and lower in TFA (MUFA:SFA = 1.31, TFA = 1.28 g/patty). The high-SFA hamburger markedly reduced HDL-C and LDL particle diameters while increasing plasma TAG; these values returned to baseline values after the high-MUFA hamburger intervention (131). Notably, while the 35% fat low-MUFA:SFA hamburger intervention in the earlier study actually contained more oleic acid per patty (15 g) than the 24% fat high-MUFA ground beef of the current study (13.2 g) it also provided 9.6 g more palmitic and 0.8 g more TFA per patty. This suggests that, in our ground beef

intervention studies, the SFA and TFA content may have been more important in altering risk factors for CVD than the oleic acid content.

None of the ground beef interventions of the current study reduced LDL particle diameters, and in fact the mid- and high-MUFA ground beef interventions increased LDL diameters. Thus, we were unable to reproduce the depression in LDL particle diameters caused by 35% fat hamburger from grass-fed cattle in our previous study (131). Similarly, serum hs-CRP was not affected by beef consumption. To our knowledge, the current and previous studies (131) are the only investigations in which the effects of supplemental beef on lipoprotein particle diameters or markers of vascular inflammation have been reported.

The current study also demonstrated a very clear reduction in HDL_3 particle diameters with all beef interventions that was independent of ground beef fatty acid composition. We know of no other study that has reported a reduction in the diameters of the individual HDL subclasses. Arsenault et al. (152) reported that HDL-C levels are the best correlates of HDL particle diameter in men ($r = 0.58$) and women ($r = 0.62$). Men and women with small HDL particles plus low HDL concentrations were at increased risk for CVD, as assessed by their cardiometabolomic risk profile (152). We observed a significant, positive correlation between HDL particle diameter and HDL-C ($r = 0.26$, $P = 0.01$) in the current study, but we also demonstrated increased HDL-C and reduced HDL diameter after the high-MUFA ground beef intervention. The relationship between HDL-C and particle diameter was true only for HDL_2, as there was no correlation between HDL-C and HDL_3 particle diameter ($r = 0.06$, $P = 0.44$). Because

depressed HDL particle diameter was accompanied by elevated HDL-C in this study, we cannot ascertain if this represents increased or decreased risk for CVD.

Plasma and beef ALA. Mente et al. (89) conducted a systematic search for prospective cohort studies or randomized trials investigating dietary exposures in relation to CVD. They found strong evidence to support valid associations of protective factors for (among others) dietary MUFA, but insufficient evidence for total fat, SFA, PUFA or ALA. The low-MUFA ground beef of this study had three times the ALA of the high-MUFA ground beef, i.e., 90 vs 30 mg per ground beef patty, but this had no significant impact on the daily ALA intake. Our data indicated an overall lower plasma ALA after consumption of the low- and mid-MUFA ground beef preparations, and neither eicosopentaenoic (20:5(n-3)) nor docosahexaenoic acid (22:6(n-3)) concentrations were affected by the ground beef interventions. These data argue strongly that 24% fat ground beef from pasture-fed cattle does not provide enough ALA to have any impact on the metabolism of n-3 fatty acids. Interestingly, the 35% fat, low-MUFA hamburger used in our earlier study provided 63 mg ALA per patty, indicating that the low-MUFA ground beef in the present study was comparatively rich in ALA.

Greater enrichment of beef with ALA can be achieved by allowing cattle to graze on pasture for much longer periods of time (up to 30 mo of age) (133), or by feeding flaxseed to cattle (153). However, the concentration of ALA in beef from these cattle would be at most 200 mg per 114-g beef serving (calculated from reported percentages of ALA) (133, 152). This is much less than the amount of ALA provided to human subjects in the form of flaxseed, margarine, or walnut oil (1 – 2 g/d) (154-156). Thus,

although supplementary ALA can lower plasma TAG (155) and hs-CRP (156), this cannot occur at the concentrations of ALA in beef from grass-fed cattle. Instead, the elevation of SFA and TFA that has been demonstrated consistently in beef from pasture-fed animals (131, 133, 139) apparently overwhelms any potentially beneficial effects of the greater amounts of ALA.

General considerations. There is convincing evidence that high-MUFA diets lower plasma cholesterol and TAG, usually by decreasing LDL-C (70, 126). Furthermore, studies that have included elevated beef intake have not demonstrated an increase in LDL-C (75, 129-131). However, it was not the intent of this study to recommend daily consumption of ground beef. It also is recognized that ground beef produced from carcasses of pasture-fed cattle typically is much leaner than the test ground beef patties developed for this study. However, none of the test ground beef patties had negative effects on risk factors for CVD, including the mid-MUFA ground beef, which was formulated to be similar to regular chub-pack ground beef. The high-MUFA ground beef actually reduced risk factors for CVD by increasing HDL-C and LDL particle diameter as well as decreasing plasma insulin. Thus, this study provided no evidence that beef from pasture-fed cattle is nutritionally superior to beef from typical commercially produced (corn-fed) cattle in products where total fat content is similar.

CHAPTER III

HIGH-OLEIC GROUND BEEF AND RISK FACTORS FOR CARDIOVASCULAR DISEASE IN POSTMENOPAUSAL WOMEN

Introduction

With cardiovascular disease (CVD) affecting the lives of over 26 million Americans, and being the leading cause of preventable death in the United States, the American Heart Association (AHA) has strived to provide "heart healthy" recommendations. The previous recommendation of the AHA was to consume a low-fat diet, especially low in saturated fatty acids (SFA). At that time, the AHA recommended that fat calories be replaced by carbohydrate. Unknowingly, consumers might have increased their risk of heart disease by increasing plasma triglycerides, Lp(a), and decreasing HDL cholesterol and LDL particle size (67-69). These findings resulted in the evaluation of fatty acids and their effect on CVD (73). While the recommendation for a reduction in saturated fat still remained, the AHA recognized diets that provided up to 40% of dietary energy in the form of unsaturated fat were as heart-healthy as low-fat diets (70, 71). An outcome of this official opinion has been the reevaluation of the nutritional properties of a number of higher fat foods such as dairy, nuts, and dietary oils such as olive oil rich in the monounsaturated fatty acid (MUFA), oleic acid (18:1n-9) (71). Although red meat is high in MUFA, especially oleic acid, it is still recommended to abstain from red meat consumption to maintain good heart health. The primary objective of this study was to establish the relationship between the MUFA:SFA ratio in

beef and plasma lipoprotein cholesterol and inflammatory markers of CVD in postmenopausal women.

Methods

Subjects. The study protocol was reviewed and approved by Texas A&M University Institutional Review Board for the use of human subjects in research (2008-125) and all participants gave written consent. Twenty-nine postmenopausal women were recruited from the local Bryan/College Station, Texas community. Nineteen women completed the study. The subjects were women whose last menstrual period was over 1 y prior to enrollment of the study by natural or surgical means with or without ovaries. Subjects were able to walk briskly for 20 min without chest pain or fatigue, were non-smokers with no history of CVD, stroke or diabetes, had normal liver function tests, normal fasting glucose, normal resting electrocardiogram, and serum total cholesterol of less than 6.72 mmol/L, and must not be taking lipid lowering drugs. Baseline characteristics of the participants are found in Table 7.

TABLE 7 Baseline characteristics for subjects[1]

Item	Mean	SE
Age, *y*	57.8	1.7
Body weight, *kg*	70.7	4.0
Body mass index, kg/m^2	26.4	1.4
Android fat, *% total fat*	45.6	2.6
Gynoid fat, *% total fat*	49.6	1.5
Total body fat, %	42.2	1.9

[1]Data are means ± SE for 19 women.

Study design. This experiment tested the hypothesis that risk factors for CVD would be lower in postmenopausal women after consumption of ground beef naturally enriched with MUFA than after consumption of ground beef enriched with SFA. The 19 women were allotted to groups for a crossover design. The first group was fed high-SFA ground beef for a 6 wk period, and following a 4 wk habitual diet washout period, was rotated to a high-MUFA ground beef. The second group was fed a high-MUFA test ground beef for a 6 wk period, and following a 4 wk habitual diet washout period, was rotated to a high-SFA test ground beef.

The beef was supplied to the participants in the form of 114 g, raw ground beef patties. The frozen, vacuum-packaged ground beef patties for an entire diet period were delivered to the participants on or before the first day of the diet period. The participants were asked to replace one of their normal meat servings with a patty 5 d/wk. No restrictions were placed on how the patty was to be prepared other than the patty should be cooked to an internal temperature of 71˚C, one patty should be prepared at a time, and

all of the patty should be consumed in one sitting. The subjects were not informed as to which test patty they had been assigned.

Baseline testing. At study entrance, each subject underwent: a complete history, physical exam by a physician, height and body weight measurements, and a DEXA scan.

Test patties. The low-MUFA ground beef patties were made from chub pack ground beef, purchased from a local retail outlet with a MUFA:SFA ratio of 0.9. The high-MUFA patties were made from Akaushi ground beef (Heartbrand Beef, Yoakum, TX) with a MUFA:SFA ratio of 1.3 (Table 8). Patties were individually vacuum-packed, quick-frozen and boxed by diet type. Patties were formulated to contain 20% fat, but after cooking, the low-MUFA patties contained 19.4 g/100g and the high-MUFA patties contained 22.5/100g

TABLE 8 Fatty acid composition of low MUFA and high MUFA ground beef patties[1]

Fatty acid	Low MUFA	High MUFA
	g/100-g cooked ground beef patty	
Total lipid	19.4	22.5
14:0 (myristic)	0.60	0.59
14:1 (myristoleic)	0.09	0.35
16:0 (palmitic)	4.88	5.30
16:1 (n-7) (palmitoleic)	0.56	0.98
18:0 (stearic)	3.41	2.73
18:1 (*trans*-11) (*trans*-vaccenic)	0.78	0.66
18:1 (n-7) (*cis*-vaccenic)	0.29	0.49
18:1 (n-9) (oleic)	7.34	10.24
18:2 (n-6) (linoleic)	0.36	0.44
MUFA:SFA ratio	0.9	1.3

[1]Data are means for three batches of ground beef per treatment group.

Collection and handling of blood samples. Blood was collected from an arm vein prior to initiation of the dietary treatments, at the end of each diet phase 24 h before and after exercise. Plasma was harvested from the blood collected with 15% EDTA. Serum was harvested from the blood with clot separation. All serum and plasma samples were stored at -80°C.

Fatty acid composition of plasma and test ground beef. Fatty acids were measured in the baseline plasma, plasma taken after 6 wk of each test beef treatment, 24 h before and 24 h after exercise. Additionally, fatty acid composition, along with total fat and moisture of the dietary ground beef patties were measured. Total lipid was extracted and methylated as described (143, 144). Fatty acid methyl esters were analyzed with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Variam Inc., Walnut Creek, CA). Separation of fatty acid methyl esters was accomplished on a fused silica capillary column CP-Sil88 [100m x 0.25mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands) with helium as the carrier gas (1.2 mL/min). Oven temperatures began at 150°C and were increased to 160°C at a rate of 1°C/min. The oven temperature rose further to 167°C at a rate of 0.2°C/min. The temperature increased a rate of 1.5°C/min to a final temperature of 225°C where it was held for 26 mins. Injector and detector temperatures were at 270°C. Individual fatty acid methyl esters were identified using genuine standards (Nu-check Prep, Inc., Elysian, MN and Sigma-Alderich Co.) and expressed as a g/100 g total fatty acid methyl esters analyzed or as g/100 g hamburger patty.

Fat and moisture of the dietary ground beef patties were measured by CEM Corp's SMART Trac Moisture and Fat Analysis system (157).

Lipoprotein analysis. Serum aliquots kept at -80°C were sent to SpectraCell Laboratories, Inc. (Houston, TX) for complete lipoprotein density and particle number analyses. A complete "Lipoprotein Particle Profile™" test was provided using the lipoprotein subgroup particle number analysis method. Lipoprotein particles were stained with a fluorescent dye and separated utilizing a patented continuous gradient over a range of d = 1.00 – 1.30 g/cm^3 generated by analytical ultracentrifugation. Once separated, the fluorescence of the lipoprotein particles was measured in a high performance liquid chromatography type flow system. For processing the fluorescence response was normalized to a cholesterol scale with a proprietary algorithm. Values corresponding to each lipoprotein subgroup at their specific densities were determined using a multiple Gaussian fit/integration routine (158).

Diet records. Prior to each diet phase, and once during each phase, participants completed a 4-d record (to include one weekend day). The diet records were analyzed for nutrient composition to establish baseline observations, and encourage compliance with total patty consumption requirement. The records were analyzed using Nutritionist Pro (Axxya Systems, Stafford, TX).

Statistical analysis. Data were analyzed as a paired *t*-test, comparing baseline (habitual) values to values after 6 wk on the test diet. Thus, each participant served as her own control. Because each participant randomly rotated through all three diets, reported values are means ± standard error of the mean (SE) for $n = 19$ observations.

Results

Dietary records indicated that when on a test diet, participants consumed a greater amount of total MUFA and oleic acid in patterns consistent with their test ground beef interventions. There were no significant differences in cholesterol, protein, or carbohydrate intake (Table 9).

TABLE 9 Total daily energy intake per day from major nutrients for habitual diets (Baseline) and for test diets of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

Nutrient	Baseline	Low MUFA	High MUFA
Total energy, kcal	1,662 ± 131	1,601 ± 98	1,688 ± 113
Protein, g	67.8 ± 4.9	74.0 ± 5	74.2 ± 5.2
Carbohydrate, g	189 ± 16	177 ± 15	188 ± 14
Fat, g	62.2 ± 6.8	65.3 ± 3.8	69.1 ± 4.7
Saturated fat, g	20.0 ± 2.3	23.6 ± 1.7	24.2 ± 1.8
Monounsaturated fat, g	15.1 ± 1.9	19.3 ± 1.1[a]	21.6 ± 1.5[a]
Polyunsaturated fat, g	8.1 ± 0.8	6.4 ± 0.5	8.0 ± 1.0
Trans-unsaturated fat, g	0.8 ± 0.2	1.0 ± 0.3	1.1 ± 0.2
Cholesterol, mg	202 ± 21	274 ± 40	241 ± 28
Oleic acid, g	12.8 ± 1.7	16.5 ± 1.0[a]	18.6 ± 1.4[a]
Linoleic acid, g	6.1 ± 0.7	4.8 ± 0.5	6.1 ± 0.8
Linolenic acid, g	0.7 ± 0.1	0.4 ± 0.1[a]	0.6 ± 0.1
Eicosapentaenoic acid, g	0.04 ± 0.02	0.06 ± 0.04	0.06 ± 0.04
Docosahexaenoic acid, g	0.05 ± 0.02	0.12 ± 0.07	0.12 ± 0.07
Fiber, g	18.3 ± 2.6	18.1 ± 2.4	16.9 ± 1.5
Sugar, g	68.3 ± 5.9	70.8 ± 8.6	81 ± 10.6
Sodium, mg	2,903 ± 205	2,4166 ± 198	2,428 ± 139[a]
Potassium, mg	1,924 ± 218	2,185 ± 242	2,262 ± 190
Vitamin A, IU	5,193 ± 798	5,886 ± 1058	6,911 ± 1455
Vitamin C, mg	94.1 ± 17.3	79.4 ± 12. 5[a]	223.6 ±137.2
Calcium, mg	750.4 ± 70.6	699.4 ± 68.7	818.4 ± 96.4
Iron, mg	13.1 ± 1.6	14.0 ± 1.4	14.2 ±1.2
Thiamin, mg	1.3 ± 0.2	1.2 ± 0.1	1.3 ± 0.2
Riboflavin, mg	1.8 ± 0.3	2.1 ± 0.3	1.9 ± 0.3
Niacin, mg	18.7 ± 3.8	23.1 ± 4.3	23.2 ± 3.9
Folate, µg	386 ± 79	322 ± 43	324 ± 50

[1]Data were derived from 4-d diet records, including one weekend day, collected during each test period. Data for the habitual diet were obtained at baseline before patty consumption began.
[a]Means without common superscripts differ ($P \leq 0.05$). Data are means ± SE for 19 women.

Serum total cholesterol, LDL, HDL, and buoyant HDL_{2b} cholesterol concentrations were significantly increased due to the consumption of the high-MUFA test patties (Figure 4 and 7; Table 10 and 13), whereas serum VLDL, RLP, and IDL particle concentrations were increased with the consumption of the low-MUFA test patties (Table 11 and Figure 5).

TABLE 10 Major lipoprotein cholesterol concentrations (mmol/L) for women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

Lipoprotein	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
Total cholesterol, mmol/L	5.09 ± 0.17	5.21 ± 0.17	5.08 ± 0.2	5.34 ± 0.19**
LDL, mmol/L	3.23 ± 0.15	3.24 ± 0.14	3.19 ± 0.16	3.35 ± 0.15*
HDL, mmol/L	1.56 ± 0.07	1.61 ± 0.06	1.56 ± 0.07	1.63 ± 0.07**

[1] Data are means ± SE for 19 women.
* $P \leq 0.10$; ** $P \leq 0.05$

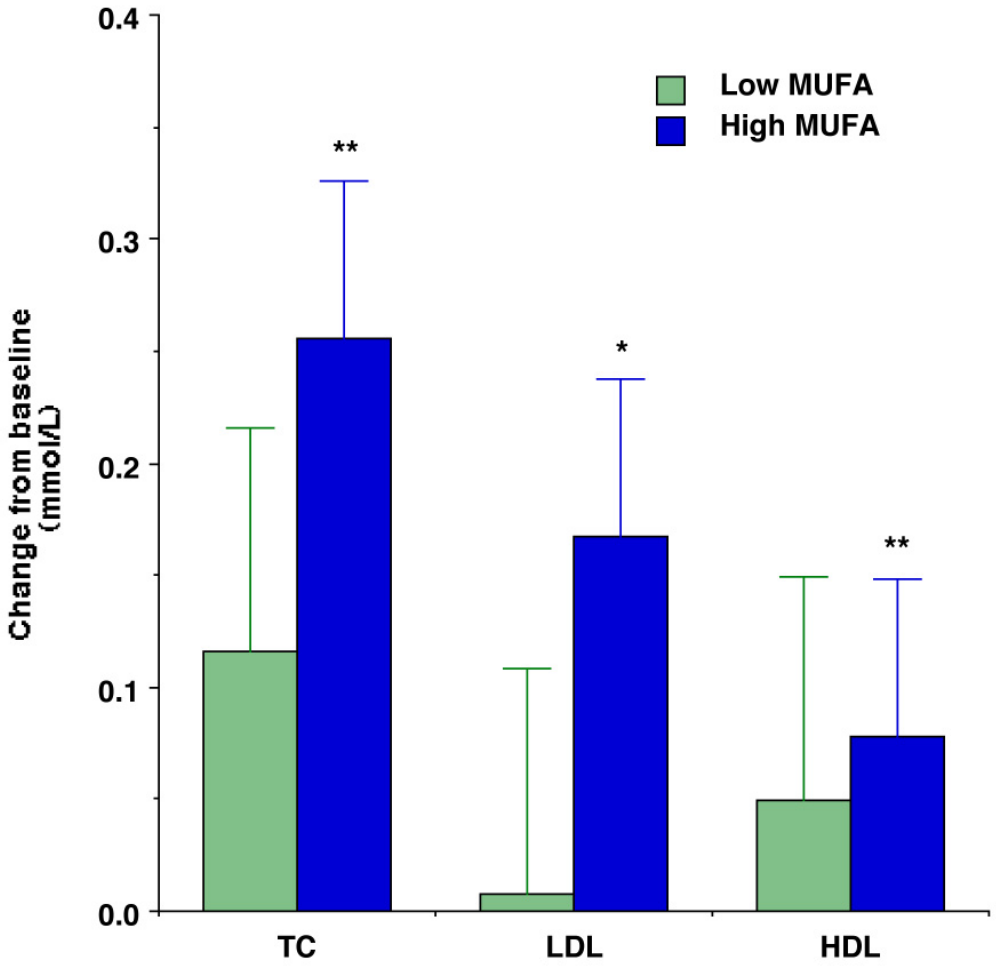

FIGURE 4 Changes from baseline in total cholesterol, LDL, and HDL cholesterol concentration (mmol/L) of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, *n* = 19. * $P \leq 0.10$; ** $P \leq 0.05$

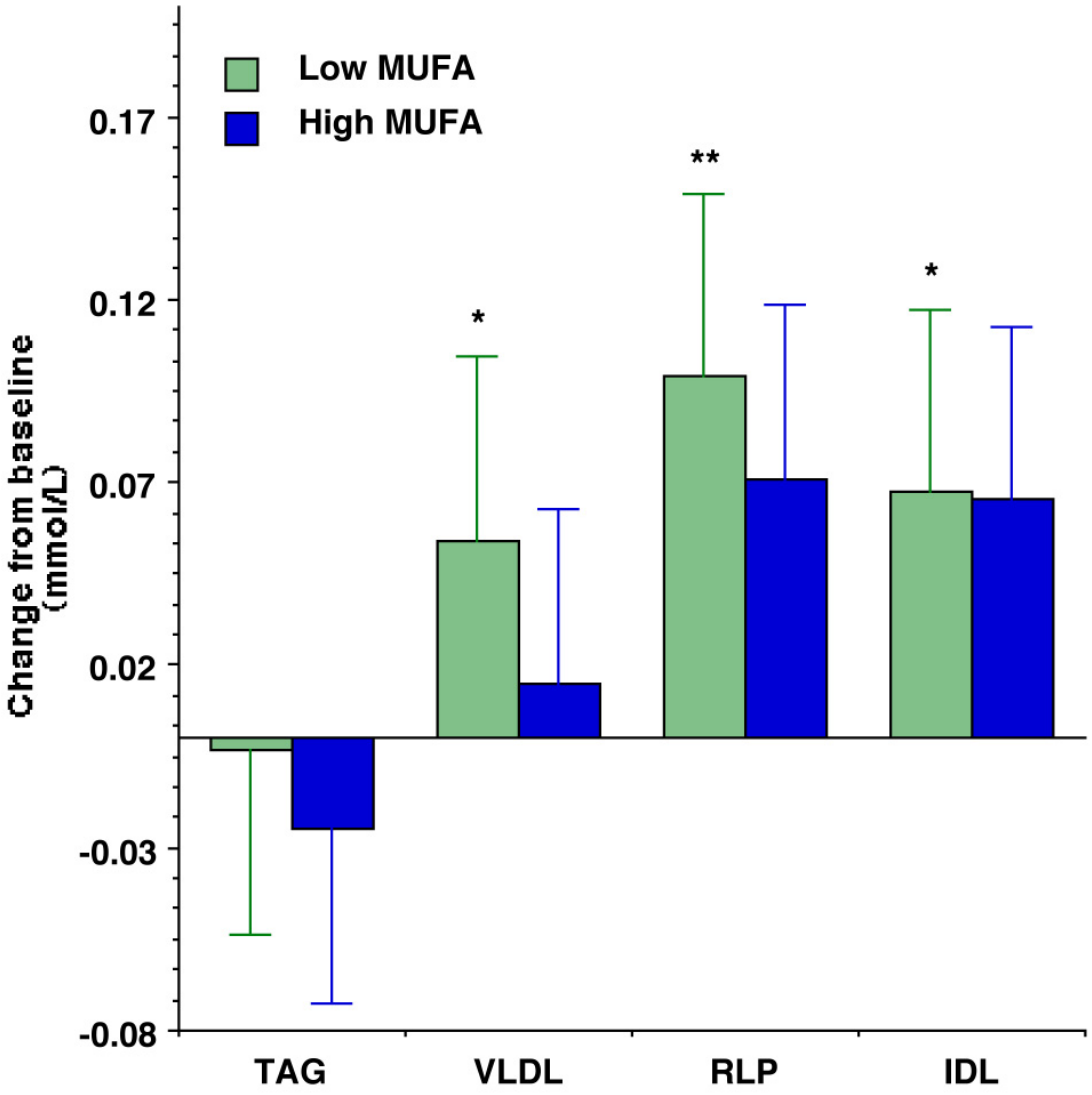

FIGURE 5 Changes from baseline in triglycerides (TAG), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL) and remnant lipoprotein (RLP) cholesterol concentration (mmol/L) of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, $n = 19$. * $P \leq 0.10$; ** $P \leq 0.05$

TABLE 11 TAG, VLDL, RLP, and IDL cholesterol concentrations for women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

Lipoprotein	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
VLDL, mmol/L	0.30 ± 0.03	0.35 ± 0.03*	0.34 ± 0.03	0.35 ± 0.05
RLP, mmol/L	0.75 ± 0.04	0.85 ± 0.05**	0.80 ± 0.05	0.87 ± 0.07
IDL, mmol/L	0.67 0.03	0.74 0.04*	0.71 0.05	0.77 0.06
TAG, mmol/L	1.12 ± 0.13	1.12 ± 0.1	1.11 ± 0.1	1.09 ± 0.10

[1] Data are means ± SE for 19 women; * $P \leq 0.10$; ** $P \leq 0.05$

Though total LDL cholesterol was increased with high-MUFA patty consumption, no significant difference was seen within LDL III, LDL IV, or Lp(a) (Figure 6 and Table 12).

