EFFECT OF CONSUMING GROUND BEEF OF DIFFERING MONOUNSATURATED FATTY ACID CONTENT ON ATHEROSCLEROTIC CARDIOVASCULAR DISEASE RISK FACTORS IN HEALTHY MEN

A Thesis

by

XIAOJUAN CAO

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

MASTER OF SCIENCE

December 2008

Major Subject: Nutrition

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ABSTRACT

Effect of Consuming Ground Beef of Differing Monounsaturated Fatty Acid Content on Atherosclerotic Cardiovascular Disease Risk Factors in Healthy Men. (December 2008)

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Chair of Advisory Committee: Dr. Rosemary L. Walzem

Atherosclerotic cardiovascular disease (ASCVD) is currently the most common cause of death in the United States. Some dietary factors contribute importantly to ASCVD and other factors can reduce risk of ASCVD. Oleic acid is a monounsaturated fatty acid (MUFA). Dietary patterns in which oleic acid contributes to a majority of dietary fatty acids are associated with reduced ASCVD risk. These beneficial effects are due to MUFA-induced lipoprotein profile changes such as decreases in low density lipoprotein (LDL) and increases in high density lipoprotein (HDL). LDL oxidation plays a central role in atherosclerosis development as it both initiates and propagates atherosclerosis. HDL is anti-atherogenic as it can attenuate LDL oxidation. HDLs are a class of diverse lipoprotein that varies in protein and enzymatic composition. The paraoxonase (PON) family of enzymes, especially PON1, is primarily expressed in the liver; PON activity in the circulatory system is associated with HDL. Both PON and HDL have been documented to be anti-atherogenic. Other factors such as homocysteine and C-reactive protein (CRP) can also be considered risk factors for ASCVD. However, studies of risk factors in healthy men who consume ground beef with a different content

of MUFA are lacking; hence, no conclusive evidence has established whether consuming a high amount of MUFA in the form of ground beef alters the development of atherosclerosis.

The overall purpose of this study was to investigate whether the provision of ground beef with a fractionally higher MUFA content could lower or improve several ASCVD risk factors in men who consume ground beef. These risk factors include the metabolic indices of glucose, insulin and homeostasis model assessment (HOMA), inflammation risk factors of CRP and homocysteine and anti-risk factor of paraoxonase. The concentration of homocysteine was determined spectrophotometrically following separation by high pressure liquid chromatography (HPLC). Enzyme-linked imminosorbent assay kits that measured the CRP and insulin concentration in plasma. The significance of the results was determined by subjecting the data to ANOVA using the general linear model for repeated measurement ($P<0.05$).

From this study, it can be concluded that MUFA has a beneficial effect of lowering risks as determined by metabolic indices and lipoprotein profile. Moreover, our study showed that different concentrations of MUFA in ground beef has no effect on PON1 activity, but that increased beef consumption generally reduces PON1 in association with increases in homocysteine concentration while improving indicators of glucose tolerance.

DEDICATION

To my father and mother, who have always stood by my side and given me strong support.

To my husband and lovely daughter for their love and support.

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Thank you.

NOMENCLATURE

ANOVA	Analysis of Variance
ASCVD	Atherosclerotic Cardiovascular Disease
ATP III	Adult Treatment Panel III
B. M.	Bachelor in Medicine
CE	Cholesteryl Ester
CRP	C-reactive Protein
CM	Chylomicron
EDTA	Ethylene Diamine Tetraacetic Acid
ELISA	Enzyme-linked ImmunoSorbent Assay
HDL	High Density Lipoprotein
HOMA	Homeostasis Model Assessment
HPLC	High Pressure Liquid Chromatography
IDL	Intermediate Density Lipoprotein
IRI	Insulin Resistance Index
GLM	General Linear Model
LDL	Low Density Lipoprotein
MUFA	Monounsaturated Fatty Acid
NCEP	The National Cholesterol Education Program
NO	Nitric Oxide
Ox-LDL	Oxidized LDL

OPA	O-phthalaldehyde
PON	Paraoxonase
RCB	Randomized Complete Block
SD	Standard