TABLE 12 LDL III, LDL IV, and Lp(a) lipoprotein cholesterol concentrations for women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

Lipoprotein	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
Dense LDL III, mmol/L	0.5517 ± 0.04	0.5603 ± 0.03	0.5097 ± 0.04	0.525 ± 0.04
Dense LDL IV, mmol/L	0.1741 ± 0.01	0.1782 ± 0.01	0.1705 ± 0.09	0.1877 ± 0.01
Lp(a), mmol/L	0.7778 ± 0.18	0.7628 ± 0.17	0.771 ± 0.17	0.7423 ± 0.18

[1] Data are means ± SE for 19 women.
There were no significant differences among phases ($P > 0.1$)

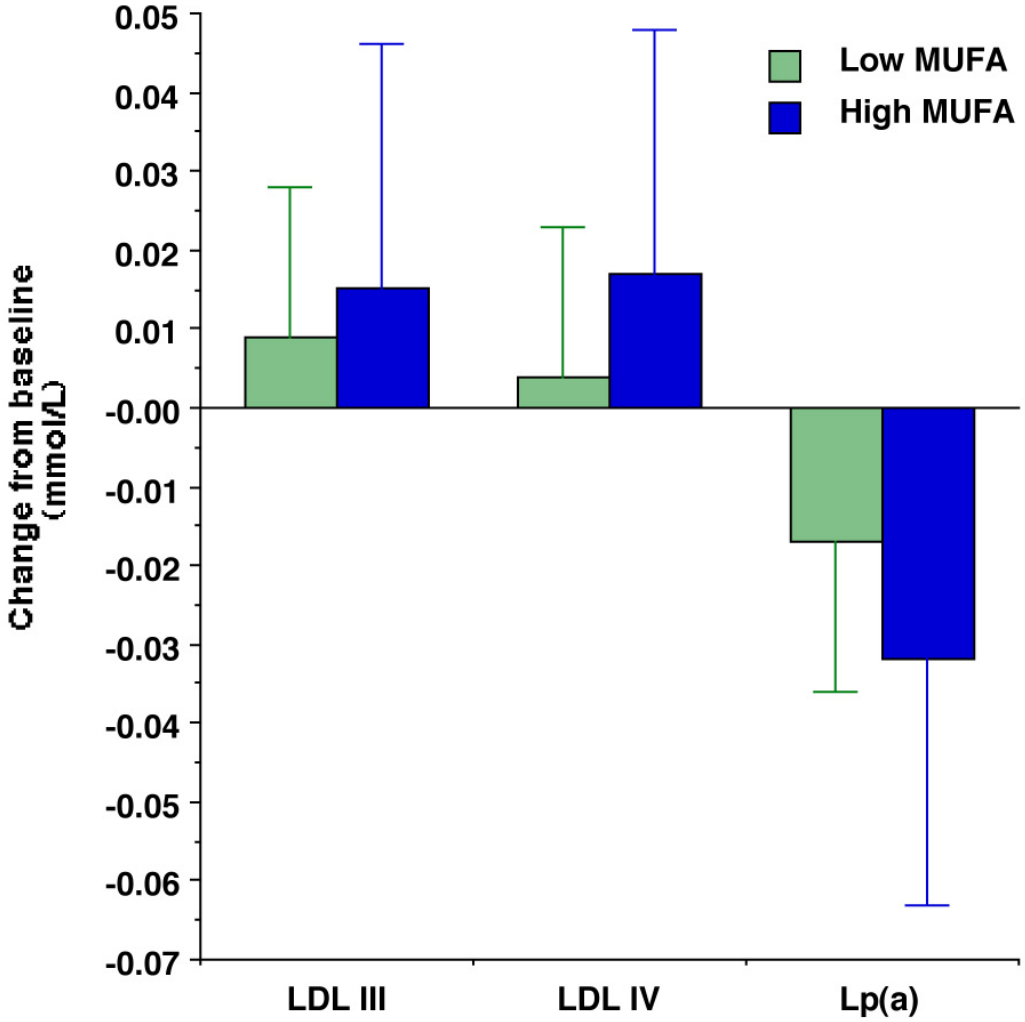

FIGURE 6 Changes from baseline in LDL III, LDL IV, and Lp(a) cholesterol concentration (mmol/L) of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, *n* = 19.

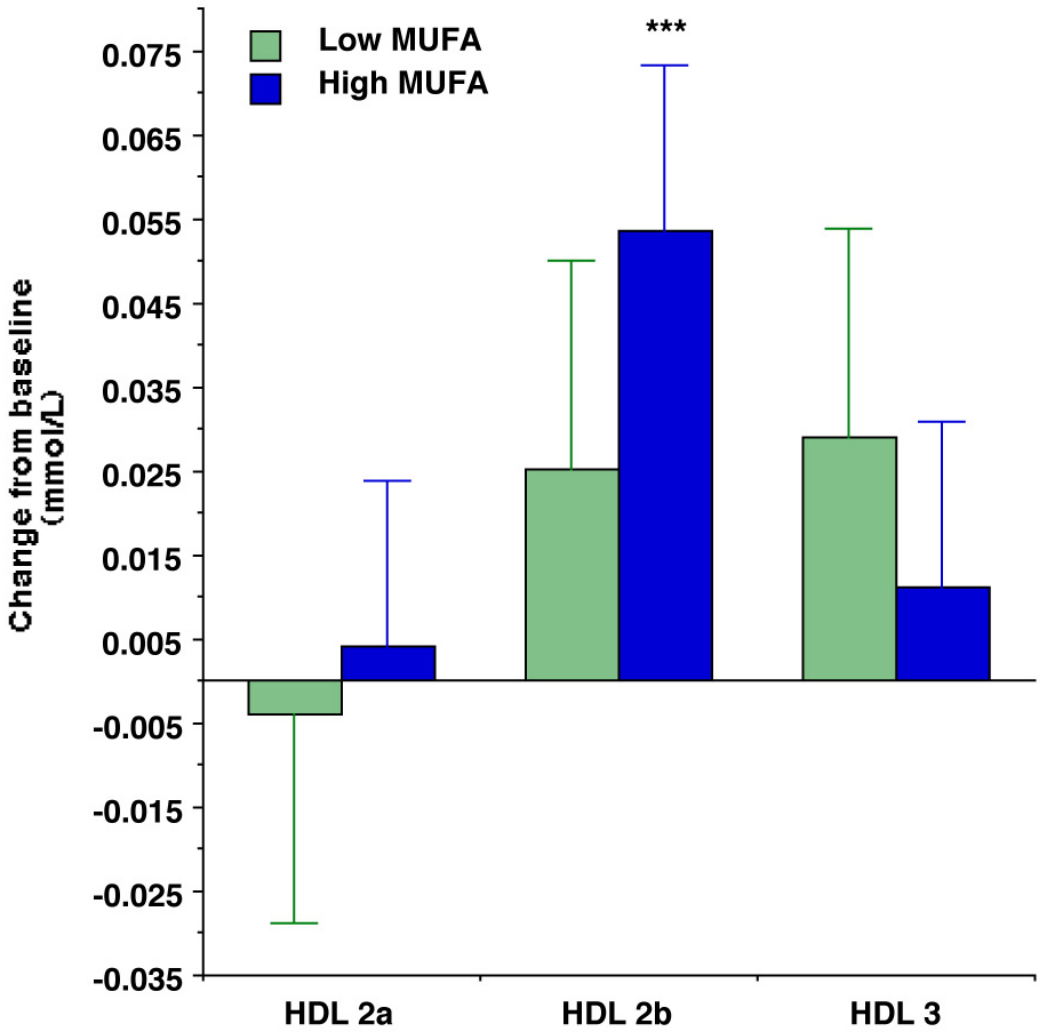

FIGURE 7 Changes from baseline in HDL subfractions cholesterol concentrations (mmol/L) of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, $n = 19$. *** $P \leq 0.01$

TABLE 13 HDL subfraction cholesterol concentrations for women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

Lipoprotein	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
HDL_{2a}, mmol/L	0.233 ± 0.02	0.2285 ± 0.02	0.2368 ± 0.02	0.2409 ± 0.02
Bouyant HDL_{2b}, mmol/L	0.6484 ± 0.05	0.6732 ± 0.04	0.6369 ± 0.05	0.6909 ± 0.05
HDL_3, mmol/L	0.6743 ± 0.02	0.7031 ± 0.02	0.6892 ± 0.02	0.7004 ± 0.02***

[1] Data are means ± SE for 19 women.
*** $P \leq 0.01$

VLDL and RLP particle concentration mirrored VLDL and RLP cholesterol increase with low-MUFA patty consumption. High-MUFA patty consumption did not have an effect on VLDL and RLP particle concentration (Figure 8 and Table 14).

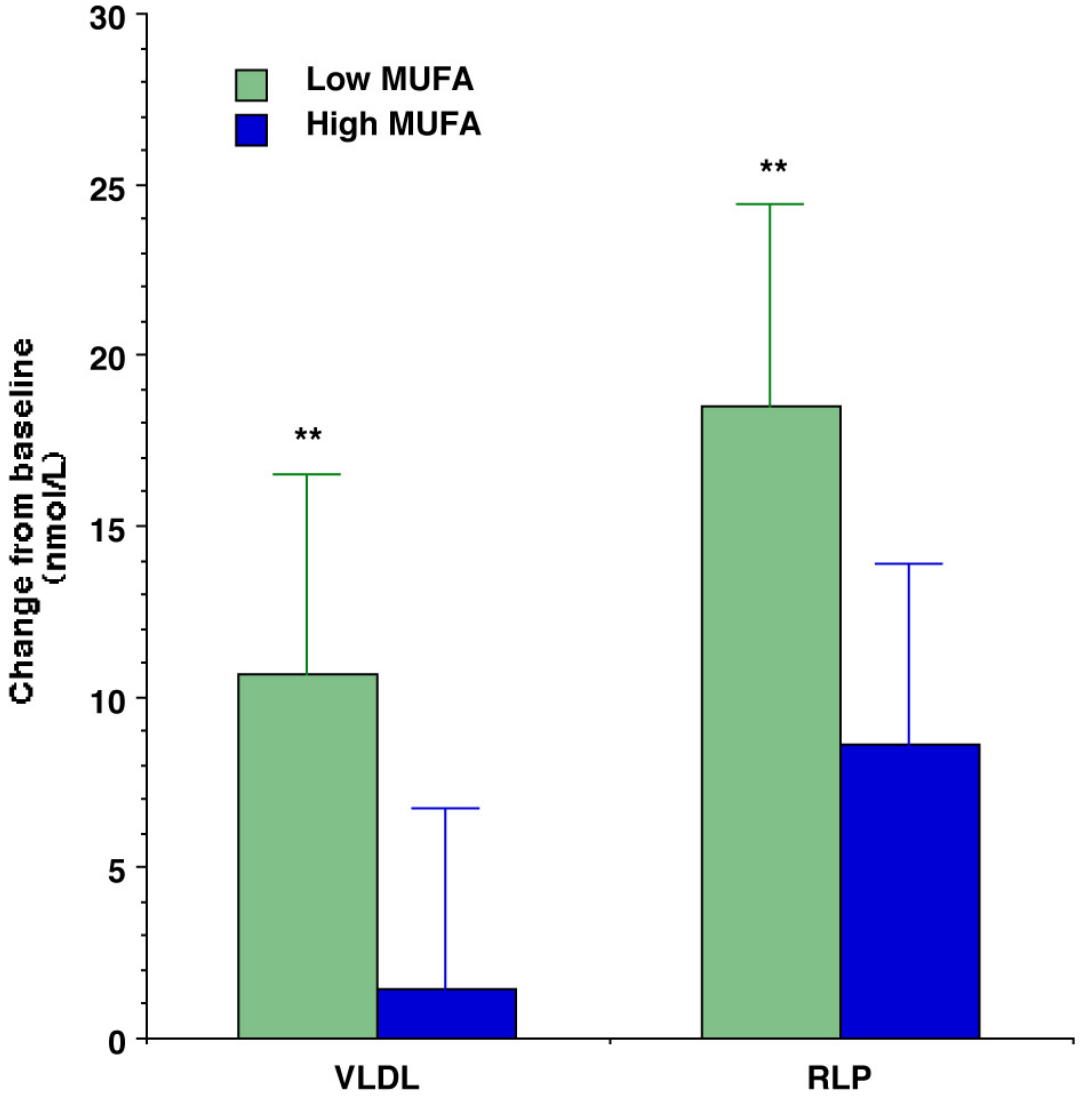

FIGURE 8 Changes from baseline in VLDL and RLP particle concentration (nmol/L) of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, $n = 19$. $^{**}P \leq 0.05$

TABLE 14 VLDL and RLP particle concentrations for women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

Lipoprotein	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
VLDL, nmol/L	42.37 ± 4.59	53 ± 5.05**	49.79 ± 4.34	51.26 ± 6.72
RLP, nmol/L	120.68 ± 6.7	139.21 ± 8.81**	128.84 ± 9.26	137.47 ± 11.03

[1] Data are means ± SE for 19 women.
** $P \leq 0.05$

Though LDL cholesterol was increased with the high-MUFA intervention, LDL, LDL III and LDL IV particle concentration, were not significantly increased (Figure 9 and Table 15). The low-MUFA intervention did not have any effect on LDL or LDL subfraction particle concentrations.

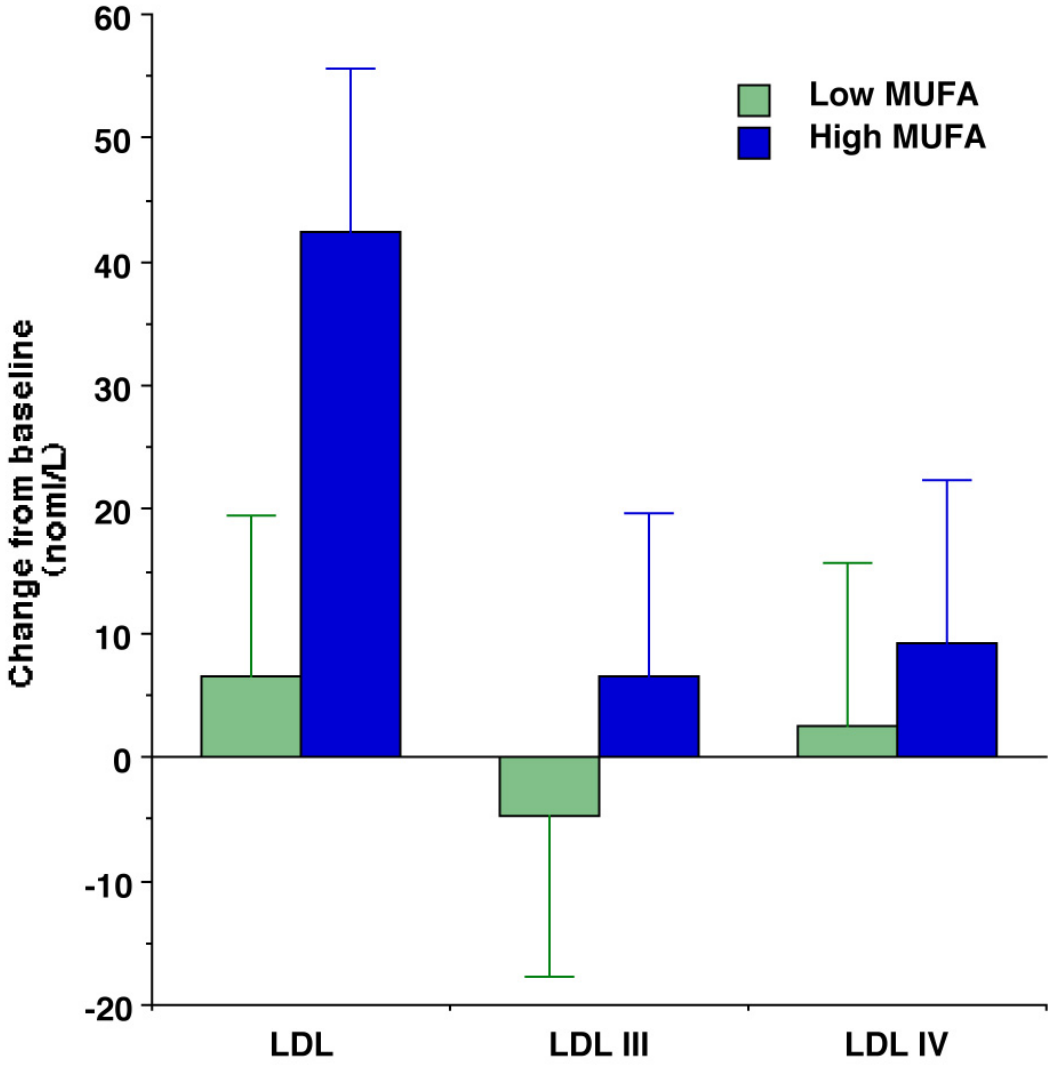

FIGURE 9 Changes from baseline in LDL subfraction particle concentration (nmol/L) of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, *n* = 19.

TABLE 15 LDL and LDL subfraction particle concentrations for women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

Lipoprotein	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
LDL Total, nmol/L	835 ± 44	842 ± 36	824 ± 46	867 ± 41
Dense LDL III, nmol/L	201 ± 15	196 ± 9.7	186 ± 16	192 ± 14
Dense LDL IV, nmol/L	81.6 ± 5.5	84.2 ± 5.2	80.3 ± 4.5	89.5 ± 7.0

[1] Data are means ± SE for 19 women.
There were no significant differences among phases ($P > 0.1$)

Total HDL and buoyant HDL_{2b} particle concentration increased with the consumption of the high-MUFA patties, while there were no significant change in HDL and HDL subfraction particle concentrations (Figure 10 and Table 16).

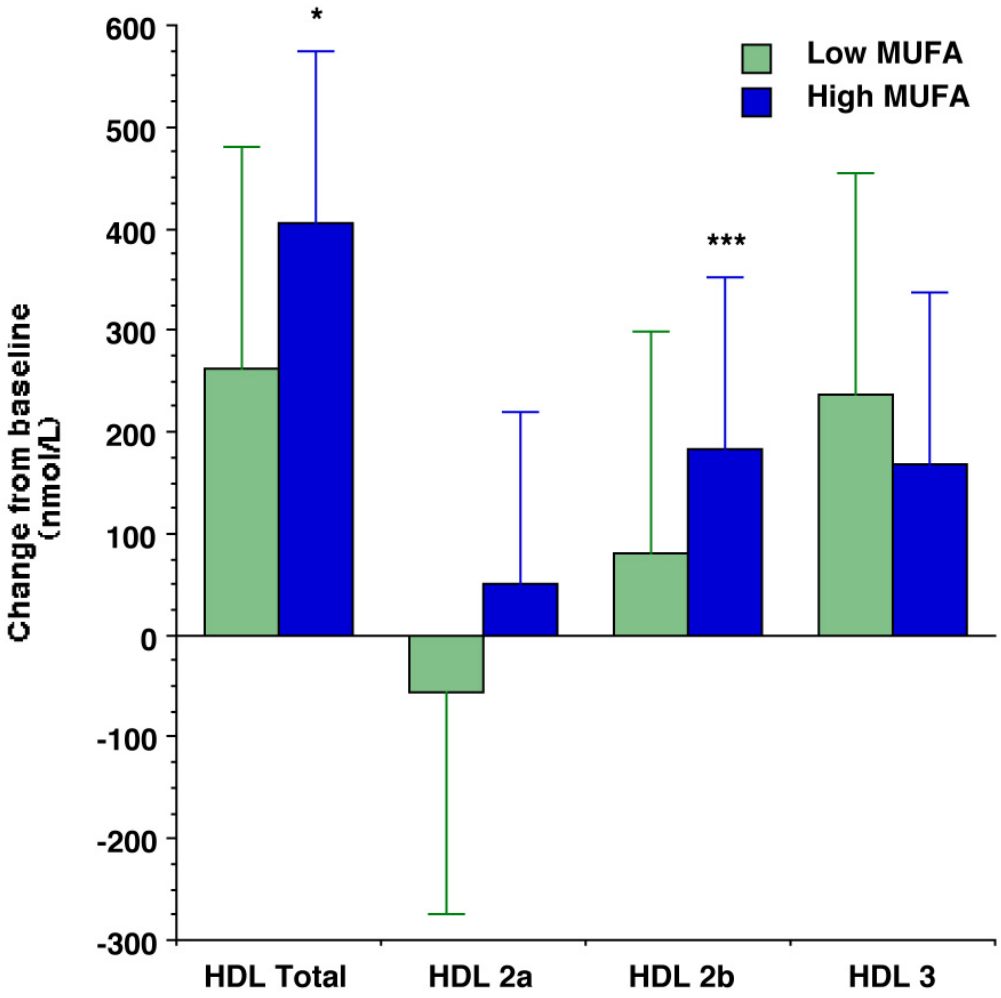

FIGURE 10 Changes from baseline in HDL particle concentration (nmol/L) of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, $n = 19$. * $P \leq 0.10$; *** $P \leq 0.01$

TABLE 16 HDL particle concentrations for women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
HDL Total, nmol/L	10,648 ± 355	10,911 ± 370	10,723 ± 377*	11,129 ± 372
HDL_{2a}, nmol/L	2,141 ± 151	2,085 ± 167	2,160 ± 183	2,212 ± 179
Bouyant HDL_{3b}, nmol/L	2,246 ± 172	2,327.05 ± 13	2,212 ± 181***	2,397 ± 165
HDL_3, nmol/L	6,262 ± 206	6,499 ± 254	6,351 ± 228	6,520 ± 219

[1] Data are means ± SE for 19 women.
* $P \leq 0.10$; *** $P \leq 0.01$

No significant differences were seen for inflammatory markers, C-reactive protein (hs-CRP) or homocystine. Insulin also remained unchanged (Table 17).

TABLE 17 C-reactive protein, homocysteine, and insulin levels pre- and post-test patty consumption[1]

	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
hs-CRP, mg/L	2.33 ± 0.51	2.57 ± 0.53	3.59 ± 0.74	2.45 ± 0.59
Insulin, uIU/ml	7.25 ± 0.85	7.31 ± 0.79	7.05 ± 0.85	7.49 ± 0.83
Homocysteine, µmol/L	9.8 ± 0.56	9.73 ± 0.57	9.61 ± 0.48	10.13 ± 0.63

[1] Data are means ± SE for 19 women.
There were no significant differences among phases ($P > 0.1$)

Along with lipoprotein concentration, particle density and size was also measured, but no significant changes were seen (Table 18).

TABLE 18 Particle density and size pre- and post-test patty consumption[1]

	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
HDL mean density	1.0922 ± 0.0008	1.0921 ± 0.0008	1.0928 ± 0.0011	1.0917 ± 0.0009
LDL mean density	1.0294 ± 0.0002	1.0296 ± 0.0002	1.0292 ± 0.0002	1.0295 ± 0.0002
LDL mean size, nm	20.18 ± 0.02	20.16 ± 0.02	20.22 ± 0.03	20.2 ± 0.02

[1] Data are means ± SE for 19 women.
There were no significant differences among phases ($P > 0.1$)

Major plasma fatty acids were unchanged due to patty consumption (Figure 11 and Table 19), while long chain fatty acids were consistently depressed by the low-MUFA test patties (Figure 12 and Table 19). *trans*-Vaccenic acid (trans-aa) increased with the high-MUFA patties and *trans*-10, *cis*-12 conjugated linolenic acid (t10,c12 CLA) increased with the low-MUFA test patties (Figure 13 and Table 19).

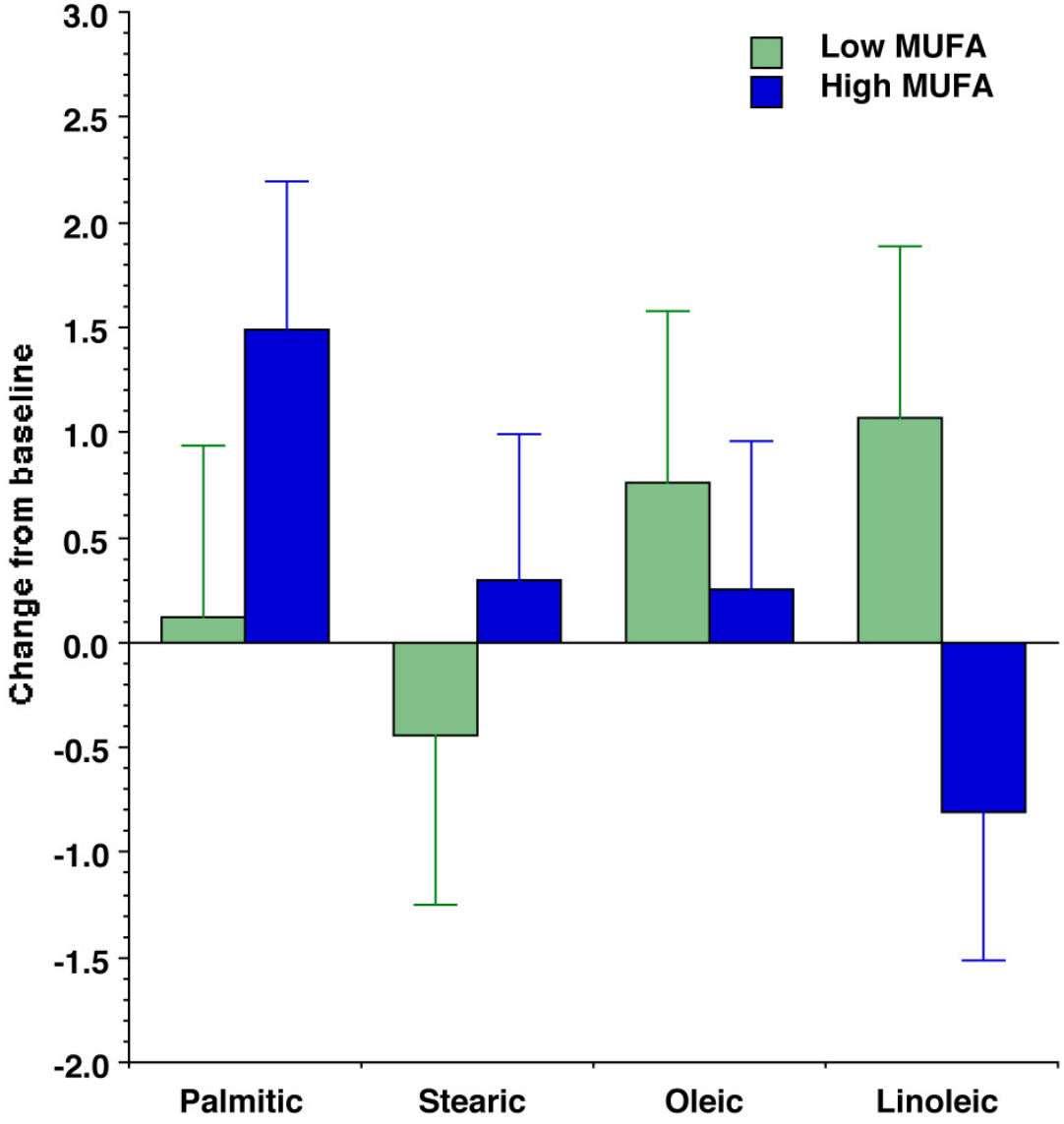

FIGURE 11 Changes from baseline in major plasma fatty acids of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, *n* = 19.

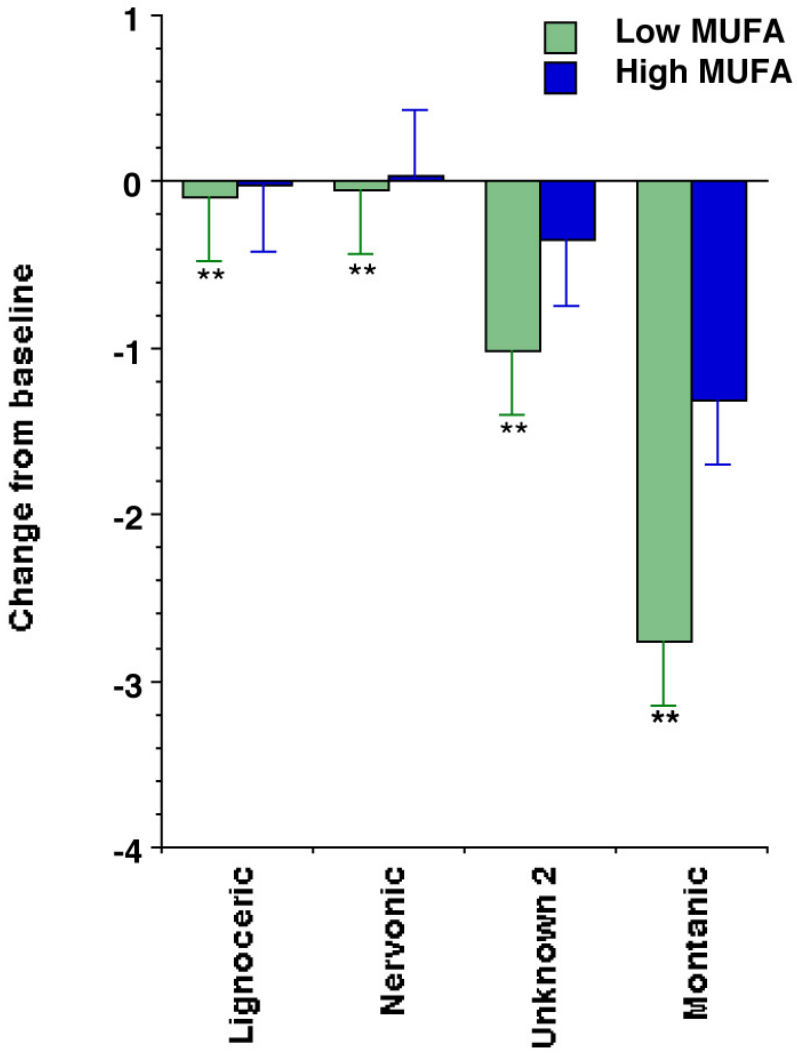

FIGURE 12 Changes from baseline in long chain plasma fatty acids of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, $n = 19$. ** $P \leq 0.05$

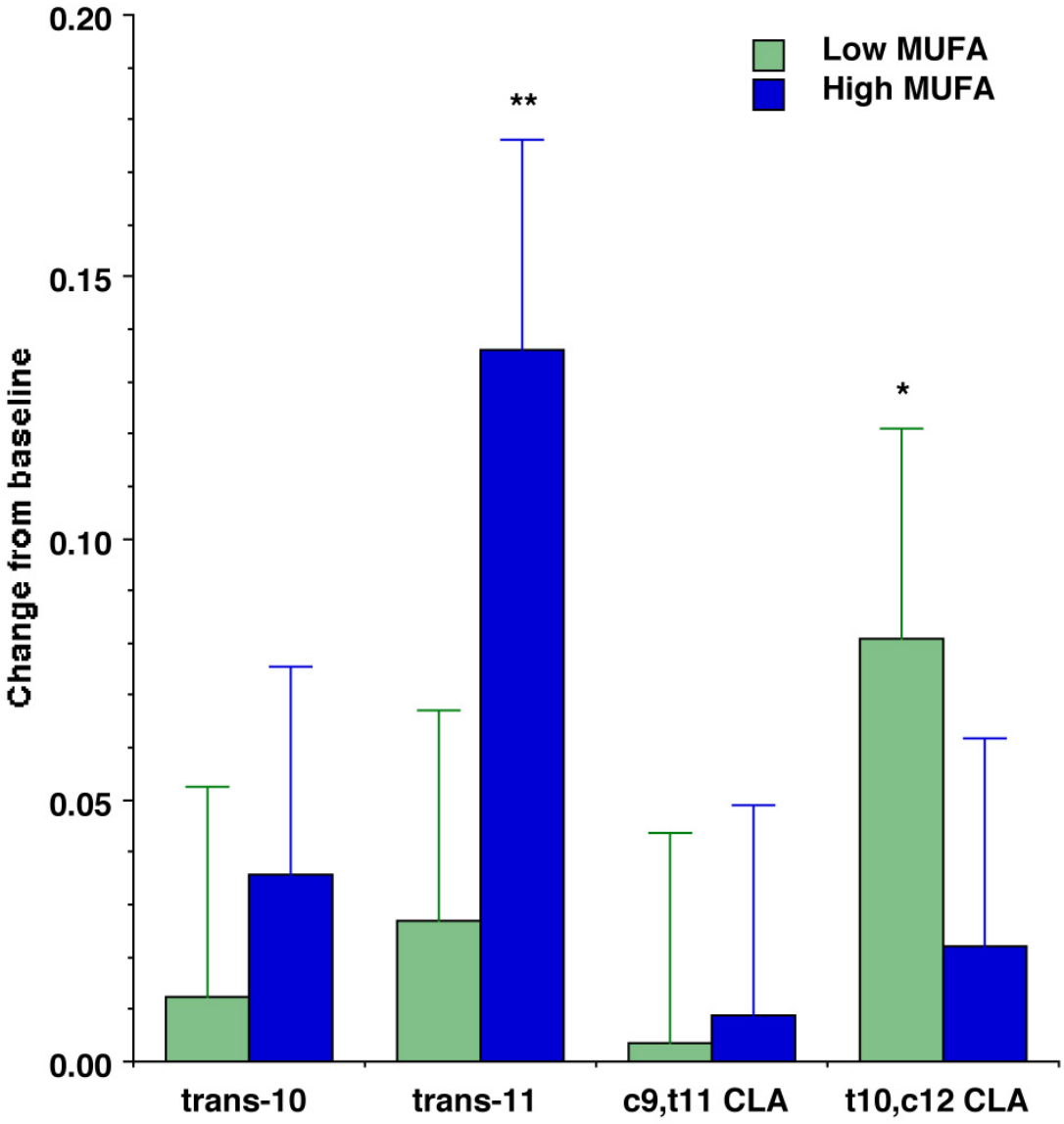

FIGURE 13 Changes from baseline in *trans* plasma fatty acids of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA). Values are expressed as mean ± SEM, $n = 19$. * $P \leq 0.10$; ** $P \leq 0.05$

TABLE 19 Plasma fatty acid concentrations (g/100 g fatty acids) of women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

Fatty Acid	Pre Low MUFA	Post Low MUFA	Pre High MUFA	Post High MUFA
14:0	0.34± 0.05	0.33 ± 0.04	0.39 ± 0.05	0.45 ± 0.06
14:1	0.06 ± 0.02	0.08 ± 0.03**	0.05 ± 0.02	0.08 ± 0.02
16:0	18.15 ± 0.79	18.27 ± 0.47	17.12 ± 1.07	18.61 ± 0.59
16:1	0.88 ± 0.19	2.07 ± 1.09	1.25 ± 0.23	1.11± 0.23
18:0	8.63 ± 0.61	8.19 ± 0.27	8.10 ± 0.19	8.39 ± 0.31
18:1t10	0.13 ± 0.05	0.14 ± 0.06	0.08 ± 0.03	0.11± 0.04
18:1t11	0.18 ± 0.05	0.21 ± 0.06	0.11 ± 0.03	0.24 ± 0.06**
18:1	17.82 ± 0.67	18.59 ± 0.59	19.0 ± 0.45	19.25 ± 0.62
18:1c11	1.38 ± 0.08	1.39 ± 0.11	1.44 ± 0.07	1.45 ± 0.05
18:2	31.20 ± 1.1	32.27 ± 1.02	32.98 ± 0.78	32.17 ± 1.11
18:3	0.54 ± 0.06	0.43 ± 0.06	0.48 ± 0.06	0.51 ± 0.05
18:2c9t11	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.02

TABLE 19 continued

Fatty Acid	Pre Low MUFA	Post Low MUFA	Pre High MUFA	Post High MUFA
18:2t10c12	0.06 ± 0.02	0.14 ± 0.04*	0.06 ± 0.02	0.08 ± 0.03
20:2	0.35 ± 0.2	0.80 ± 0.35	0.56± 0.31	0.68 ± 0.29
Unkn 1	2.03 ± 0.24	1.88 ± 0.11	1.88 ± 0.1	1.84 ± 0.11
20:4	7.42 ± 0.46	7.80 ± 0.49	7.50± 0.38	7.36 ± 0.6541
22:0	0.21 ± 0.12	0.45 ± 0.2	0.65± 0.3	0.34 ± 0.2*
20:5	0.44 ± 0.08	0.48 ± 0.07	0.49 ± 0.07	0.56 ± 0.07
24:0	0.31 ± 0.04	0.21 ± 0.04***	0.29 ± 0.04	0.26 ± 0.04
24:1	0.12 ± 0.03	0.05 ± 0.02**	0.05 ± 0.02	0.08 ± 0.02
22:6	1.41 ± 0.16	1.20 ± 0.14	1.23 ± 0.14	1.26 ± 0.09
Unkn 2	2.09 ± 0.57	1.06 ± 0.35**	1.62 ± 0.41	1.26 ± 0.35
28:0	6.93 ± 1.47	4.16 ± 0.93**	6.17 ± 1.15	4.85 ± 1.04

[1] Data are means ± SE for 19 women.

* $P \leq 0.10$; ** $P \leq 0.05$; ***$P \leq 0.01$

Tables 20 and 21 present correlations between plasma fatty acids and lipoprotein cholesterol concentrations, LDL particle and size, HDL particle density, triglycerides, insulin, homocysteine and hs-CRP. Palmitoleic acid was significantly increased with low-MUFA patty consumption. and was positively correlated with homocysteine, VLDL, RLP and IDL, but negatively correlated with Lp(a). t10c12 CLA also was increased with the low-MUFA intervention and was correlated positively with triglycerides, hs-crp, insulin, VLDL, RLP, and LDL and negatively correlated with HDL cholesterol. Plasma *trans*-vaccenic acid was increased with high-MUFA patty consumption, and was correlated positively with total cholesterol, LDL, VLDL, RLP, IDL, LDL III and LDL IV. The low-MUFA intervention decreased 24:0, 24:1, Unknown 2 and 28:0 plasma concentrations (Figure 12). Lignocenic acid (24:0) was negatively correlated with LDL IV and Lp(a), nervonic acid (24:1) was negatively correlated with triglycerides, LDL III, LDL IV, LDL mean density and Lp(a). Unknown 2 was negatively correlated with total cholesterol, LDL, LDL III, LDL IV, HDL 2b and Lp(a) and motanic acid (28:0) was negatively correlated with total cholesterol, LDL IV and HDL 2b and positively correlated with hs-CRP and insulin.

TABLE 20 Simple correlations between plasma fatty acids and cholesterol fractions, triglycerides, hs-CRP, insulin and homocysteine[1]

	Total Cholesterol	LDL	HDL	TAG	hs-CRP	Insulin	Homocysteine	VLDL	RLP	IDL
16:0	0.28	0.29	NC	NC	NC	NC	NC	NC	NC	NC
16:1	0.37	0.34	NC	NC	NC	NC	0.39	0.23	0.23	NC
18:1	NC	0.22	NC	0.44	NC	NC	NC	0.35	0.29	0.28
18:1t11	NC	NC	NC	NC	NC	NC	NC	0.24	0.26	0.28
18:1c11	0.33	0.33	NC	0.28	NC	NC	NC	NC	NC	NC
18:2	NC	NC	0.27	-0.23	-0.30	NC	NC	-0.29	NC	NC
18:2 c9t11	NC	NC	NC	NC	NC	0.23	NC	NC	NC	NC
18:2 t10c12	NC	NC	-0.24	0.42	0.27	0.29	NC	0.35	0.25	0.23
20:4	NC	NC	0.30	-0.47	-0.22	-0.27	NC	-0.32	NC	NC
22:6	NC	NC	NC	-0.28	NC	-0.26	-0.25	-0.35	-0.28	-0.27
28:0	-0.26	NC	NC	NC	0.25	0.23	NC	NC	NC	NC

[1]Correlations data pooled 76 measurements.
All r values stated are significant, $P \leq 0.05$; NC = no correlation

TABLE 21 Simple correlations between plasma fatty acids and cholesterol subfractions, HDL mean density, LDL particle size and density[1]

	LDL III	LDL IV	HDL 2b	HDL 2a	HDL 3	HDL mean density	LDL mean density	LDL mean size	Lp(a)
16:0	0.27	NC	NC	NC	NC	NC	NC	NC	NC
16:1	0.30	NC	NC	NC	0.23	NC	NC	NC	NC
18:0	NC	NC	NC	NC	NC	NC	-0.23	NC	0.34
18:1t10	NC	NC	NC	NC	0.24	NC	NC	NC	NC
18:1t11	NC	-0.31	NC	0.25	NC	NC	NC	NC	NC
18:1c11	0.45	0.38	NC	NC	NC	NC	NC	NC	NC
18:2	NC	NC	0.30	NC	NC	NC	NC	NC	NC
20:4	NC	NC	0.32	NC	NC	NC	NC	NC	NC
28:0	NC	-0.25	-0.24	NC	NC	NC	NC	NC	NC

[1]Correlations data pooled 76 measurements.
All r values stated are significant, $P \leq 0.05$; NC = no correlation

Discussion

This study focused on the relationship between the ground beef MUFA:SFA ratio impact on total lipoproteins, lipoprotein subfractions and inflammatory markers associated with increased CVD risk. LDL and HDL particle diameter measurement were used to identify the presence of particularly atherogenic LDL or antiatherogenic HDL. Small, dense LDL are recognized as a risk factor for CVD, as this form of LDL is more susceptible to oxidative damage (6, 159) and promotes vascular inflammation (160, 161). Measurement of HDL particle diameter is important, as it can be diagnostic of metabolic changes leading to small dense LDL and the antioxidative capacity of HDL (160).

Because of recent advances in understanding of how inflammation instigates CVD (6), we also measured hs-CRP (162). Although a cross-sectional study showed a positive association between red meat and hs-CRP (163), the current intervention study showed no effect of ground beef consumption and hs-CRP levels.

The high-MUFA ground beef contained nearly 4 g more MUFA per patty than did the low-MUFA ground beef, mainly in the form of oleic acid. Each participant on the high-MUFA diet consumed nearly 20 g more MUFA per week than those consuming the low-MUFA ground beef. This pattern of intake was documented in the dietary records. We chose not to strictly control dietary intake of nutrients by participants. We stipulated only that the ground beef patties replaced an equal portion of meat (beef or otherwise) that they normally would have consumed. This encouraged compliance and allowed us to evaluate the effects of the test ground beef patties in a more natural, free-living

setting. The data indicate that the increase in HDL-C without a significant increase in LDL cholesterol was due to the increased consumption of MUFA, probably oleic acid.

Changes in plasma fatty acids supported the diet dependent changes in lipoprotein metabolism. Overall the low-MUFA intervention negatively impacted plasma triglycerides and increased 14:1 and t10c12 CLA, and decreased 24:0, 24:1, Unknown 2, and 28:0. For the most part these changes in plasma fatty acids correlated with undesirable effects such as increased VLDL, RLP, IDL, triglycerides, total cholesterol and decreased HDL-C although not all were observed after the low-MUFA intervention. It is possible that the increase in HDL-Cl, specifically HDL_{2b}, seen with the high-MUFA intervention was due in part to the increase in plasma 18:1t11 and decrease in 22:0, as these fatty acids were positively correlated with HDL-C. Few studies have identified 28:0 in plasma, but the negative correlation seen between 28:0 (and Unknown 2, a possible desaturation product of 28:0) and total cholesterol is consistent with the cholesterol lowering effects of D003. D003 is a mixture of very long chain fatty acids from sugar cane wax with octaconsanoic acid (28:0) as its main component. This mixture inhibits cholesterol metabolism in fibroblasts by modulating 3-hydroxy-3-methyl coenzyme A (HMG-CoA) reductase activity and possibly HMG-CoA synthase activity (164).

We recently reported the effects of two high-fat, hamburger preparations on cholesterol metabolism in a group of mildly hypercholesterolemic men (131). In that study, 10 men were first fed 35% fat hamburger high in SFA and *trans*-fat (MUFA:SFA = 0.95), and then were rotated to 35% hamburger high in MUFA (MUFA:SFA = 1.31).

As in the current study, neither of the test hamburger patties increased LDL-C. However, the high-SFA hamburger significantly reduced HDL-C and increased plasma TAG; these values returned to baseline values after consumption of the high-MUFA hamburger. The high-SFA ground beef also decreased LDL particle diameter, which was reversed by the high-MUFA ground beef. In this study, neither the low-MUFA ground beef nor the high-MUFA ground beef affected LDL diameters (nm), density (g/gm^3), or concentration (mmol/dL), which indicates that neither ground beef type affected LDL risk factors for CVD. However, the low-MUFA ground beef increased the concentrations of VLDL and RLP particles, effects that were not observed in women who consumed high-MUFA ground beef. RLP cholesterol is derived from VLDL remnants in fasting humans (165), and an increase in the concentration of RLP particles is considered a risk factor for CVD (166, 167). A unique and positive finding of this study is that the high-MUFA ground beef increased buoyant HDL_{2b} cholesterol concentration and HDL_{2b} particle concentration. There is convincing evidence that, of the subclasses of HDL particles, HDL_{2b} cholesterol is cardioprotective because patients with premature CVD have reduced HDL_{2b} (168), and families with low HDL_{2b} have increased carotid intima-media thickness (associated with CVD) (27). Thus, high-MUFA ground beef not only increased HDL cholesterol, it increased the most cardioprotective subclass of HDL particles.

CHAPTER IV

HIGH-OLEIC GROUND BEEF, EXERCISE, AND RISK FACTORS FOR CARDIOVASCULAR DISEASE IN POSTMENOPAUSAL WOMEN

Introduction

Postmenopausal women are at high risk for cardiovascular disease (CVD) (169, 170). Dietary interventions with low total fat consumption in postmenopausal women have not demonstrated significant reduction in CVD risk (171). While optimal dietary content continues to be debated and red meat consumption is discourage by the American Heart Association, the major monounsaturated fatty acids (MUFA) in ground beef, oleic acid, has been studied in more detail and found to lower LDL-cholesterol without affecting the beneficial HDL-cholesterol (HDL-C) (125, 126). In contrast to wavering dietary recommendations, existing literature broadly supports the beneficial effects of a single session of exercise on blood lipids in postmenopausal women (62, 172). Exercise results in a decrease in LDL cholesterol (LDL-C) and an increase in HDL-C (118, 173, 174). Even a single session of endurance exercise can beneficially alter serum lipids (62, 175). Additionally, studies have found a reduction in hs-CRP and fasting glucose levels in response to exercise (162, 176, 177). Few studies to date have examined the metabolic and inflammatory response to exercise in combination with an increase in the monounsaturated:saturated fatty acid (MUFA:SFA) ratio of beef. Therefore, the objective of this study was to establish the relation between the

MUFA:SFA ratio of ground beef and the metabolic and inflammatory responses to a single session of aerobic exercise in postmenopausal women.

Methods

Subjects. Twenty-nine postmenopausal women were recruited from the local Bryan/College Station, Texas community. Nineteen women completed the diet portion of the study, while seventeen completed both the exercise and diet portions. Detailed subject information can be found in Chapter III.

Study design. Detailed study design can be found in Chapter III. Briefly, 17 women were allotted to groups for a crossover design. The participants were asked to consume one low- or high-MUFA patty 5d/wk during the 6 wk test periods. Patty composition is listed in Table 2.

Baseline testing. At study entrance, each subject underwent a complete history, a DEXA scan, height and body weight measurements, and peak oxygen consumption assessed with an automated metabolic gas-analysis system during a maximal effort graded exercise test according to the Bruce protocol (178). Heart rate and rhythm were monitored continuously throughout the graded exercise by a 12-lead electrocardiogram (EKG) and a rating of perceived exertion and manual blood pressures were obtained during the last 30 s of each stage and at maximal exercise. Review of graded exercise, EKG, and health history along with a physical exam were performed by a cardiologist.

Exercise component. At the completion of each diet phase and once in the washout period, the participants underwent a bout of exercise. After abstaining from any

physical exercise for at least 3 d and fasting for 12 h, each subject reported to the J.L. Huffines Institute at Texas A&M University for a 24-h pre-exercise blood sampling. The following day subjects returned to the laboratory to complete the sub-maximal, experimental exercise session. Specifically, the subjects were asked to walk on a motor-driven treadmill at 75% of their predetermined VO_{2peak} for the duration required to expend 500 kcal of energy. Heart rate was monitored continuously and expired gases were measured every 10 min of exercise with a portable metabolic system (Medical Graphics VO2000) to ensure that the prescribed intensity and caloric expenditure were maintained. The speed and grade of the treadmill was adjusted as necessary to maintain the required intensity and caloric expenditure. Twenty-four hours after the acute exercise session, fasting blood samples were obtained for post-exercise analysis.

Collection and handling of blood samples. Blood was collected from an arm vein prior to initiation of the dietary treatments, at the end of each diet phase 24 h before and after exercise. Plasma was harvested from the blood collected with 15% EDTA. Serum was harvested from the blood with clot separation. All serum and plasma samples were stored at -80°C.

Fatty acid composition of plasma and test ground beef. Fatty acids were measured in the baseline plasma, plasma taken after 6 wk of each test beef treatment, 24 h before and 24 h after exercise and expressed as a g/100 g total fatty acids analyzed (see Chapter III for procedure details).

Blood chemistry and complete blood count. Serum samples were sent on the same day as the draw to St. Joseph Regional Health Center Bryan, TX for a complete

chemistry profile. Whole blood collected in a vaccutainer tube treated with 15% EDTA was sent on the same day as the draw to St. Joseph Regional Health Center for a complete blood count. The complete blood count was performed on the blood draws done 24 h before and after exercise to calculate the plasma volume shift caused by the acute exercise session.

Lipoprotein analysis. Serum aliquots kept at -80°C were sent to SpectraCell Laboratories, Inc. (Houston, TX) for complete lipoprotein density and particle number analyses. A complete "Lipoprotein Particle Profile™" test was provided using the lipoprotein subgroup particle number analysis method (179).

Diet records. Prior to each diet phase, and once during each phase, participants completed a 4-d record (to include one weekend day). The diet records were analyzed for nutrient composition to establish baseline observations, and encourage compliance with total patty consumption requirement. The records were analyzed using Nutritionist Pro (Axxya Systems, Stafford, TX).

Statistical analysis. Data were analyzed as a paired *t*-test, comparing baseline (habitual) values to values after 6 wk on the test diet. Thus, each participant served as her own control. Because each participant randomly rotated through all three diets, reported values are means ± standard error of the mean (SE) for $n = 17$ observations.

Results

Diet records indicated a change in monounsaturated fatty acids with no difference in energy percentage from protein or carbohydrate (Table 22). Detailed diet record analysis can be found in Chapter III.

TABLE 22 Percent energy from fat, carbohydrate, and protein[1]

	Baseline	Low MUFA	High MUFA
Fat, %	34 ± 2.3	37 ± 1.6	37 ± 1.5
Carbohydrate, %	48 ± 2.7	44 ± 2.1	45 ± 2.5
Protein, %	17 ± 0.8	19 ± 0.9	18 ± 0.8

[1]Data were derived from 4-d, including one weekend day, diet records collected during each test period. Data for habitual diet were obtained at baseline before patty consumption began. Means ± SE for 19 women.

The average energy expenditure for the three acute exercise bouts were 502 ± 0.58 kcal which lasted an average of 77 ± 1.35 min; there were no significant differences in energy expenditure among bouts (Table 23).

TABLE 23 Average energy expenditure and exercise duration with each bout of exercise[1]

	Acute bout 1	Acute bout 2	Acute bout 3
Energy expenditure, kcals	501 ± 0.73	502 ± 0.54	503 ± 1.5
Duration, m	79 ± 2.7	77 ± 2.4	76 ± 2.1

[1]Data are means ± SE for 17 women.
There were no significant differences among phases ($P > 0.1$)

Plasma volume increased significantly between pre-exercise and 24-h post-exercise for acute bout during the high-MUFA intervention (Table 24). Therefore results are presented as raw data and those values based on plasma volume are also presented as shifted to account for plasma volume changes due to exercise.

TABLE 24 Plasma volume shifts due to exercise[1]

Exercise bout	Mean plasma shift, %
Exercise bout during Low-MUFA intervention	1.6 ± 0.92
Exercise bout during washout	2.4 ± 1.15
Exercise bout during high-MUFA intervention	3.6 ± 1**

[1]Data are means ± SE for 17 women.
**$P \leq 0.05$

Activity records reflected no significant change in activity throughout the three phases (Table 25).

TABLE 25 Average energy expenditure per day estimated from self reported 7-d activity records for each study phase[1]

	Phase 1	Phase 2	Phase 3
Energy expended, kcal/d	2,323 ± 85	2,399 ± 104	2,413 ± 110

[1]Data are means ± SE for 14 women.
There were no significant differences among phases ($P > 0.1$)

TABLE 26 Cholesterol concentrations in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

	Low MUFA pre-exercise	Low MUFA post-exercise	High MUFA pre-exercise	High MUFA post-exercise	Washout pre-exercise	Washout post-exercise
Raw total cholesterol, mmol/L	5.30 ± 0.17	5.31 ± 0.21	5.26 ± 0.22	5.18 ± 0.22	5.02 ± 0.15	5.09 ± 0.22
Adjusted total cholesterol, mmol/L	5.30 ± 0.17	5.39 ± 0.22	5.26 ± 0.22	5.36 ± 0.22	5.02 ± 0.15	5.21 ± 0.22
Raw LDL-C, mmol/L	3.30 ± 0.15	3.45 ± 0.19	3.34 ± 0.17	3.36 ± 0.16	3.16 ± 0.14	3.26 ± 0.18
Adjusted LDL-C, mmol/L	3.30 ± 0.15	3.50 ± 0.19*	3.34 ± 0.17	3.48 ± 0.18*	3.16 ± 0.14	3.34 ± 0.18*
Raw HDL-C, mmol/L	1.65 ± 0.06	1.58 ± 0.07	1.64 ± 0.08	1.62 ± 0.05	1.53 ± 0.06	1.51 ± 0.06
Adjusted HDL-C, mmol/L	1.65 ± 0.06	1.61 ± 0.07	1.64 ± 0.08	1.68 ± 0.05	1.53 ± 0.06	1.54 ± 0.06

[1]Data are means ± SE for 17 women.
*$P \leq 0.1$

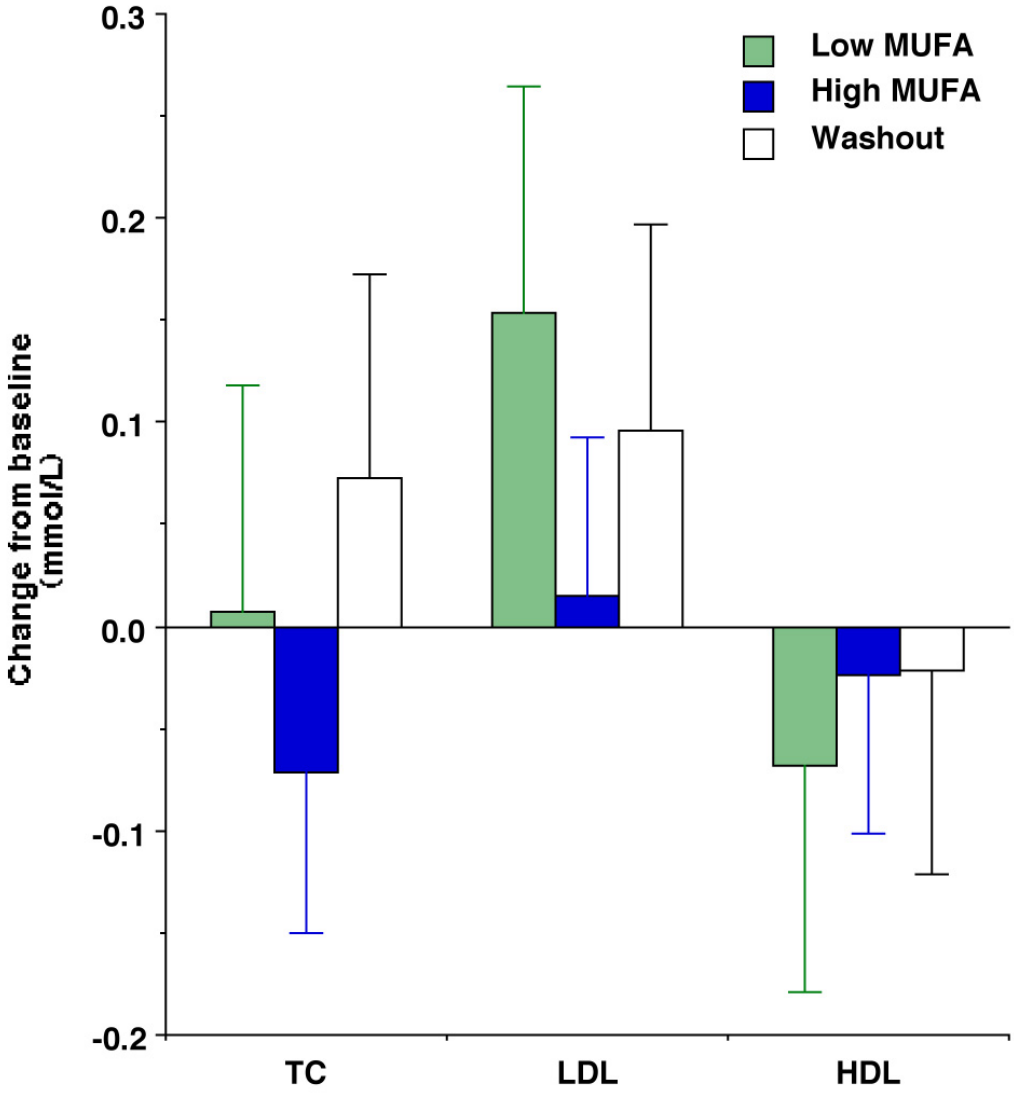

FIGURE 14 Changes from baseline in cholesterol (mmol/L) in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, *n* = 17.

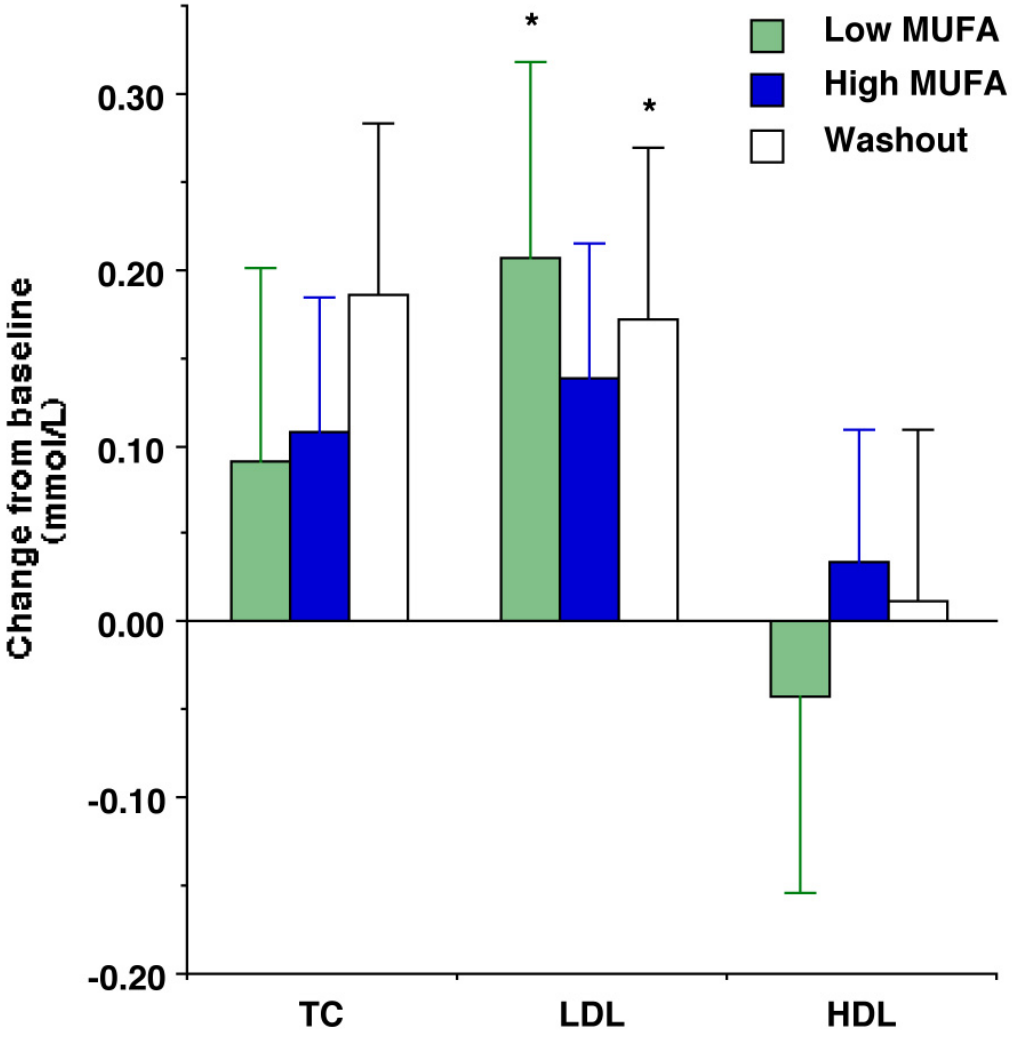

FIGURE 15 Changes from baseline in cholesterol (mmol/L) when adjusted for plasma volume shifts of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, $n = 17$. $*P \leq 0.01$.

The exercise bouts had no significant effect on raw total cholesterol, LDL-C, or HDL-C, but when values are adjusted for plasma volume shifts, an increase in LDL-C 24 h after the exercise bout was seen during the low-MUFA intervention and the washout period (Table 26; Figures 14 and 15).

Raw triglycerides were decreased after each exercise bout with the greatest response during the washout period. After adjustment for plasma volume shifts only the decrease in triglycerides seen after exercise during the washout period remained significant. Both raw and shifted VLDL cholesterol levels showed a significant ground beef effect as levels were decreased after exercise only during ground beef intervention. Raw RLP cholesterol was decreased after exercise during the high-MUFA intervention, but this effect was not seen when values were adjusted for plasma volume shifts. No changes in IDL cholesterol were observed after exercise (Table 27; Figures 16 and 17).

Both raw and shifted LDL III and LDL IV cholesterol were left unchanged after the exercise bout in all phases. When adjusted for plasma volume shifts, Lp(a) was significantly increased after exercise during the washout phase and the high-MUFA intervention (Table 28; Figures 18 and 19).

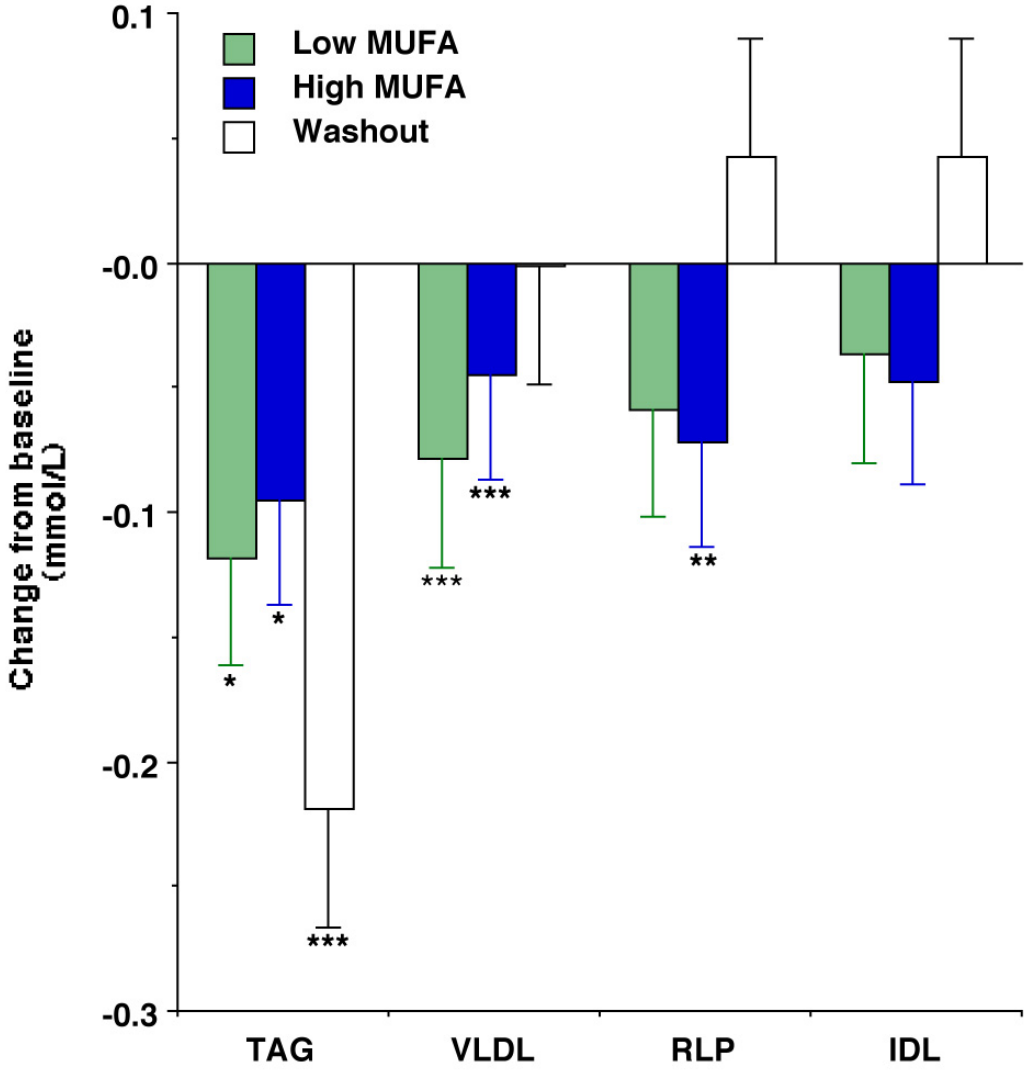

FIGURE 16 Changes from baseline in triglycerides (TAG), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL), and remnant lipoprotein (RLP) cholesterol concentration (mmol/L) of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, $n = 17$. *$P < 0.01$; **$P \leq 0.05$; ***$P \leq 0.001$.

TABLE 27 TAG, VLDL, RLP, and IDL cholesterol concentrations of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

	Low MUFA pre-exercise	Low MUFA post-exercise	High MUFA pre-exercise	High MUFA post-exercise	Washout pre-exercise	Washout post-exercise
Raw TAG, mmol/L	1.06 ± 0.11	0.94 ± 0.1*	1.11 ± 0.11	1.01 ± 0.1*	1.16 ± 0.12	0.94 ± 0.07***
Adjusted TAG, mmol/L	1.06 ± 0.11	0.96 ± 0.1	1.11 ± 0.11	1.05 ± 0.11	1.16 ± 0.12	0.96 ± 0.07**
Raw VLDL, mmol/L	0.35 ± 0.04	0.27 ± 0.03***	0.38 ± 0.05	0.31 ± 0.05***	0.32 ± 0.03	0.32 ± 0.04
Adjusted VLDL, mmol/L	0.35 ± 0.04	0.28 ± 0.03***	0.36 ± 0.05	0.32 ± 0.0487	0.32 ± 0.03	0.33 ± 0.04
Raw RLP, mmol/L	0.86 ± 0.06	0.80 ± 0.06	0.86 ± 0.08	0.79 ± 0.08**	0.72 ± 0.05	0.76 ± 0.09
Adjusted RLP, mmol/L	0.86 ± 0.06	0.81 ± 0.06	0.86 ± 0.08	0.81 ± 0.08	0.72 ± 0.05	0.78 ± 0.08
Raw IDL, mmol/L	0.75 ± 0.05	0.71 ± 0.05	0.75 ± 0.06	0.70 ± 0.07	0.66 ± 0.04	0.71 ± 0.07
Adjusted IDL, mmol/L	0.75 ± 0.05	0.73 ± 0.05	0.75 ± 0.06	0.72 ± 0.07	0.66 ± 0.04	0.72 ± 0.07

[1]Data are means ± SE for 17 women.
*$P \leq 0.01$; **$P \leq 0.05$; ***$P \leq 0.01$

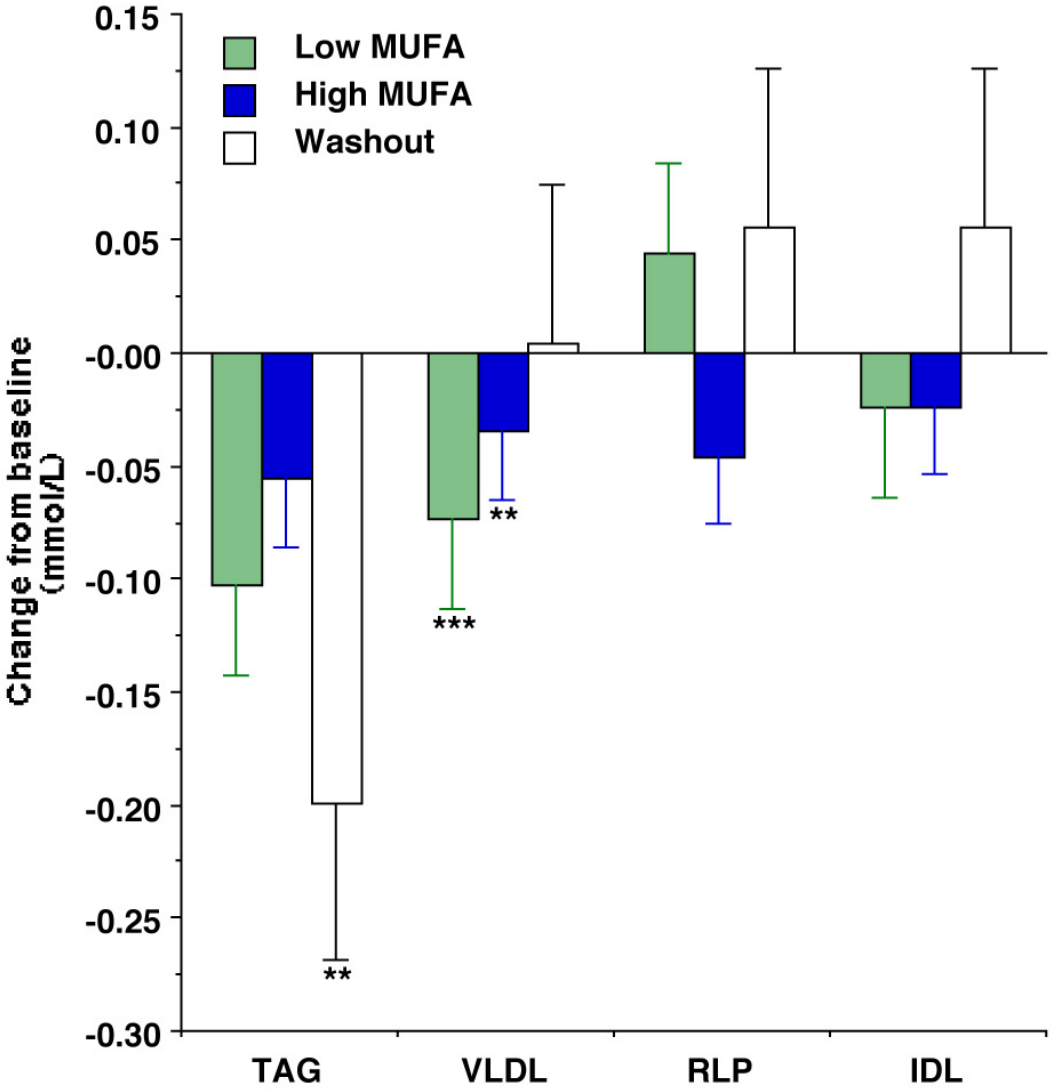

FIGURE 17 Changes from baseline in triglycerides (TAG), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL), and remnant lipoprotein (RLP) cholesterol concentration (mmol/L) when adjusted for plasma volume shift of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, $n = 17$. $^{**}P \leq 0.05$; $^{***}P \leq 0.001$.

TABLE 28 LDL subfraction cholesterol concentrations of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

	Low MUFA pre-exercise	Low MUFA post-exercise	High MUFA pre-exercise	High MUFA post-exercise	Washout pre-exercise	Washout post-exercise
Raw LDL III, mmol/L	0.54 ± 0.03	0.51 ± 0.03	0.53 ± 0.04	0.53 ± 0.03	0.51 ± 0.04	0.52 ± 0.03
Adjusted LDL III, mmol/L	0.54 ± 0.03	0.52 ± 0.03	0.53 ± 0.04	0.55± 0.03	0.51 ± 0.04	0.53 ± 0.03
Raw LDL IV, mmol/L	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
Adjusted LDL IV, mmol/L	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.20 ± 0.01	0.18 ± 0.01	0.19 ± 0.01
Raw Lp(a), mmol/L	0.71 ± 0.19	0.71± 0.19	0.67 ± 0.19	0.68 ± 0.18	0.69 ± 0.19	0.68 ± 0.18
Adjusted Lp(a), mmol/L	0.71 ± 0.19	0.72 ± 0.19	0.67 ± 0.19	0.71 ± 0.19**	0.69 ± 0.19	0.71 ± 0.19*

[1]Data are means ± SE for 17 women.
*$P \leq 0.1$; **$P \leq 0.05$

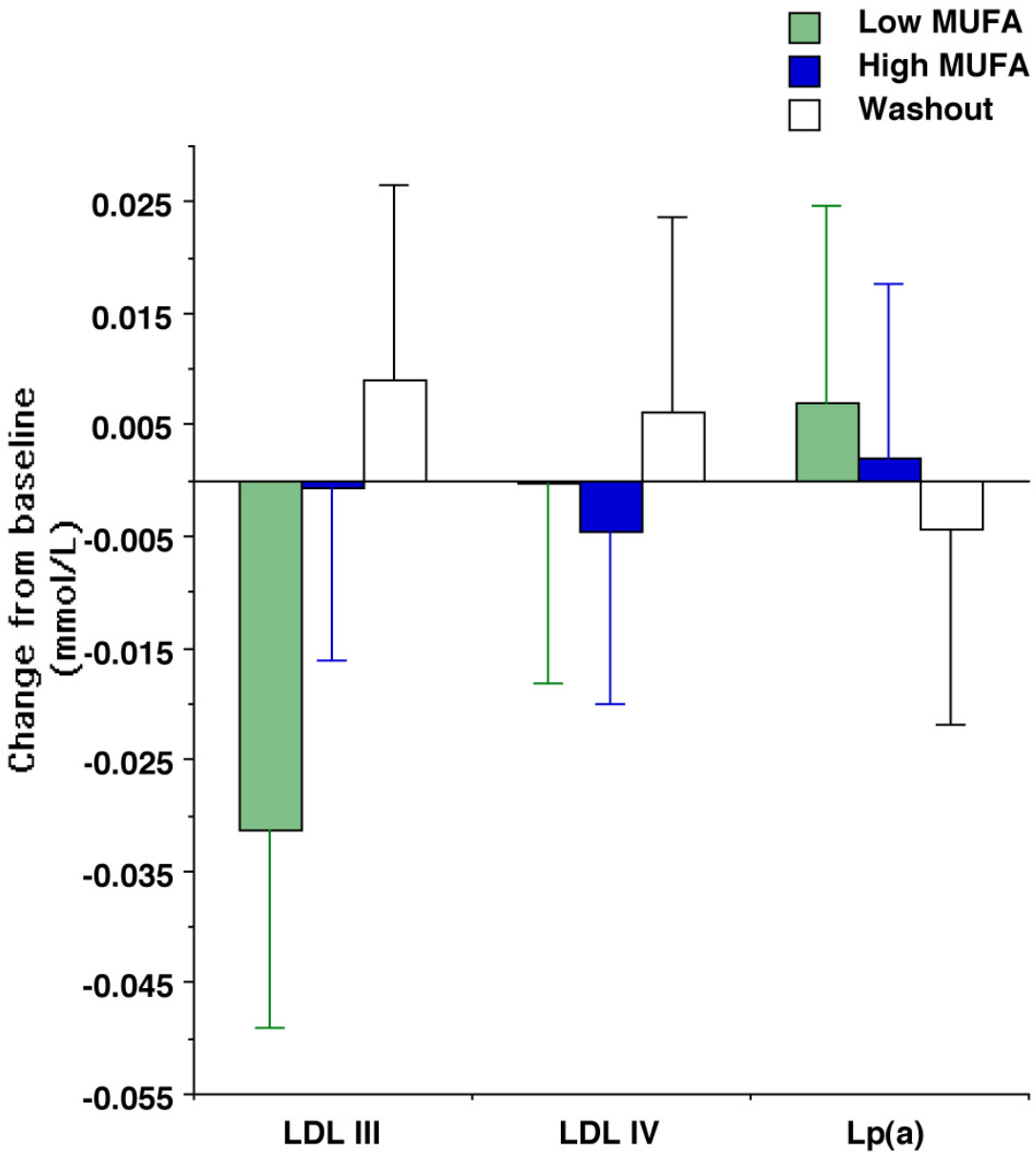

FIGURE 18 Changes from baseline in LDL subfraction cholesterol concentrations (mmol/L) of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, *n* = 17.

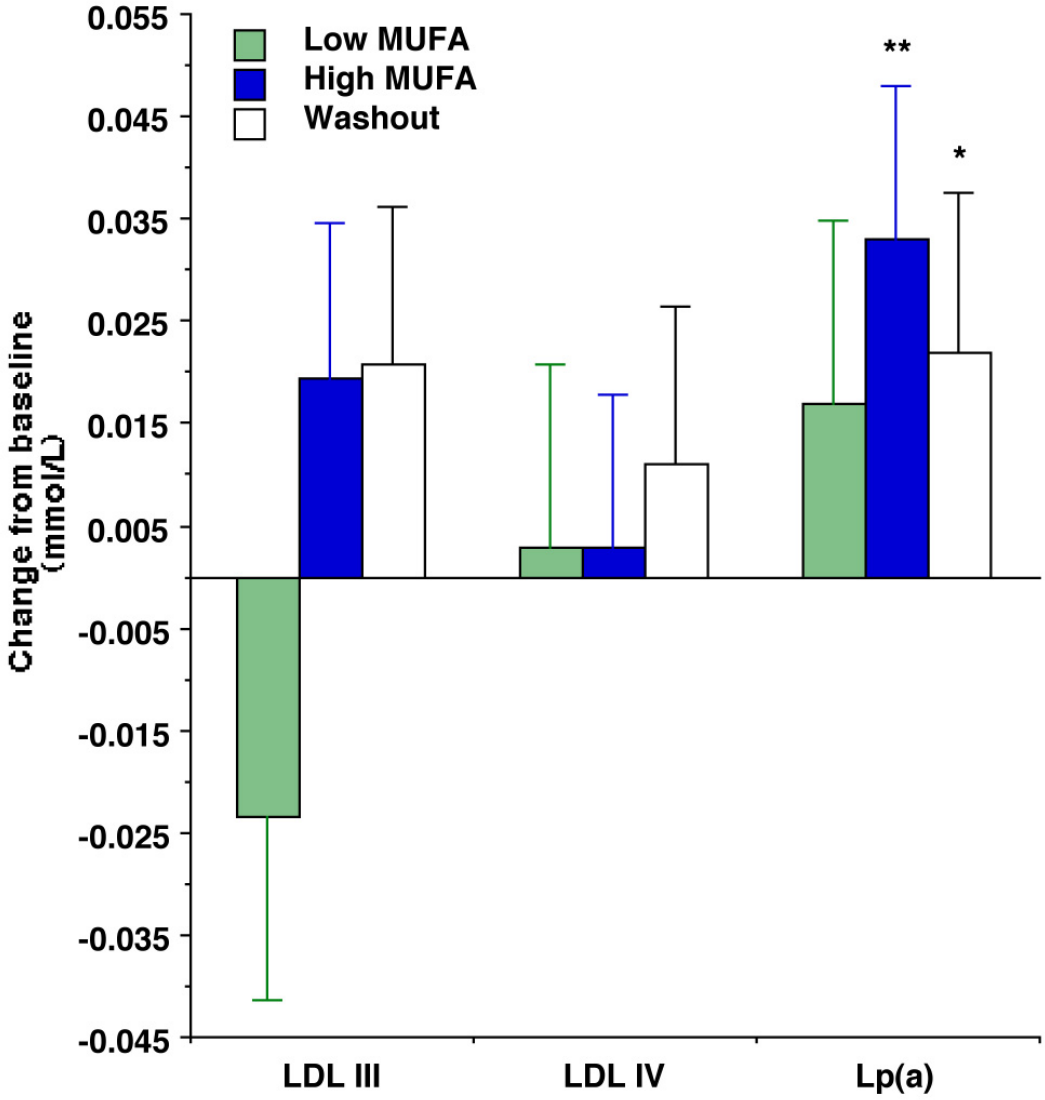

FIGURE 19 Changes from baseline in LDL subfraction cholesterol concentrations (mmol/L) when adjusted for plasma volume shift of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, *n* = 17. **P* ≤ 0.01; ***P* ≤ 0.05.

Raw buoyant HDL_{2b} was decreased as a result of a high-MUFA exercise interaction, but when adjusted for plasma volume shifts no effect of exercise was observed (Table 29; Figures 20 and 21).

TABLE 29 HDL subfraction cholesterol concentrations of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

	Low MUFA pre-exercise	Low MUFA post-exercise	High MUFA pre-exercise	High MUFA post-exercise	Washout pre-exercise	Washout post-exercise
Raw HDL_{2a}, mmol/L	0.24 ± 0.02	0.22 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.21 ± 0.02	0.20 ± 0.02
Adjusted HDL_{2a}, mmol/L	0.24 ± 0.02	0.23 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.21 ± 0.02	0.21 ± 0.02
Raw HDL_{2b}, mmol/L	0.70 ± 0.03	0.67 ± 0.04	0.72 ± 0.05	0.67 ± 0.04*	0.64 ± 0.05	0.63 ± 0.04
Adjusted HDL_{2b}, mmol/L	0.70 ± 0.03	0.68 ± 0.04	0.72 ± 0.05	0.70 ± 0.04	0.64 ± 0.05	0.64 ± 0.04
HDL_3, mmol/L	0.70 ± 0.03	0.69 ± 0.02	0.71 ± 0.02	0.70 ± 0.02	0.69 ± 0.02	0.67 ± 0.02
HDL_3, mmol/L	0.70± 0.03	0.70 ± 0.02	0.71 ± 0.02	0.72 ± 0.02	0.69± 0.02	0.69 ± 0.02

[1]Data are means ± SE for 17 women.
*$P \leq 0.01$

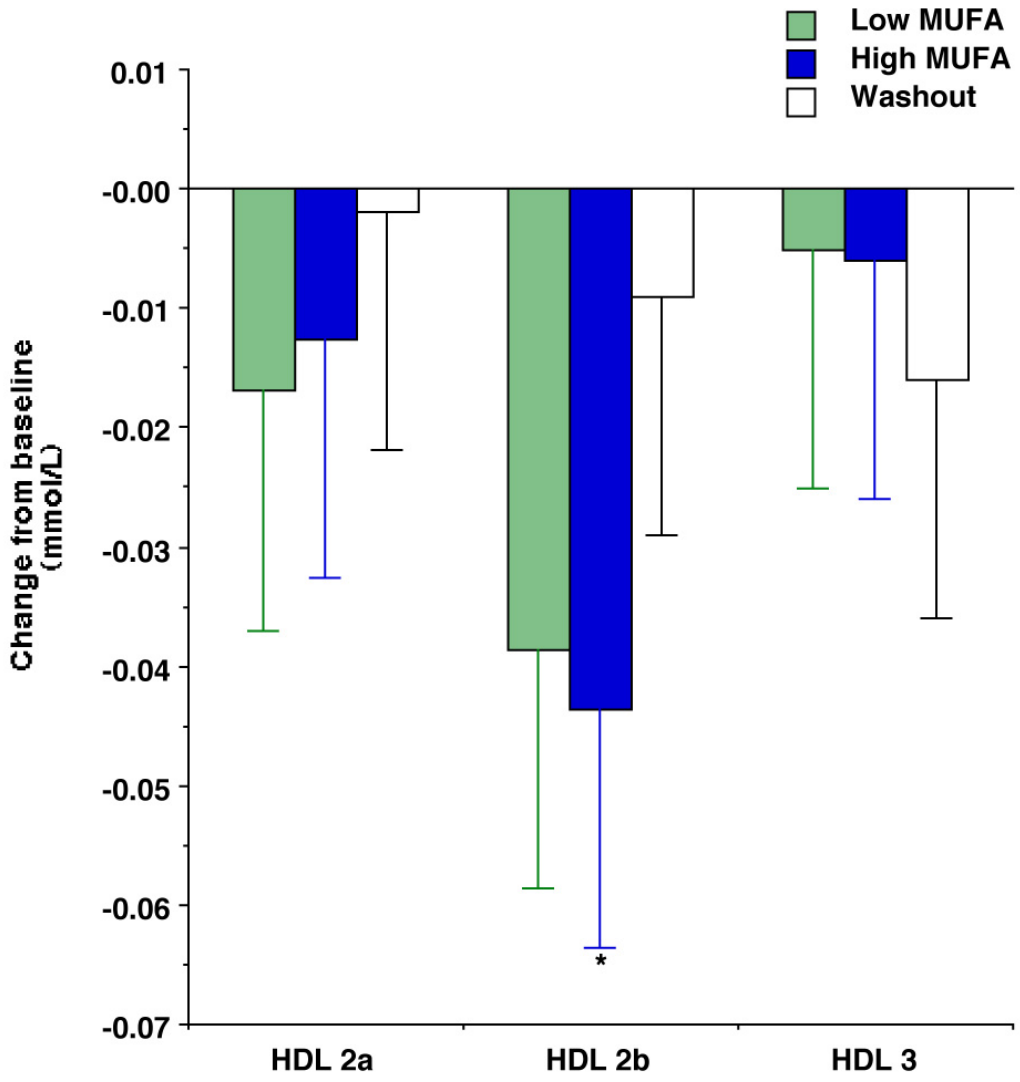

FIGURE 20 Changes from baseline in HDL subfraction cholesterol concentrations (mmol/L) of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, $n = 17$. *$P \leq 0.01$

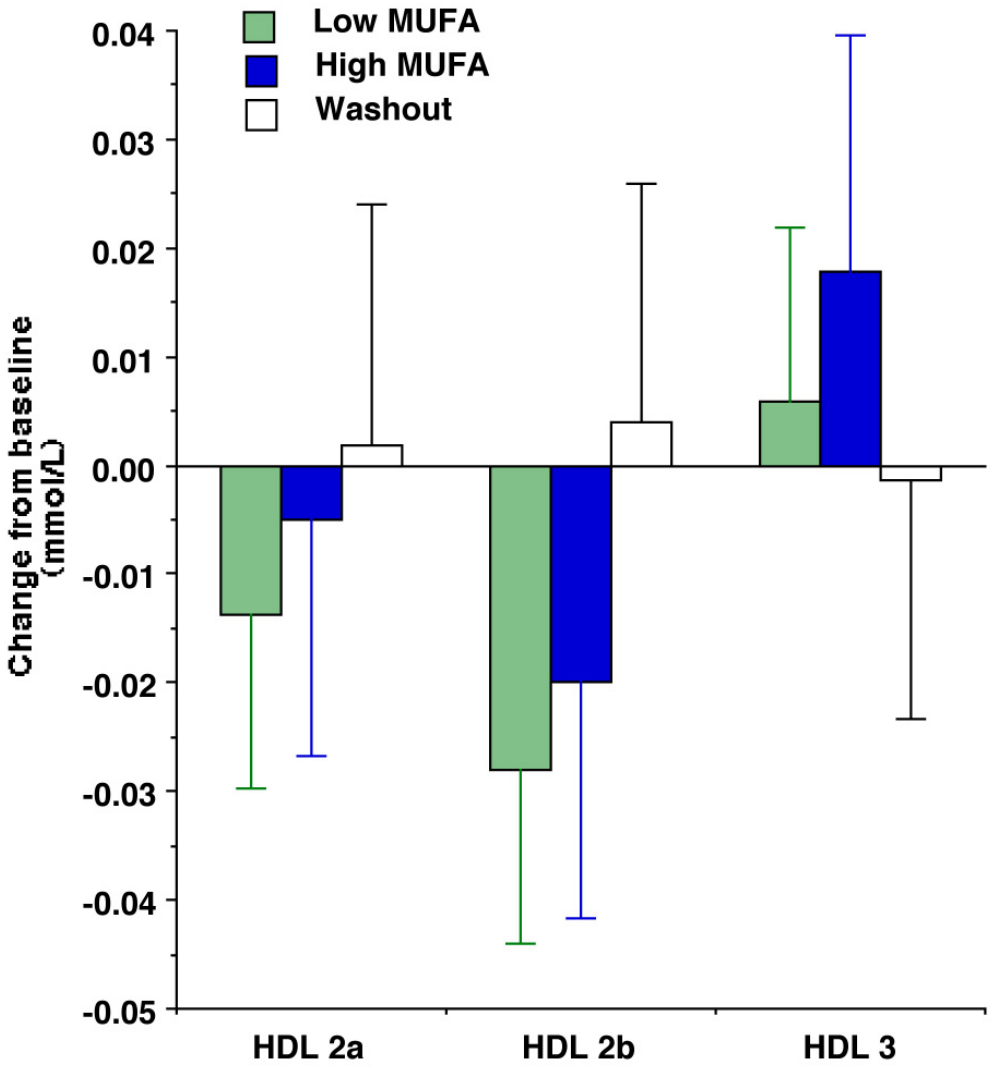

FIGURE 21 Changes from baseline in HDL subfraction cholesterol concentrations (mmol/L) when adjusted for plasma volume shift of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, *n* = 17.

Raw VLDL particle concentrations mirrored concentrations of RLP cholesterol and were decreased after exercise during both low- and high-MUFA interventions, but not during the washout period. This effect remained when values were adjusted for plasma volume shifts. Raw RLP particle concentration is also decreased after exercise with both beef interventions, but not during the washout period. When adjusted for plasma volume shifts, no effect was observed for RLP particle concentrations (Table 30; Figures 22 and 23).

TABLE 30 VLDL and RLP particle concentrations of women in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

	Low MUFA pre-exercise	Low MUFA post-exercise	High MUFA pre-exercise	High MUFA post-exercise	Washout pre-exercise	Washout post-exercise
Raw VLDL, nmol/L	53.41 ± 5.53	39.12 ± 4.6***	53.41 ± 7.4	46.29 ± 7.18***	48.29 ± 4.2	48.35 ± 6.35
Adjusted VLDL, nmol/L	53.41 ± 5.53	39.87 ± 4.73***	53.41 ± 7.4	47.80 ± 7.26**	48.29 ± 4.2	49.13 ± 6.17
Raw RLP, nmol/L	141.82 ± 9.67	127.41± 10.14**	139.47± 12.59	127.59 ± 13.64**	121.76 ± 8.48	127.76 ± 14.24
Adjusted RLP, nmol/L	141.82 ± 9.67	129.85 ± 10.63	139.47± 12.59	131.88 ± 13.76	121.76 ± 8.48	130.01 ± 14.08

[1]Data are means ± SE for 17 women.
**$P \leq 0.05$; ***$P \leq 0.01$

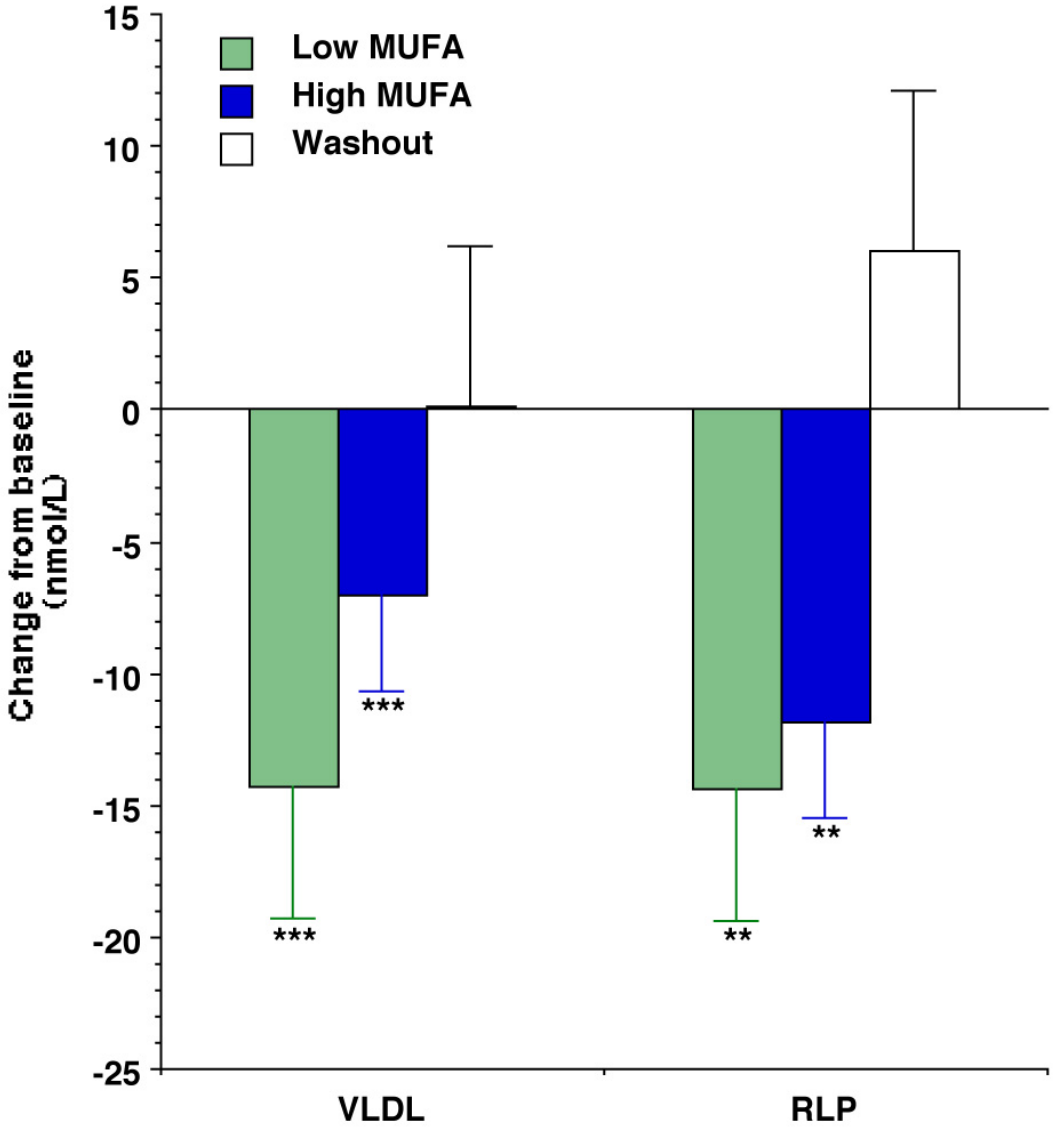

FIGURE 22 Changes from baseline in VLDL and RLP particle concentrations (nmol/L) in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, $n = 17$. $^{**}P \leq 0.05$; $^{***}P \leq 0.001$.

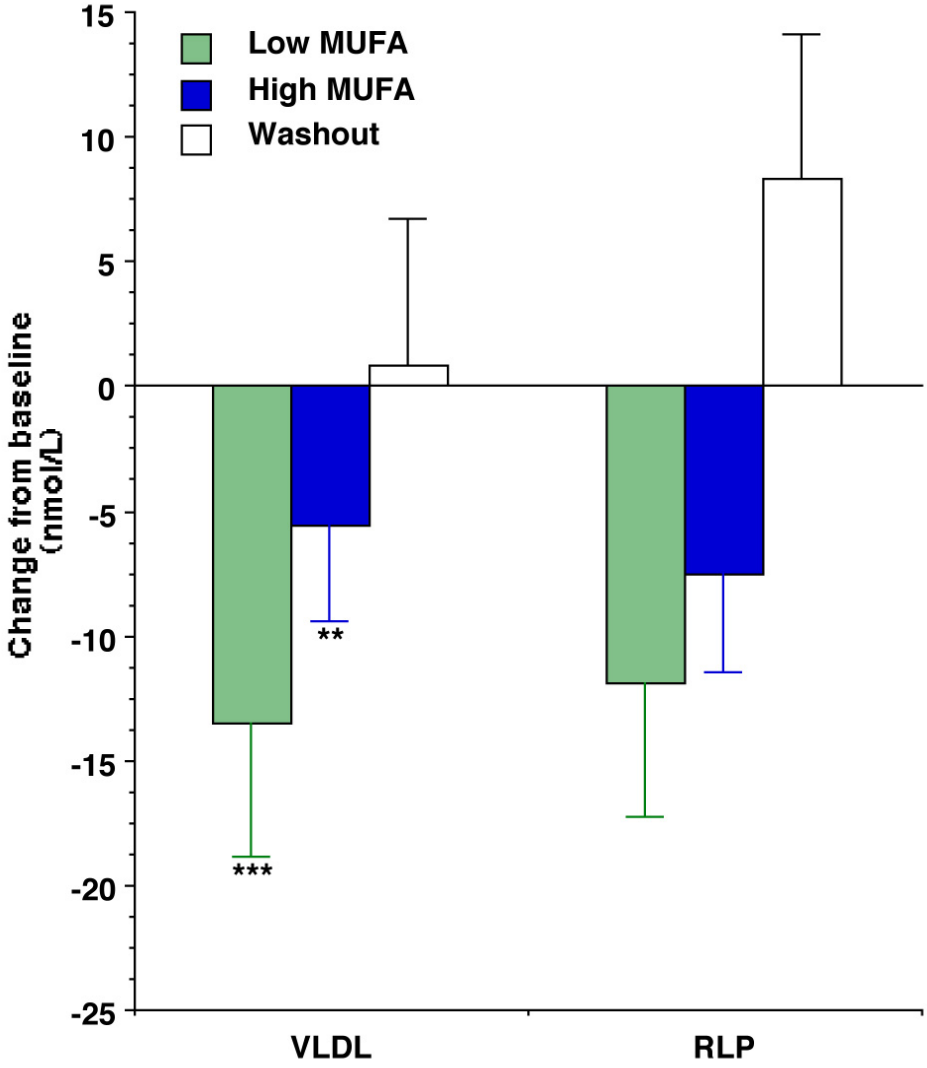

FIGURE 23 Changes from baseline in VLDL and RLP particle concentrations (nmol/L) changes from baseline when adjusted for plasma volume shifts in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, *n* = 17. *****P* **≤ 0.05; ******P*** **≤ 0.001.**

LDL particle concentration, when adjusted for plasma volume shifts, was increased after exercise during the high-MUFA intervention. No other effects were seen for LDL total, LDL III, and LDL particle concentrations (Table 31; Figures 24 and 25).

TABLE 31 LDL particle concentrations in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

	Low MUFA pre-exercise	Low MUFA post-exercise	High MUFA pre-exercise	High MUFA post-exercise	Washout pre-exercise	Washout post-exercise
Raw LDL, nmol/L	869.4± 37.3	893.9 ± 48.8	880.9± 43.8	882.3± 42.3	824.5 ± 41.8	843.2± 48.3
Adjusted LDL, nmol/L	869.4 ± 37.3	908.2 ± 50.3	880.9 ± 43.8	915.2 ± 46.5*	824.5 ± 41.8	862.3 ± 49.6
Raw LDL III, nmol/L	199 ± 10.4	186.9± 11.9	194.8 ± 15.4	193.18± 11.5	185.3 ± 13.4	179.7 ± 10.5
Adjusted LDL III, nmol/L	199.0 ± 10.4	189.9 ± 12.3	194.7 ± 15.4	200.6 ± 12.7	185.3 ± 13.4	183.8 ± 10.8
Raw LDL IV, nmol/L	86 ± 5.4	86.3 ± 4.9	92.7 ± 7.5	89.18 ± 6.4	85.1 ± 5.3	88.2 ± 4.5
LDL IV, nmol/L	86 ± 5.4	87.7 ± 5.0	92.7 ± 7.5	92.6 ± 6.9	85.1 ± 5.3	90.4 ± 4.8

[1]Data are means ± SE for 17 women.
*$P \leq 0.1$

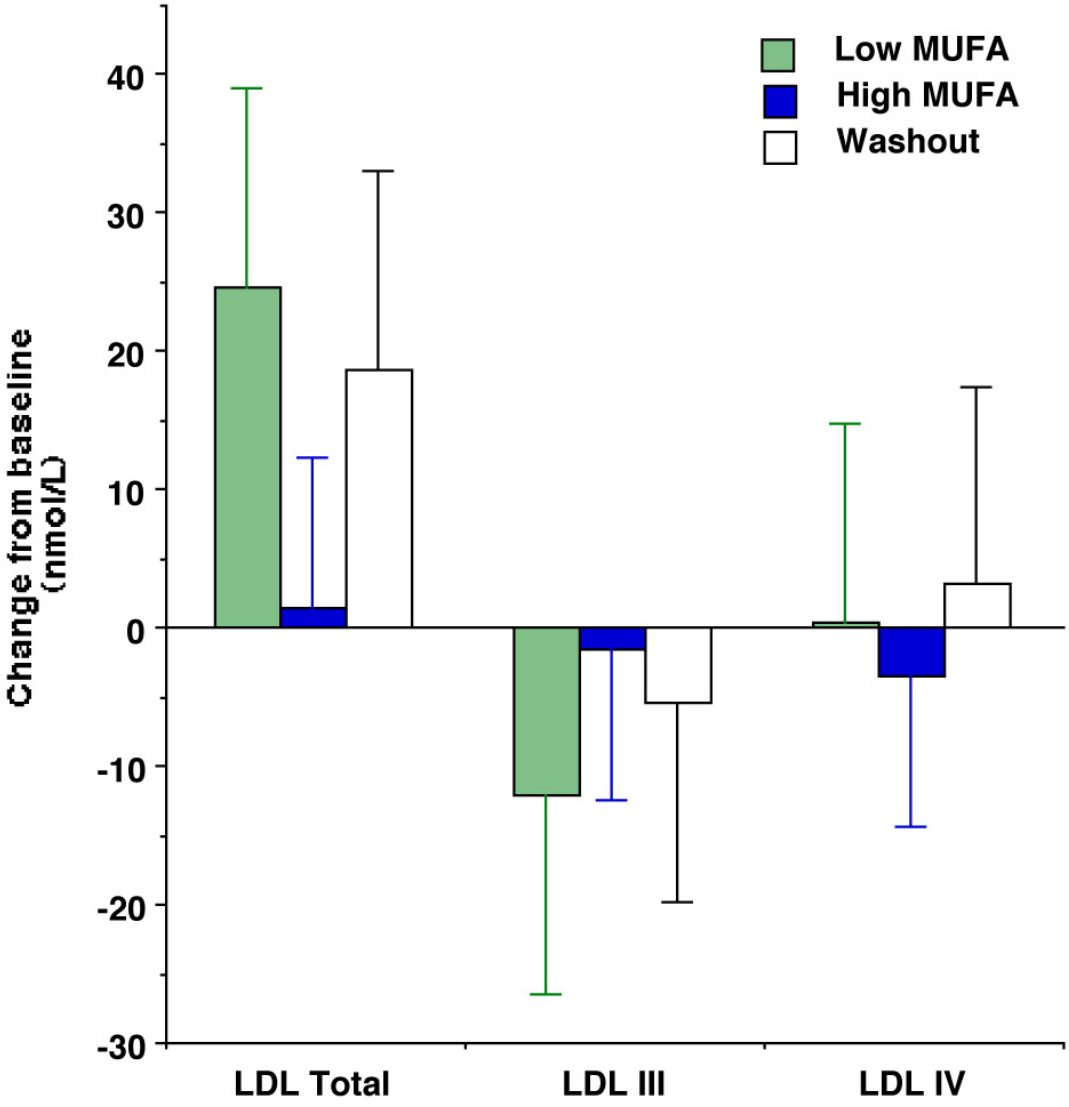

FIGURE 24 Changes from baseline in LDL subfraction particle concentrations (nmol/L) in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, *n* = 17.

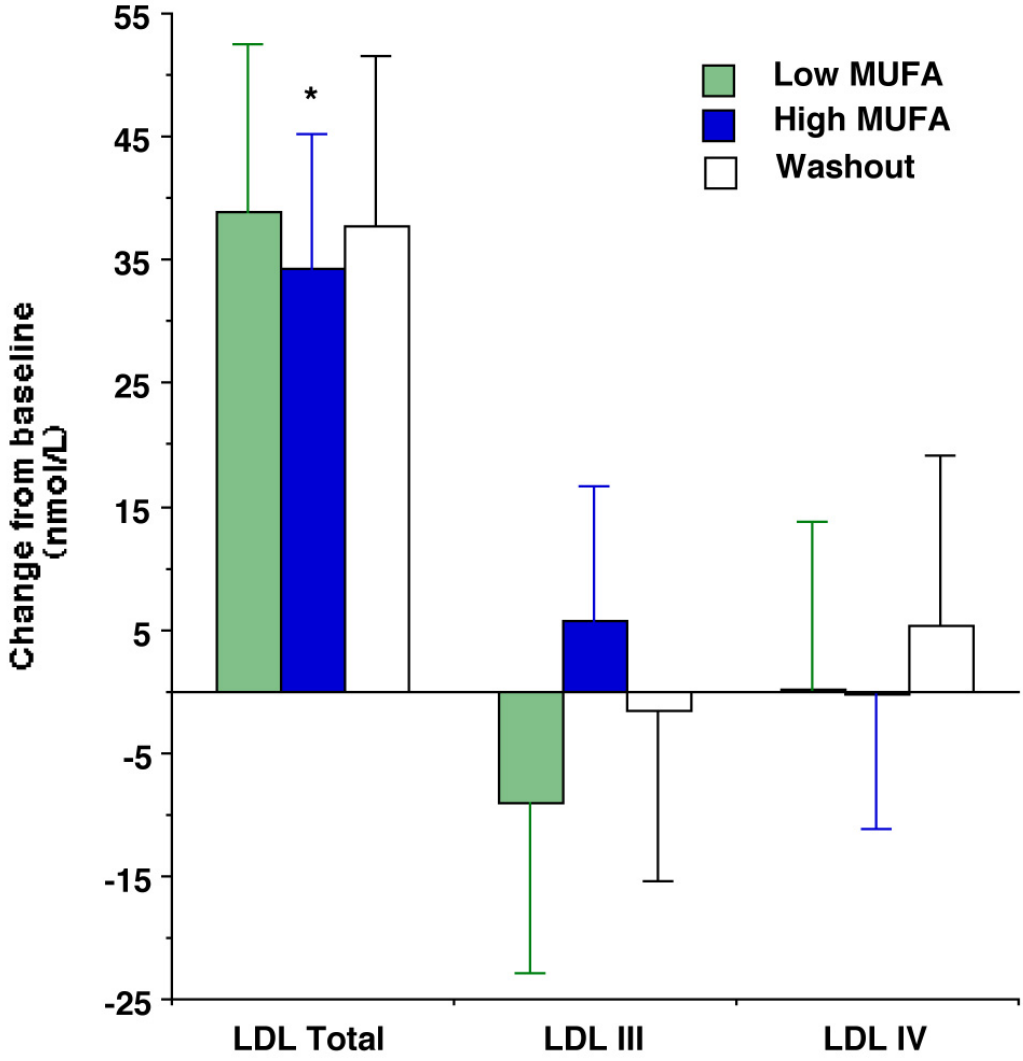

FIGURE 25 Changes from baseline in LDL subfraction particle concentration (nmol/L) when adjusted for plasma volume shifts in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, $n = 17$. *$P \leq 0.10$.

Exercise and exercise diet interactions had no significant effect on HDL total and subclasses particle concentrations (Table 32; Figures 26 and 27).

TABLE 32 HDL particle concentrations in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

	Low MUFA pre-exercise	Low MUFA post-exercise	High MUFA pre-exercise	High MUFA post-exercise	Washout pre-exercise	Washout post-exercise
Raw HDL, nmol/L	11,285± 348	10,879 ± 358	11,370 ± 390	11,107 ± 265	10,612 ± 318	10,434± 346
Adjusted HDL, nmol/L	11,285 ± 348	11,050 ± 383	11,370 ± 390	11,486 ± 256	10,612 ± 318	10,661 ± 344
Raw HDL_{2a}, nmol/L	2,268 ± 173	2,083 ± 182	2,399 ± 177	2,294 ± 150	1,937 ± 147	1,921 ± 142
Adjusted HDL_{2a}, nmol/L	2,268 ± 173	2,115 ± 187	2,399 ± 177	2,365 ± 147	1,937 ± 147	1,961 ± 143
Raw HDL_{2b}, nmol/L	2,424 ± 131	2,320 ± 146	2,475 ± 173	2,361 ± 136	2,206 ± 166	2,202 ± 141
Adjusted HDL_{2b}, nmol/L	2,423 ± 131	2,356 ± 150	2,474 ± 173	2,444 ± 142	2,206 ± 166	2,248 ± 142
Raw HDL_3, nmol/L	6,593 ± 246	6,476 ± 213	6,496 ± 235	6,452 ± 181	6,470 ± 209	6,311 ± 245
Adjusted HDL_3, nmol/L	6,593 ± 246	6,579 ± 231	6,496 ± 235	6,678 ± 189	6,470 ± 209	6,452 ± 248

[1]Data are means ± SE for 17 women.
There were no significant differences among phases ($P > 0.1$)

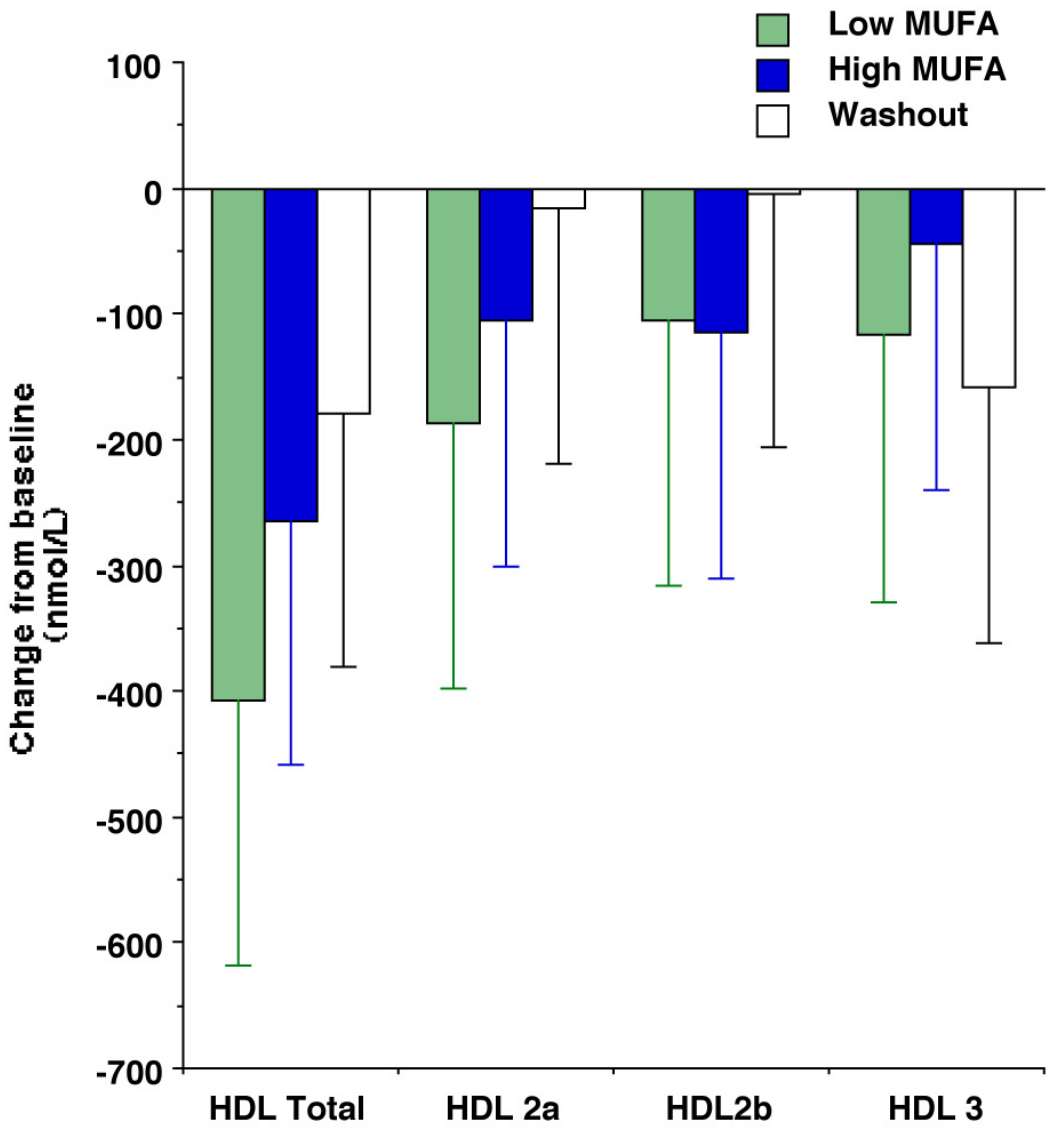

FIGURE 26 Changes from baseline in HDL lipoprotein particle concentration (nmol/L) in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, *n* = 17.

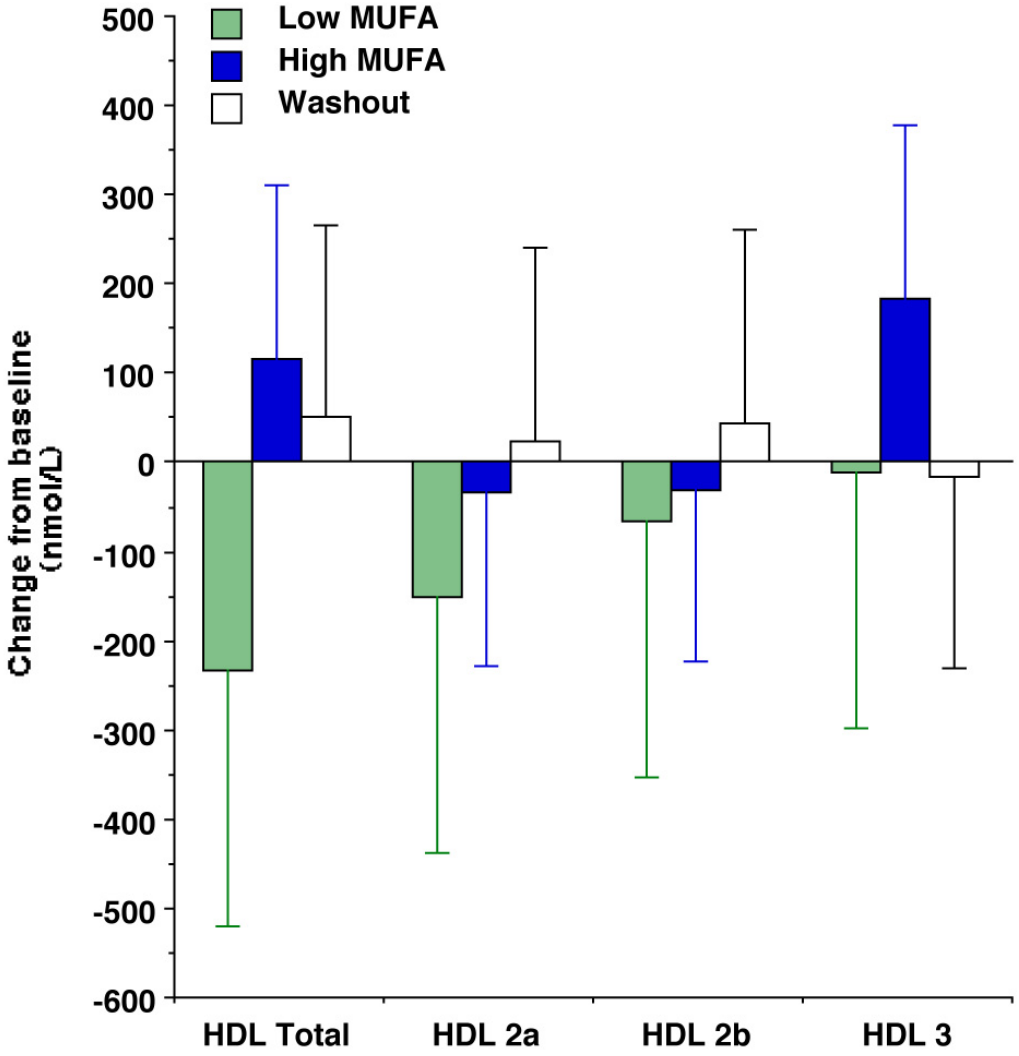

FIGURE 27 Changes from baseline in HDL lipoprotein particle concentration (nmol/L) when adjusted for plasma volume in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, *n* = 17.

hs-CRP was significantly increased after the exercise bout in all three phases. This effect remained after adjustments for plasma volume shift were made. Homocysteine was significantly increased after exercise during the washout phase. After adjustment for plasma volume shifts, this effect became more significant. Insulin was unchanged after exercise (Table 33).

TABLE 33 C-reactive protein, homocysteine and insulin levels in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

	Low MUFA Pre Exercise	Low MUFA Post Exercise	High MUFA Pre Exercise	High MUFA Post Exercise	Washout Pre Exercise	Washout Post Exercise
Raw hs-CRP, mg/L	2.32 ± 0.45	2.98 ± 0.72*	2.261 ± 0.58	3.39 ± 0.73**	1.98 ± 0.42	2.91 ± 0.69**
Adjusted hs-CRP, mg/L	2.32 ± 0.45	3.26 ± 0.74*	2.26 ± 0.58	3.78 ± 0.73**	1.98 ± 0.42	2.97 ± 0.71**
Raw insulin, µIU/ml	6.88 ± 0.79	6.18 ± 0.73	7.28 ± 0.9	6.36 ± 0.51	6.51 ± 0.57	6.78 ± 0.63
Adjusted insulin, µIU/ml	6.88 ± 0.79	6.30 ± 0.77	7.28 ± 0.9	6.57 ± 0.52	6.51 ± 0.57	6.92 ± 0.63
Raw homocysteine, µmol/L	9.71 ± 0.62	9.49 ± 0.44	9.85 ± 0.72	9.78 ± 0.78	9.28 ± 0.48	9.94 ± 0.64*
Adjusted homocysteine, µmol/L	9.71 ± 0.62	9.64 ± 0.47	9.85 ± 0.72	10.11 ± 0.78	9.28 ± 0.48	10.16 ± 0.65**

[1]Data are means ± SE for 17 women.
*$P \leq 0.1$; **$P \leq 0.05$;

HDL mean density and LDL size increased while LDL mean density decreased after exercise during the low-MUFA intervention. HDL density was increased after exercise during the high-MUFA intervention. There were no changes in HDL or LDL mean density or LDL size after exercise during the washout period (Table 34).

TABLE 34 Particle density and size in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

	Low MUFA Pre Exercise	Low MUFA Post Exercise	High MUFA Pre Exercise	High MUFA Post Exercise	Washout Pre Exercise	Washout Post Exercise
HDL mean density	1.0918 ± 0.0008	1.0938 ± 0.0011***	1.0912 ± 0.0009	1.0931 ± 0.001**	1.0877 ± 0.005	1.0916 ± 0.0009
LDL mean density	1.0295 ± 0.0002	1.0287 ± 0.0002**	1.0296 ± 0.0002	1.0293 ± 0.0002	1.0292 ± 0.0003	1.0295 ± 0.0003
LDL mean size, nm	20.17 ± 0.02	20.27 ± 0.03***	20.19 ± 0.02	20.22 ± 0.02	20.22 ± 0.03	20.20 ± 0.03

[1]Data are means ± SE for 17 women.
**$P \leq 0.05$; ***$P \leq 0.01$

Plasma fatty acids are expressed as g/100 g fatty acids (Table 35). Because the values have no dependence on plasma volume, no adjustment for plasma volume shifts was needed. Palmitoleic acid, *cis*-vaccenic, and α-linolenic were decreased after exercise only during the high-MUFA intervention. Oleic acid was decreased after exercise during the washout period and high-MUFA intervention. Plasma 20:2 was increased with exercise during the washout period. c9t11 CLA, unknown 1 and 20:5 were decreased during the low-MUFA intervention after the exercise bout (Figures 28 and 29; Table 35).

TABLE 35 Plasma fatty acid levels in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout)[1]

Fatty Acid	Pre Low MUFA	Post Low MUFA	Pre Washout	Post Washout	Pre High MUFA	Post High MUFA
14:0	0.32 ± 0.05	0.31 ± 0.05	0.42 ± 0.05	0.36 ± 0.06	0.43 ± 0.02	0.38 ± 0.03
14:1	0.07 ± 0.02	0.08 ± 0.03	0.05 ± 0.02	1.28 ± 1.21	0.09 ± 0.01	0.08 ± 0.01
16:0	18.35 ± 0.52	18.32 ± 0.78	17.71 ± 1.27	16.14 ± 1.33	18.56 ± 0.24	17.66 ± 0.25
16:1	2.15 ± 1.22	0.99 ± 0.22	1.09 ± 0.21	0.95 ± 0.19	1.12 ± 0.09	1.0 ± 0.08*
18:0	8.20 ± 0.30	8.20 ± 0.30	8.62 ± 0.71	8.19 ± 0.32	8.37 ± 0.12	8.0 ± 0.09
18:1t10	0.13 ± 0.06	0.09 ± 0.05	0.10 ± 0.04	0.10 ± 0.04	0.12 ± 0.02	0.09 ± 0.01
18:1t11	0.22 ± 0.06	0.11 ± 0.03	0.15 ± 0.05	0.21 ± 0.07	0.26 ± 0.03	0.24 ± 0.02
18:1	18.57 ± 0.66	18.13 ± 0.75	19.0 ± 0.38	17.44 ± 0.58**	19.28 ± 0.25	17.35 ± 0.18***
18:1c11	1.37 ± 0.12	1.42 ± 0.09	1.45 ± 0.08	1.42 ± 0.09	1.45 ± 0.02	1.35 ± 0.03
18:2	32.14 ± 1.13	31.57 ± 1.17	31.71 ± 1.23	30.75 ± 1.06	31.94 ± 0.44	31.55 ± 0.44

TABLE 35 Continued

Fatty Acid	Pre Low MUFA	Post Low MUFA	Pre Washout	Post Washout	Pre High MUFA	Post High MUFA
18:3	0.42 ± 0.07	0.37 ± 0.05	0.54 ± 0.07	0.45 ± 0.03	0.52 ± 0.02	0.45 ± 0.03*
18:2c9t11	0.07 ± 0.02	0.04 ± 0.02*	0.06 ± 0.02	0.05 ± 0.03	0.07 ± 0.01	0.04 ± 0.01
18:2t10c12	0.11 ± 0.04	0.05 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.08 ± 0.01	0.10 ± 0.01
20:2	0.67 ± 0.37	0.28 ± 0.13	0.32 ± 0.20	0.91 ± 0.37*	0.72 ± 0.12	0.47 ± 0.11
Unkn 1	1.88 ± 0.12	1.69 ± 0.11***	2.13 ± 0.27	1.86 ± 0.14	1.86 ± 0.04	1.79 ± 0.04
20:4	8.05 ± 0.51	10.53 ± 2.38	7.17 ± 0.28	7.83 ± 0.79	7.36 ± 0.26	7.78 ± 0.19
22:0	0.19 ± 0.12	0.07 ± 0.03	0.53 ± 0.31	0.42 ± 0.25	0.36 ± 0.08	0.43 ± 0.09
20:5	0.50 ± 0.08	0.41 ± 0.08**	0.54 ± 0.11	0.45 ± 0.08	0.57 ± 0.03	0.51 ± 0.03
24:0	0.22 ± 0.04	0.25 ± 0.04	0.27 ± 0.05	0.38 ± 0.09	0.26 ± 0.02	0.25 ± 0.02
24:1	0.06 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.11 ± 0.04	0.09 ± 0.01	0.07 ± 0.01
22:6	1.29 ± 0.15	1.28 ± 0.13	1.34 ± 0.14	1.25 ± 0.19	1.25 ± 0.04	1.16 ± 0.06
Unkn 2	1.11 ± 0.39	1.15 ± 0.23	1.67 ± 0.46	2.18 ± 0.53	1.31 ± 0.14	2.13 ± 0.19
28:0	4.19 ± 1.04	4.58 ± 0.70	6.21 ± 1.26	7.21 ± 1.49	5.0 ± 0.42	7.14 ± 0.55

[1]Data are means ± SE for 17 women.
*$P \leq 0.01$; **$P \leq 0.05$; ***$P \leq 0.01$

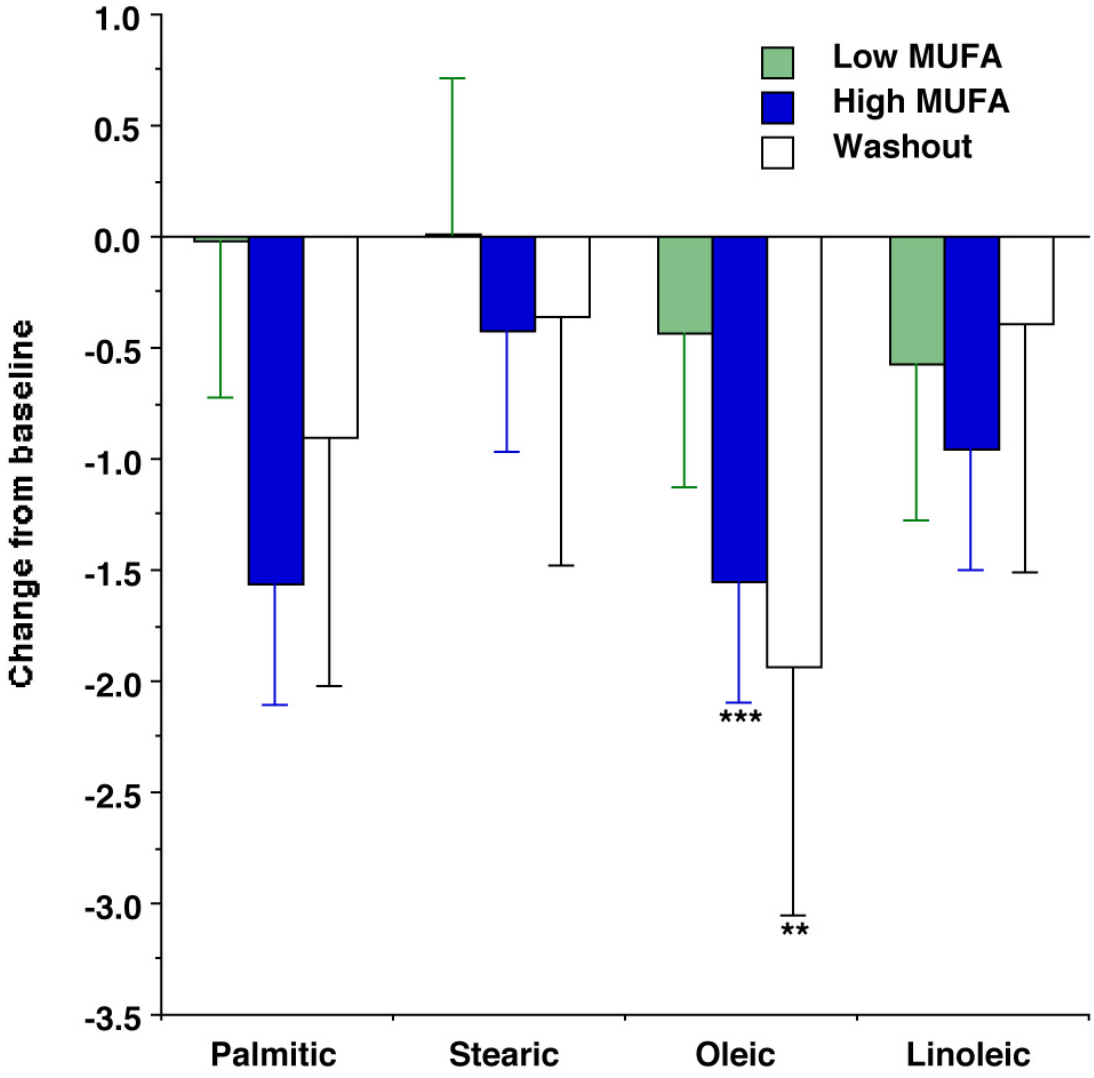

FIGURE 28 Changes from baseline in major plasma fatty acids in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, *n* = 17. *****P* **≤ 0.05; *P* ≤ 0.01**

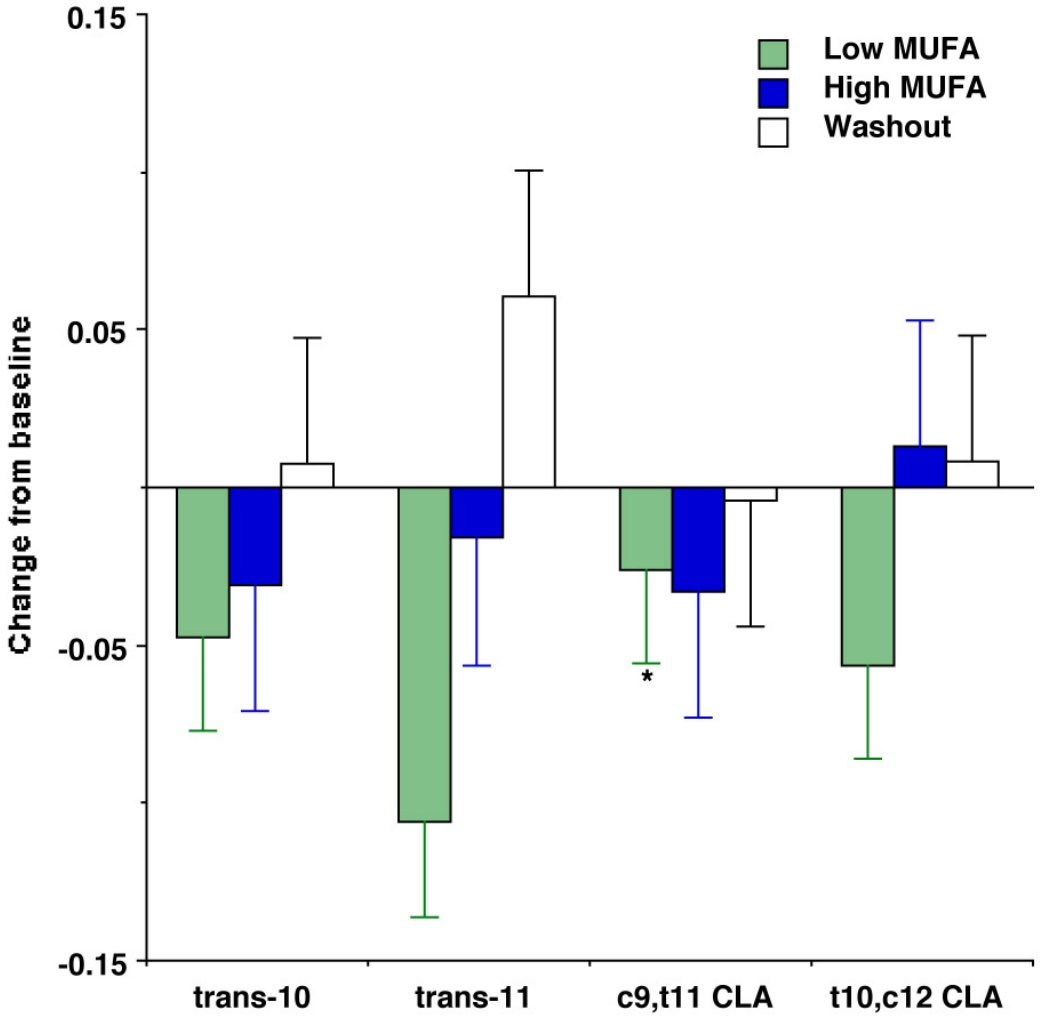

FIGURE 29 Change from baseline in *trans* plasma fatty acids in response to a single exercise session as the women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA), high in monounsaturated fatty acids (High MUFA), or habitual diet (Washout). Values are expressed as mean ± SEM, $n = 17$. $P \leq 0.10$

Discussion

Adjusting values due to plasma volume shifts is controversial. Some consider the adjustment for plasma volume changes essential when comparing pre- and post-exercise markers so as to not confuse exercise effects with simple changes in plasma volume. Others confirm that plasma volume shifts as a result of exercise, but argue that it is the concentration of the parameter that is of most biological significance, irrespective of plasma volume shifts. To address both exercise effects independent of volume shifts and potential biological effects, both raw and adjusted data were presented. Whereas most exercise reduces plasma volume, many other factors like hydration, heat stress, physical training, exercise duration and exercise intensity can influence plasma volume. Frequently, variation in findings can be attributed to plasma volume shifts and whether or notadjustments were made (180). In this study, an average increase in plasma volume was observed in each phase, but was statistically significant only after exercise during the high-MUFA intervention. This increase in plasma volume most likely was due to access to water during exercise and encouraged fluid consumption post-exercise.

Activity records reported desired consistent activity throughout the three study phases. When estimated calories burned from the activity records are compared to caloric intake, a negative energy balance was suggested. In light of the suggested negative energy balance, stable weight was maintained throughout the study. The discrepancy may be due to under-reporting or estimating dietary intake and over-reporting or estimating activity.

Although exercise is a common recommendation for increasing HDL-C, no change in HDL-C or HDL particle number with exercise was seen in this study. Also, no changes in the HDL subfractions were observed except a decrease in buoyant HDL_{2b} after exercise during the high-MUFA intervention. Measurements were taken only at 24-h post-exercise and many of studies reported an increase in HDL-C either immediately after exercise (62, 122) or at 48-h post-exercise (122). Gordon et al. (122) attributes the delayed response in adjusted HDL-C to an exercise session lasting less than 2 h which was the case for the current study. The women in this study, though postmenopausal, had higher than average baseline HDL-C. It is possible a larger volume of work is required to elicit a post-exercise increase in HDL-C when baseline levels are high (181). During a diet intervention x exercise interaction study in which sedentary men were supplemented omega-3 fatty acids, no change in total HDL-C or HDL-C subfractions was observed after the 3 d of exercise. The current study only utilized one isolated bout of exercise, and no change in HDL particle distribution was observed.

Raw and adjusted triglycerides were decreased after exercise along with a reduction in raw VLDL after exercise during both ground beef interventions. An increase in lipoprotein lipase after exercise may be responsible for the reduction in VLDL, other triglyceride rich lipoproteins, and triglycerides (182). A beneficial increase in LDL diameter was observed after exercise during the low-MUFA intervention, which was consistent to the exercise effects seen after three consecutive days of exercise (181).

An increase in inflammatory markers is viewed as a negative (8). Raw hs-CRP was increased after every bout of exercise, but was elevated acutely as a response to the

stress of exercise. When individuals train over time, the inflammatory response to exercise is reduced (183). Treadmill walking induces microtrauma to the muscle fibers resulting in an acute inflammatory state and leads to muscle repair and hypertrophy (184). Cholesterol and fatty acids are an important component of cellular membranes. As muscles repair and grow, the demand for cholesterol increases. The increased cellular need for cholesterol and fatty acids after exercise for muscle repair and growth (185), may explain the shift in lipoprotein metabolism seen in this study. LDL cholesterol and LDL particle size increased and triglycerides and VLDL decreased. That mirrors the increased extrahepatic tissue demand for cholesterol and fatty acids necessary for muscle repair and growth. The reduction in HDL_{2b} supports this notion as reverse cholesterol transport is likely reduced during this time of skeletal muscle repair and growth.

In summary, an acute bout of exercise independent and in conjunction with dietary interventions can elicit beneficial effects such as a reduction in plasma triglycerides, VLDL, and RLP along with an increase in LDL diameter.

CHAPTER V

CONCLUSIONS

As time progresses more diseases stemming from poor lifestyle choices, like CVD, are plaguing industrialized nations. The evaluation of diet and exercise regimens to prevent such diseases would therefore seem important. Epidemiological studies have shown that red meat consumption is associated with an increased risk of CVD (186, 187), but the epidemiological data remain unclear (188) Red meat contributes important nutrients to the diet, including iron, vitamin B12, and high-quality protein (189), but recommendations for a "heart healthy" diet do not encourage red meat consumption likely due to its saturated fat content (190). The fatty acid profiles in beef can be altered through production methods including feed type, length of feeding, age at time of slaughter and genetics of the cattle (131, 132, 139). The present studies aimed to evaluate the effect of different fatty acids in beef on CVD risk factors. An earlier, recently published study was designed similarly to the present studies, and evaluated the effects of the MUFA:SFA ratio of hamburger on lipoprotein and lipid profiles in mildly hypercholesterolemic men. When looking at the overall effects of the MUFA:SFA ratio of ground beef on major cholesterol fractions and triglycerides, one effect remains consistent through three independent studies: the high-MUFA ground beef (MUFA:SFA $\geq$ 1.10) significantly increased HDL cholesterol (Table 36). While many other risk factor are associated with CVD and are evaluated in the present studies total HDL cholesterol is most consistently negatively associated with CVD risk (191).

TABLE 36 Overall effects of MUFA:SFA ratio of ground beef on major cholesterol fractions and triglycerides[1]

	MUFA:SFA effect on cholesterol fractions and triglycerides					
Study 1	0.71		0.83		1.10	
	Total	±[2]	Total	±	Total	±
	LDL	±	LDL	±	LDL	±
	HDL	±	HDL	±	HDL	↑
	TAG	±	TAG	±	TAG	±
Study 2			0.90		1.3	
			Total	±	Total	↑
			LDL	±	LDL	↑
			HDL	±	HDL	↑
			TAG	±	TAG	±
Study 3			0.95		1.31	
			Total	---	Total	---
			LDL	±	LDL	±
			HDL	↓	HDL	↑
			TAG	↑	TAG	↓

[1]Study 1 = Study from Chapter II, Study 2 = Study from Chapter III, Study 3 = Adams et al. (131).
[2]± = no change, --- = not reported, ↑ = increased, ↓ = decreased

It is not the intention of the study to recommend daily consumption of one high-MUFA ground beef serving, but these studies failed to show consistent negative effects of ground beef intake. These studies argue that ground beef, especially high-MUFA ground beef, can be incorporated into a healthy diet to provide beneficial health effects with exercise or diet alone. To further evaluate the inconsistent negative effects of ground beef seen in these studies such as, increased HDL density, LDL, VLDL, RLP and IDL cholesterol additional studies which measure theses biomarkers are needed.

When diet influenced lipid metabolism, exercise attenuated the diet effects returning the cholesterol concentrations to baseline.

The area of lipoprotein metabolism, especially that of HDL subfractions and functionality, has many unanswered questions (12). It is known total HDL cholesterol is a strong negative predictor of CVD events. Desired characteristics of HDL and HDL subfractions are equivocal. Even less is known about the effects of dietary interventions on HDL functionality. As more is learned about lipoprotein metabolism and functionality, how it relates to the CVD process and the impacts of diet and exercise, reliable recommendations to improve or preserve health can be given.

LITERATURE CITED

1. Ruffer M. On arterial lesions found in Egyptian mummies. J Pathol Bacteriol. 1911;15:453-62.

2. Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. Annu Rev Immunol. 2009;27:165-97.

3. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993;362:801-9.

4. Chilton R. Pathophysiology of coronary heart disease: a brief review. J Am Osteopath Assoc 2004 September 1, 2004;104:5S-8.

5. Ross R. Atherosclerosis is an inflammatory disease. Am Heart J. 1999;138:S419-20.

6. Chait A, Han C, Oram J, Heinecke J. Lipoprotein-associated inflammatory proteins: markers or mediators of cardiovascular disease? J Lipid Res. 2005;46:389-403.

7. Cesari M, Penninx B, Newman A, Kritcheversusky S, Nicklas B. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation. 2003;108:2317-22.

8. Ridker P, Hennekens C, Buring J, Rifia N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342:836-43.

9. Hansson G, Libby P, Schonbeck U, Yan Z. Innate and adaptive immunity in the pathogenesis of atherosclerosis. Circ Res. 2002;91:281-91.

10. Newton L, Sandhu K, Livingstone C, Leslie R, Davis J. Clinical diagnostics for homocysteine: a rogue amino acid? Expert Rev Mol Diagn. 2010;10:489-500.

11. Arant C, Wessel T, Ridker P, Olson M, Reis S, Johnson B, Sharaf B, Pauly D, Handberg E, et al. Multimarker approach predicts adverse cardiovascular events in women evaluated for suspected ischemia: results from the National Heart, Lung, and Blood Institute-sponsored women's ischemia syndrome evaluation. Clin Cardiol 2009;32:244-50.

12. Barter P, Kaselein J, Nunn A, Hobbs R, Board FFE. High density lipoproteins (HDLs) and atherosclerosis; the unanswered questions. Atherosclerosis. 2003;168:195-211.

13. Moriyama Y, Okamura T, Inazu A, Doi M, Iso H, Mouri Y, Ishikawa Y, Suzuki H, Iida M, et al. A low prevalence of coronary heart disease among subjects with increased high-density lipoprotein cholesterol levels, including those with plasma cholesteyl ester transfer protein deficiency. Prev Med. 1998;27:659-67.

14. Ohashi R, Mu H, Wang X, Yao Q, Chen C. Reverse cholesterol transport and cholesterol efflux in atherosclerosis. Q J Med. 2005;98:845-56.

15. Webb N, DeBeer M, Asztalos B, Whitaker N, Van der Westhuyzen D, DeBeer F. Remodeling of HDL remnants generated by scavenger receptor class B type 1. J Lipid Res. 2004;45:1666-73.

16. Yancey P, Asztalos B, Stettler N, Piccoli D, Williams D, Connely M, Rothblat G. Sr-B1- and ABCA-1 mediatied cholesterol efflux to serum from patients with Alagille syndrome. J Lipid Res. 2004;45:1724-32.

17. Wang N, Silver D, Costet P, Tall A. Specific binding of ApoA-1 enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. J Biol Chem. 2000;275:33053-8.

18. Wang N, Lan D, Chen W, Matsuura F, Tall A. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high density lipoproteins. Proc Natl Acad Sci U S A. 2004;101:9774-79.

19. de Roos N, Schouten E, Katan M. Trans fatty acids, HDL-cholesterol, and cardiovascular disease. Effects of dietary changes on vascular reactivity. Eur J Med Res. 2003;8:355-7.

20. Barter P, Nicholls S, Rye K, Anantharamaiah G, Navab M, Fogelman A. Antiinflammatory properties of HDL. Circ Res. 2004;95:764-72.

21. Navab M, Hama S, Anantharamaiah G, Hassan K, Hough G, Watson A, Reddy S, Sevanian A, Fonarrow G, Fogelman A. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. J Lipid Res. 2000;41:1495-508.

22. Bérard A, Dabadie H, Palos-Pinto A, Dumon M, Darmon M. Reduction of dietary saturated fatty acids correlates with increased plasma lecithin cholesterol acltransferase activity in humans. Eur J Clin Nutr. 2004;58:881-7.

23. Yang Y, Yan B, Fu M, Xu Y, Tian Y. Relationship between plasma lipid concentrations and HDL subclasses. Clin Chim Acta. 2005;354:49-58.

24. Festa A, Williams K, Hanley A. Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the insulin resistance atherosclerosis study. Circulation. 2005;111:3465-72.

25. Garvey W, Kwon S, Zheng D. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes. 2003;52:453-62.

26. Medina-Urrutia A, Juarez-Rojas J, Martinez-Alvarado R, Jorge-Galarza E. High-density lipoprotein subclasses distribution and composition in Mexican adolescents with low HDL cholesterol and/or high triglyceride concentrations, and its association with insulin and c-reactive protein. Atherosclerosis. 2008;201.

27. Watanabe H, Soderlund S, Soro-Paavonen A, Hiukka A, Leinonen E, Alagona C, Salonen R, Tuomainen T, Ehnholm C, et al. Decreased high-density lipoprotein (HDL) particle size, prebeta-, and large HDL subspecies concentration in Finnish low-HDL families: relationship with intima-media thickness. Arterioscler Thromb Vasc Biol. 2006;26:897-902.

28. Johansson J, Carlson L, Landou C, Hamsten A. A strong inverse relation with the largest particles is confined to normotriglyceridemic patients. Arterioscler Thromb 1991;11:174-82.

29. Kontush A, Chantepie S, Chapman M. Small, dense HDL particles exert potent protection of atherogenic LDL against oxidative stress. Arterioscler Thromb Vasc Biol. 2003;23:1881-8.

30. Brites F, Bonavita C, De Geitere C. Alterations in the main steps of reverse cholesterol transport in male patients with primary hypertriglyceridemia and low HDL-cholesterol transport. Atherosclerosis. 2000;152:181-92.

31. Calabresi L, Gomarashchi M, Villa B. Elevated soluble cellular adhesion moleculs in subjects with low HDL-cholesterol. Arterioscl Throm Vas. 2002;22:656-61.

32. Hansel B, Philippe G, Nobecourt E, Chantepie S, Bruckert E, Chapman M, Kontush A. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. J Clin Endocrinol Metab. 2004;89:4963-71.

33. Heinecke J. The HDL proteome: a marker-and perhaps mediator of coronary artery disease. J Lipid Res. 2009;50:S167-71.

34. Navab M, Berliner J, Subbanagounder G, Hama S, Lusis AJ, Castellani L, Reddy S, Shih D, Shi W, et al. HDL and the inflammatory response induced by LDL-derived oxidized phospholipids. Arterioscler Thromb Vasc Biol. 2001;21:481-8.

35. Krauss R. The tangled web of coronary risk factors. Am J Med. 1991;90:2A-36S-41.

36. Goldstein J, Brown M. The low density lipoprotein pathway and its relation to atherosclerosis. Annu Rev Biochem. 1977;47:897-930.

37. Brown M, Goldstein J. Regulation of the activity of the low density lipoprotein receptor in human fibroblasts Cell. 1975;6:307-16.

38. Brown M, Goldstein J. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. Annu Rev Biochem. 1983;52:223-61.

39. Krauss R, Lindgren F, Ray R. Interrelationships among subgroups of serum lipoproteins in normal human subjects. Clin Chim Acta. 1980;104:275-90.

40. Krauss R, Williams P, Lindgren F, Wood P. Coordinate changes in levels of human serum low and high density lipoprotein subclasses in healthy men. Arterioscelrosis. 1988;8:155-62.

41. National Cholesterol Education Program. Second report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). Bethesda, MD: National Heart, Lung and Blood Institute. (NIH publication no 93-3095). 1993.

42. Krauss R, Blanche P. Detection and quantitation of LDL subfractions. Curr Opin Lipidol. 1992;3:377-83.

43. Krauss R. Dietary and genetic effects on low-density lipoprotein heterogeneity. Annu Rev Nutr. 2001;21:283-95.

44. Nigon F, Lesnik P, Rouis M, Champman M. Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with cellular LDL receptor. J Lipid Res. 1991;32:1741-53.

45. Bjornheden T, Babyi A, Bondjers G, Wiklund O. Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. Atherosclerosis. 1996;123:43-56.

46. Chait A, Brazg R, Tribble D, Krauss R. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Am J Med. 1993;94.

47. Cromwell W, Otvos J. Low-density lipoprotein particle number and risk for cardiovascular disease. Curr Atheroscler Rep. 2004;6:381-7.

48. Superko H, Gadesam R. Is it LDL particle size or number that correlates with risk for cardiovascular disease. Curr Atheroscler Rep. 2008;10:377-85.

49. Parthasarathy S, Rankin S. Role of oxidized low density lipoprotein in atherogenesis. Prog Lipid Res. 1992;31:127-43.

50. Otvos J, Jeyarajah E, Cromwell W. Measurement issues related to lipoprotein heterogeneity. Am J Cardiol. 2002;90(Suppl):22i-9.

51. Kiechl S, Willeit J. The mysteries of lipoprotein(a) and cardiovascular disease revisited. J Am Coll Cardiol. 2010;55:2168-70.

52. Kiechl S, Willeit J, Mayr M, Viehweider B, Oberhollenzer M, Kronenberg F, Wiedermann C, Oberthaler S, Xu Q, et al. Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes. Arterioscler Thromb Vasc Biol. 2007;27:1788-95.

53. Berglund L, Ramakrishnan R. Lipoprotein(a) an elusive cardiovascular risk factor. Arterioscl Throm Vas. 2004;24:2219-26.

54. Ruíz-Gutiérrez V, Muriana F, Guerrero A, Cert A, Villar J. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. J Hypertens. 1996;14:1483-90.

55. Attie A, Krauss R, Gray-Keller M, Brownlie A, Miyazaki M, Kastelein J, Lusis A, Stalenhoef A, Stoehr J, et al. Relationship between stearoyl-CoA desaturase activity

and plasma triglycerides in human and mouse hypertriglyceridemia. Journal of Lipid Research. 2002;43:1899-907.

56. Ntambi J, Miyazaki M. Recent insights into stearoyl-CoA desaturase-1. Curr Opin Lipidol. 2003;14:255-61.

57. Chu K, Miyazaki M, Man W, Ntambi J. Steaoyl-coenzyme A Desaturase 1 deficiency protects against hypertriglyceridemia and increases plasma high-density lipoprotein cholesterol induced by Liver X Receptor activation. Mol Cell Biol. 2006;26:6786-98.

58. Flowers M, Ntambi J. Role of stearoyl-coenzyme A desaturase om regulating lipid metabolism. Curr Opin Lipidol 2008;19:248-56.

59. MacDonald M, van Eck M, Hildebrand R, Wong B, Bissada N, Ruddle P, Kontush A, Hussein H, Pouladi M, et al. Despite antiatherogenic metabolic characteristics, SCD1-deficient mice have increased inflammation and atherosclerosis. Arterioscler Thromb Vasc Biol. 2009;29:341-7.

60. Erbay E, Babaev V, Mayers J, Makowski L, Charles K, Snitow M, Fazio S, Wiest M, Watkins S, et al. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. Nat Med. 2009;15:1383-91.

61. Monda K, Ballantyne C, North K. Longitudinal impact of physical antivity on lipid profiles in middle-aged adults: the atherosclerosis risk in communities study. J Lipid Res. 2009;50:1685-91.

62. Weise S, Grandjean P, Rohack J, Womack J, Crouse S. Acute changes in blood lipids and enzymes in postmenopausal women after exercise. J Appl Physiol. 2005;99:609-15.

63. Grandjean P, Crouse S, O'Brien B, Rohack J, Brown J. The effects of menopausal status and exercise training on serum lipids and the activities of intravascular enzymes related to lipid transport. Metabolism. 1998;47:377-83.

64. Li Z, McNamara J, Fruchart J, Luc G, Bard J, Ordovas J, Wilson P, Schaefer E. Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes. J Lipid Res. 1996;37:1886-96.

65. Lawler J, Hu Z, Green J, Crouse S, Grandjean P, Bounds R. Combination of estrogen replacement and exercise protects against HDL oxidation in post-menopausal women. Int J Sports Med. 2002;23:477-83.

66. Stone N, Nicolosi R, Kris-Etherton P. Summary of the scientific conference on the efficacy of hypocholeserolemic dietary interventions. Circulation. 1996;94:3388-91.

67. Campos H, Willett WC, Peterson R, Siles X, Bailey S. Nutrient intake comparisons between Framingham and rural and urban Puriscal, Costa Rica. Associations with lipoproteins and low density lipoprotein particle size. Atheroscler Thromb. 1991;11:1089-99.

68. Müller H, Lindman AS, Brantsæter AL, Pedersen J. The serum LDL/HDL cholesterol ratio is influenced more favorably by exchanging saturated with unsaturated fat than by reducing saturated fat in the diet of women. J Nutr. 2003;133:78-83.

69. Hu F, Stampfer M, Manson J, Rimm E, Colditz G, Rosner B, Hennekens C, Willett W. Dietary fat intake and the risk of coronary heart disease in women. N Engl J Med. 1997;337:1491-9.

70. Kris-Etherton P, Pearson T, Wan Y, Hargrove R, Moriarty K, Fishell V. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am J Clin Nutr. 1999;70:1009-15.

71. Krauss R, Eckel R, Howard B, Appel L, Danielsl S, Deckelbaum R, Erdman J, Jr. AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation. 2000;102:2284-99.

72. Mozaffarian D, Katan M, Ascherio A, Stampfer M, Willett W. Trans fatty acids and cardiovascular disease. New Engl J Med. 2006;354:1601-13.

73. Kris-Etherton P, Yu S. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. Am J Clin Nutr. 1997;65:1628S-44.

74. Reiser, Probstfield J, Silvers A, Scott L, Shorney M, Wood R, O'Brien B, Gotto A, Jr, Phil D, Insull W, Jr. Plasma lipid and lipoprotein response of humans to beef, coconut oil and safflower oil. Am J Clin Nutr. 1985;42:190-7.

75. O'Brien B, Reiser R. Human plasma lipid responses to red meat, poultry, fish, and eggs. Am J Clin Nutr. 1980;33:2573-80.

76. Griel A, Kris-Etherton P. Beyound saturated fat: the importance of the dietary fatty acid profile on cardiovascular disease. Nutr Rev. 2006;64:257-62.

77. Keys A, Anderson J, Grande F. Serum cholesterol response to diet. Metabolism. 1965;14:747-87.

78. Hegsted D, McGandy R, Myers M, Stare F. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr. 1965;17:281-95.

79. Mozaffarian D, Rimm E, Herrington D. Dietary fats, carbohydrate, and the progression of coronary atheosclerosis in postmenopausal women. Am J Clin Nutr. 2004;80:1175-84.

80. St John L, Young C, Knabe D, Schelling G, Grundy S, Smith S. Fatty acid profiles and sensory and carcass traits of tissues from steers and swine fed an elevated monounsaturated fat diet. J Anim Sci. 1987;64:1441-7.

81. Muller H, Kirkhus B, Pedersen J. Serum cholesterol predictive equation with special emphasis on trans and saturated fatty acids. An analysis from designed controlled studies. Lipids. 2001;36:783-91.

82. Mensink R, Zock P, Kester A, Katan M. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003;77:1146-55.

83. Watts G, Jackson P, Burke V, Lewis B. Dietary fatty acids and progression of coronary artery disease in men Am J Clin Nutr. 1996;64:202-9.

84. Kris-Etherton P, Griel A, Psota T, Gebauer S, Zhang J, Etherton T. Dietary stearic acid and risk of cardiovascular disease: intake, sources, digestion and absorption. Lipids. 2005;40:1193-200.

85. Grundy S. Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. Am J Clin Nutr. 1994;60:986S-90.

86. Loison C, Mendy F, Serougne C, Lutton C. Dietary myristic acid modifies the HDL-concentration and liver scavenger receptor BI expression in the hamster. Brit J Nutr. 2002;87:199-210.

87. Nicholls S, Lundman P, Harmer J. Consumption of saturated fat impairs the anti-inflammatory properties of high-density lipoproteins and endothelial function. Am J Clin Nutr. 2006.

88. Patel S, Puranik R, Nakhla S, Lundman P, Stocker R, Wang X, Lambert G, Rye K-A, Barter PJ, et al. Acute hypertriglyceridaemia in humans increases the triglyceride content and decreased the anti-inflammatory capacity of high density lipoproteins. Atherosclerosis 2009;204:424-8.

89. Mente A, de Koning L, Shannon H, Anand S. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Arch Intern Med. 2009;169:659-69.

90. Lecerf J. Fatty acids and cardiovascular disease. Nutr Rev. 2009;67:273-83.

91. Pérez-Jiménez F, Ruano J, Perez-Mendez P, Lopez-Segura F, Lopez-Miranda J. The influence of olive oil on human health: not a question of fat alone. Mol Nutr Food Res. 2007;51:1199-208.

92. Benkhalti F, Prost J, Paz E, Perez-Jimenez F. Effects of feeding extra virgin olive oil or their polyphenols on lipid of rat liver. Nutr Res. 2002;22:1067-75.

93. Jakobsen M, O'Reilly E, Heitmann B, Pereira M, Balter K, Fraser G, Goldbourt U, Hallmans G, Knekt P, et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. Am J Clin Nutr. 2009;89:1425-32.

94. Kris-Etherton P, Hecker KB, AE. Polyunsaturated fatty acids and cardiovascular health. Nutr Rev. 2004;62:414-26.

95. GISSI-Prevenzione-Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet. 1999;354:447-55.

96. Lemaitre R, King I, Mozaffarian D, Kuller L, Tracy R, Siscovick D. n-3 Polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the Cardiovascular Health Study. Am J Clin Nutr. 2003;77.

97. Kelley D, Siegel D, Vemuri M, Chung G, Mackey B. Docosahexaenoic acid supplementation decreases remnant-like particle-cholesterol and increases the (n-3) index in hypertriglyceridemic men. J Nutr. 2008;138:30-5.

98. Goyens P, Mensink R. Effects of alpha-linolenic acid versus those of EPA/DHA on cardiovascular risk markers in healthy elderly subjects. Eur J Clin Nutr. 2006;60:978-84.

99. Wood R, Kubena K, O'Brien B, Tseng S, Martin G. Effect of butter, mono- and polyunsaturated fatty acid-enriched butter, trans fatty acid margarine, and zero trans fatty acid margarine on serum lipids and lipoproteins in healthy men. J Lipid Res. 1993;34:1-11.

100. Gebauer S, Psota T, Kris-Etherton P. The diversity of health effects of individual trans fatty acid isomers. Lipids. 2007;42:787-99.

101. Willett W. Trans fatty acids and cardiovascular disease-epidemiological data. Atheroscler Suppl. 2006;7:5-8.

102. Motard-Belanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lenieux S, Couture P, Lamarche B. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for caridovascular disease. Am J Clin Nutr. 2008;87:593-9.

103. Chardigny J, Destaillats F, Malpuech-Brugere C, Moulin J, Bauman D, Lock A, Barbano D, Mensink R, Bezelgues J, et al. Do *trans* fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the *trans* Fatty Acids Collaboration (TRANSFACT) study. Am J Clin Nutr. 2008;87:558-66.

104. Mozaffarian D. *Trans* fatty acids - effects on systemic inflammation and endothelial function. Atheroscler Suppl. 2006;7:29-32.

105. Han S, Leka LL, AH. Effect of hydrogenated and saturated , relative to polyunsatured, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. J Lipid Res. 2002;79:606-12.

106. Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K, Straarup E. Effects of butter high in ruminant trans and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. Am J Clin Nutr. 2006;83:237-43.

107. Chin S, Liu W, Storkson J, Ha Y, Pariza M. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Compost Anal. 1992;5:185-97.

108. Tricon S, Burdge G, Kew S, Banerjee T, Russell J, Jones E, Grimble R, Williams C, Yaqoob P, Calder P. Opposing effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic acid on blood lipids in healthy humans. Am J Clin Nutr. 2004;80:614-20.

109. Ringseis R, Wen G, Saal D, Eder K. Conjugated linoleic acid isomers reduce cholesterol accumulation in acetylated LDL-induced mouse RAW264.7 macrophage-derived foam cells. Lipids. 2008;43:913-23.

110. Grundy S, Denke M. Dietary influences on serum lipids and lipoproteins. J Lipid Res. 1990;31:1149-72.

111. Jones P, Pappu A, Hathcher L, Li Z, Illingworth R, Connor W. Dietary cholesterol feeding suppresses human cholesterol synthesis measured by deuterium incorporation and urinary mevalonic acid levels. Arterioscler Thromb Vasc Biol. 1996;16:1222-8.

112. Brown M, Goldstein J. Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. J Lipid Res. 2009;50:S15-27.

113. Brown M, Goldstein J. A receptor mediated pathway for cholesterol homeostasis. Science. 1986;232:34-47.

114. Rumsey S, Galeano N, Lipschitz B, Deckelbaum R. Oleate and other long chain fatty acids stimulate low density lipoprotein receptor activity by enhancing acyl

coenzyme a:cholesterol acyltransferase activity and altering intracellular regulatory cholesterol pools in cultured cells. J Biol Chem. 1995;270:10008-16.

115. Nelson M, Rejeski J, Blair S, Duncan P, Judge J, King A, Macera C, Castaneda-Sceppa C. Physical Activity and Public Health in Older Adults: Recommendation From the American College of Sports Medicine and the American Heart Association. 2007. p. 1094-105.

116. Lee I, Rexrode K, Cook N, Manson J, Buring J. Physical activity and coronary heart disease in women: is "no pain, no gain" passé? JAMA. 2001;285:1447-54.

117. Bergeron J, Couillard C, Despres J, Gagnon, Leon A, Rao D, Skinner J, Wilmore J, Bouchard C. Race differences in the response of postheparin plasma lipoprotein lipase and hepatic lipase activities to endurance exercise training in men. Atherosclerosis. 2001;159:399-406.

118. Kelley G, Kelley K, Tran Z. Aerobic exercise and lipids and lipoproteins in women:a meta-analysis of randomized controlled trials. J Womens Health. 2004;13:1148-64.

119. Leon A, Sanchez O. Response of blood lipids to exercise training alone or combined with dietary intervention. Med Sci Sports Exerc. 2001;33:S502-15.

120. Thompson P, Crouse S, Goodpaster B, Kelley D, Moyna M, Pescatello L. The acute versus the chronic response to exercise. Med Sci Sports Exerc. 2001;33:S438-45.

121. Crouse S, O'Brien B, Rohack J, Lowe R, Green J, Tolson H, Reed J. Changes in serum lipids and apolipoproteins after exercise in men with high cholesterol: influence of intensity. J Appl Physiol. 1995;79:279-86.

122. Gordon P, Fowler S, Warty V, Danduran M, Visich P, Keteyian S. Effects of acute exercise on high density lipoprotein cholesterol and high density lipoprotein subfractions in moderately trained females. Brit J Sport Med. 1998;32:63-7.

123. Diekman C, Elmadfa I, Koletzko B, Pusk P, Uauy R, Zevenbergen H. Summary statement of the international expert meeting: health significance of fat quality of the diet. Ann Nutr Metab. 2009;54S:39-40.

124. Smit L, Moxafarian D, Willeit W. Review of fat and fatty acid requirements and criteria for developing dietary guidelines. Ann Nutr Metab. 2009;55:44-55.

125. Baggio G, Pagan A, Muraca M, Marini S, Opportuno A, Bonanome A, Amrosio G. Olive-oil-enriched diet: effect on serum lipoprotein levels and bilary cholesterol saturation. Am J Clin Nutr. 1988;47:960-4.

126. Grundy S, Florentin L, Nix D, Whelan M. Comparison of monounsaturated fatty acids and carbohydrates for reducing raised levels of plasma cholesterol in man. Am J Clin Nutr. 1988;47:965-9.

127. Sturdivant C, Lunt D, Smith C, Smith S. Fatty acid composition of subcutaneous and intramuscular adipose tissues and *M. longissimus dorsi* of Wagyu cattle. Meat Sci. 1992;32:449-58.

128. O'Dea K, Traianedes K, Chisholm K, Leyden H, Sinclair A. Cholesterol-lowering effect of low-fat diet containing lean beef is reversed by the addition of beef fat. Am J Clin Nutr. 1990;52:491-4.

129. Scott L, Kimball K, Wittels E, Dunn K, Brauchi D, Pownall H, Herd J. Effects of a lean beef diet and of a chicken and fish diet on lipoprotein profiles. Nutr Metab Cardiovasc Dis. 1991;1:25-30.

130. Smith D, Wood R, Tseng S, Smith S. Increased beef consumption increases apolipoprotein A-1 but not serum cholesterol of mildly hypercholesterolesterolemic men with different levels of habitual beef intake. Exp Biol Med. 2002;227:266-75.

131. Adams T, Walzem R, Smith D, Tseng S, Smith S. Ground beef high in total, saturated and trans-fatty acids decreases HDL cholesterol and LDL particle diameter, and increases plasma TAG, in mildly hypercholesterolaemic men. Brit J Nutr. 2010;103:91-8.

132. Turk S, Smith S. Carcass fatty acid mapping. Meat Sci. 2009;81:658-63.

133. Leheska J, Thompson L, Howe J, Hentges E, Boyce J, Brooks J, Shriver B, Hoover L, Miller M. Effects of conventional and grass-feeding systems on the nutrient composition of beef. J Anim Sci. 2008;86:3575-85.

134. Rhoades R, Saywer J, Ponce C, Lunt D, Choi C, Smith S. Substrate utilization and dose response to insulin by subcutaneous adipose tissue of Angus steers fed corn- or hay- based dierts. J Anim Sci. 2009;87:2338-45.

135. Smith S, Smith D, Lunt D. Chemical and physical characteristics of meat: adipose tissue. In: Jenson W, Devine C, Dikemann M, editors. Encyclopedia of Meat Sciences: Elsevier Science Publishers, Oxford; 2004. p. 225-38.

136. United States Department of Agriculture. Factors affecting U.S. beef consumption. 2005 [accessed 2010 September]; Available from: http://www.ers.usda.gov/publications/ldp/Oct05/ldpm13502/ldpm13502.pdf

137. USDA-FSIS. Fact sheet-meat prepartion-focus on ground beef-"What's the difference between "hamburger" and "ground beef?" 2008 [accessed 2010 September]; Available from: http://www.fsis.usda.gov/Factsheets/Ground_Beef_and_Food_Safety/

138. Retail B. Ground beef catagory breakdown. 2010 [accessed 2010 September]; Available from: http://www.beefretail.org/GroundBeefCategoryBreakdown.aspx/

139. Chung K, Lunt D, Choi C, Chae S, Rhoades R, Adams T, Booren B, Smith S. Lipid characteristics of subcutaneous adipose tissue and *M. longissimus thoracis* of Angus and Wagyu steers fed to US and Japanese endpoints. Meat Sci. 2006;73:432-41.

140. Walzem R, Watkins S, Frankel E, Hansen R, German J. Older plasma lipoproteins are more susceptible to oxidation: a linking mechanism for the lipid and oxidation theories of atherosclerotic cardiovascular disease. Proc Natl Acad Sci U S A. 1995;92:7460-4.

141. Nichols A, Krauss R, Musliner T. Nondenaturing polyacrylamide gradient gel electrophoresis. Method Enzymol. 1986;128:417-31.

142. Gong E, Nichols A, Forte T, Blanche P, Shore V. Transformation of large discoidal complexes of apolipoprotein A-I and phosphatidylcholine by lecithin-cholesterol acyltransferase. Biochim Biophys Acta. 1988;961:73-85.

143. Folch J, Lees M, Sloane Stanley G. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957;226:497-509.

144. Morrison W, Smith L. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res. 1964;5:600-8.

145. Nicklas T, Hampl J, Taylor C, Thompson V, Heird W. Monounsaturated fatty acid intake by children and adults: temporal trands and demographic differences. Nutr Rev. 2004;62:132-41.

146. Bonanome A, Grundy S. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. New Engl J Med. 1998;318:1244-8.

147. Attie A, Krauss R, Gray-Keller M, Brownlie A, Miyazaki M, Kaselein J, Lusis A, Stalenhoef A, Stoehr R, et al. Relationship between stearoyl-CoA desaturase activity and plasma triglycerides in human and mouse hypertriglyceridemia. J Lipid Res. 2002;43:1899-907.

148. Miyazaki M, YC K, Gray-Keller M, Attie A, Ntambi J. The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase. J Biol Chem. 2000;301:32-8.

149. Sampath H, Miyazaki M, Dobrzyn A, Ntambi J. Stearoyl-CoA desaturase-1 mediates the pro-lipogenic effects of dietary saturated fat. J Biol Chem. 2007;282:2483-93.

150. Warensjo E, Sundstrom J, Vessby B, Cederholm T, Riserus U. Markers of dietary fat quality and fatty acid desaturation as predictors of total and cardiovascular mortality: a population-based prospective study. Am J Clin Nutr. 2008;88:203-9.

151. German J, Gibson R, Krauss R, Nestel P, Lamarche B, van Staveren W, Steijns J, de Groot L, Lock A, Destaillats F. A reappraisal of the impact of dairy foods and milk fat on cardiovascular disease risk. Eur J Nutr. 2009;48:191-203.

152. Arsenault B, Lemieux I, Despres J, Gagnon P, Wareham N, Stroes E, Kastelein J, Khaw K, Boekholdt S. HDL particle size and the risk for coronary heart disease in apparently healthy men and women: The EPIC-Norfolk prospective population study. Atherosclerosis. 2009;206:276-81.

153. Archibeque S, Lunt D, Gilbert C, Tume R, Smith S. Fatty acid indices of stearoyl-CoA desaturase do not reflect actual stearoyl-CoA desaturase enzyme activies in adipose tissues of beef steers finished with corn-, flaxseed-, or sorghum-based diets. J Anim Sci. 2005;83:1153-66.

154. Bloedon L, Balikai S, Chittams J, Nunnane S, Berlin J, Rader D, Szapary P. Flaxseed and cardiovascular risk factors: result from a double blind, randomized, controlled clinical trial. J Am Coll Nutr 2008;27:65-74.

155. Egert S, Kannenberg F, Somoza V, Erbersdobler H, Wahrburg U. Dietary α-linolenic acid, EPA, and DHA have differential effects on LDL fatty acid composition but similar effects on serum lipid profiles in normolipidemic humans. J Nutr. 2009;139:861-8.

156. Zhao G, Etherton T, Martin K, West S, Gillies P, Kris-Etherton P. Dietary α-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. J Nutr. 2004;134:2991-7.

157. Leffler T, Moser C, McManus B, Urh J, Keeton J, Claflin A. Determination of moisture and fat in meats by microwave and nuclear magnetic resonance analysis: collaborative study. J AOAC Int. 2008;91:802-10.

158. Troup J; SpectraCell Laboratories, Inc. Method for analyzing blood for lipoprotein components. United States patent application 20080038763, 2007.

159. Chait A, Brazg R, Tribble D, Krauss R. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Am J Med. 1993;94:350-6.

160. Libby P. Atherosclerosis: the new view. Sci Am. 2002;286:46-55.

161. Veniant M, Sullivan M, Kim S, Ambroziak P, Chu A, Wilson M, Hellerstein M. Defining the atherogenicity of large and smalllipoproteins containing apolipoprotein B100. J Clin Invest. 2000;106:1501-10.

162. Villareal D, Miller B, Banks M, Fontana L, Sinacore D, Klein S. Effect of lifestyle intervention on metabolic coronary heart disease risk factors in obese older adults. Am J Clin Nutr. 2006;84:1317-23.

163. Azadbakht L, Esmailzadeh A. Red meat intake is associated with metabolic syndrome and the plasma c-reactive protein concentration in women. J Nutr. 2009;139:335-9.

164. Menendez R, R M, Amor A, Rodeiros I, Gonzalez R, Alfonso J. Inhibition of cholesterol biosynthesis in cultured fibroblasts by D003, a mixture of very long chain saturated fatty acids. Pharmacol Res. 2001;44:299-303.

165. Nakajima K, Nakano T, Moon H, Nagamine T, Stanhope K, Havel P, Warnick G. The correlation between TG vs remnant lipoproteins in the fasting and postprandial plasma of 23 volunteers. Clin Chim Acta. 2009;404:124-7.

166. Karpe F, Boquist S, Tang R, Bond G, de Faire U, Hamsten A. Remnant lipoproteins are related to intima-media thickness of the carotid artery independently of LDL cholesterol and plasma triglycerides. J Lipid Res. 2001;42:17-21.

167. Twickler T, Dallinga-Thie G, Chapman M, Cohn J. Remnant lipoproteins and atherosclerosis. Curr Atheroscler Rep. 2005;7:140-7.

168. Barbagallo C, Rizzo M, Noto D, Frasheri A, Pernice V, Rubino A, Pieri D, Pinto V, Cafalu A, et al. Accumulation of apoE-enriched triglyceride-rich lipoproteins in patients with coronary heart disease. Metabolism. 2006;55:662-8.

169. Kannel W, Hfortland M, McNamara E, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med. 1976;85:447-52.

170. American Heart Association. Heart disease and stroke statistics-2003 update. Dallas, TX; 2002.

171. Howard B, Van Horn L, Hsia J. Low-fat dietary pattern and risk of cardiovascular disease: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA. 2006;295:655-66.

172. Pronk N, Crouse S, O'Brien B, Rohack J. Acute effects of walking on serum lipids and lipoproteins in women. J Sports Med Phys Fitness. 1995;35:50-8.

173. Nolte L, Nowson C, Dyke A. Effect of dietary fat reduction and increased aerobic exercise on cardiovascular risk factors. Clin Exp Pharmacol Physiol. 1997;24:901-3.

174. Young D, Haskell W, Jatulis D, Fortmann S. Associations between changes in physical activity and risk factors for coronary heart disease in a community-based sample of men and women: the Standord Five-City Project. Am J Epidemiol. 1993;138:205-16.

175. Crouse S, O'Brien B, Grandjean P, Lowe R, Rohack J, Green J. Effects of training and a single session of exercise on lipids and apolipoproteins in hypercholesterolemic men. J Appl Physiol. 1997;83:2019-28.

176. Okura T, Makata Y, Tanaka K. Effects of exercise intensity on physical fitness and risk factors for coronary heart disease. Obes Res. 2003;11:1131-9.

177. Panagiotalos D, Pitsavos C, Chrysohoou C, Kavouras S, Stefanadis C. The associations between leisure-time physical activity and inflammatory and coagulation markers related to cardiovascular disease: the ATTICA Study. Prev Med. 2005;40:432-7.

178. Bruce R. Exercise testing of patients with coronary heart disease. Ann of Clin Res 1971;3:323-32.

179. Troup J; SpectraCell Laboratories, Inc. Method for analyzing blood for lipoprotein components. United States2007.

180. Kargotich S, Goodman C, Keast D, Morton A. The influence of exercise-induced plasma volume changes on the interpretation of biochemical parameters used for monitoring exercise, training and sport. Sport Med. 1998;26:101-17.

181. Durstine J, Haskell W. Effects of exercise training on plasma lipids and lipoproteins. Exerc Sport Sci Rev. 1994;22:477-521.

182. Angelopoulos T, Robertson R, Goss R, Metz K, LaPorte R. Effect of repeated exercise bouts on high density lipoprotein-cholesterol and its subfractions HDL_2-C and HDL_3-C. Int J Sports Med. 1993;14:196-201.

183. Lara Fernandes J, Serrano C, Toledo F, Hunziker M, Zamperini A, Teo F, Oliveira R, Blotta M, Rondon M, Negrao C. Acute and chronic effects of exercise on inflammatory markers and B-type natriuretic peptide in patients with coronary artery disease. Clin Res Cardiol. 2010 ahead of print.

184. Ozaki H, Miyachi M, Nakajima T, Abe T. Effects of 10-week walk training with leg blood flow reduction on carotid arterial compliance and muscle size in the elderly adults. Angiology 2010;OnlineFirst:1-6.

185. Riechman S, Lee C, Chikani G, Chen V, Lee T. Cholesterol and skeletal muscle health. World Rev Nutr Diet. 2009;100:71-9.

186. Fraser GE. Associations between diet and cancer, ishemic heart disease and all-cause mortality in non-hispanic white California Seventh-day Adventists. Am J Clin Nutr. 1999;70:532s-8s.

187. Snowdon D, Phillips R, Fraser G. Meat consumption and fatal ischemic heart disease. Prev Med. 1984;13:490-500.

188. Micha R, Wallace S, Mozaffarian D. Red and processed meat consumption and risk of incident coronary heart disease, stroke and diabetes mellitus a systemic review and meta-analysis. Circulation. 2010;121:2271-83.

189. U.S. Department of Agriculture, Agricultural Research Service. USDA national nutrient database for standard reference, release 22. 2009 [accessed; Available from: Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata

190. Lichtenstein A, Appel L, Brands M, Carnethon M, Daniels S, Franch H, Franklin B, Kris-Etherton P, Harris W, et al. Diet and lifestyle recommendation revision 2006. A scientific statement from the American Heart Association Nutrition Committee. Circulation. 2006;114:82-96.

191. Sviridov D, Mukhamedova N, Remaley A, Chin-Dusting J, Nestel P. Antiatherogenic functionality of high density lipoprotein: how much versus how good. J Atheroscler Thromb. 2008;15:52-62.

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Publications

Gilmore, L.A., Walzem, R.L., Crouse, S.F., and Smith, S.B. Ground beef from corn-fed cattle provides greater health benefits than ground beef from pasture-fed cattle. FASEB J. 2010; 24: abstract 336.2.

Gilmore, L. A., Carbuhn, A. F., Crouse, S. F., Walzem, R.L., Smith, S.B.High-oleic ground beef, exercise, and risk factors for cardiovascular disease in postmenopausal women. FASEB J. 2009; 23: abstract 553.4.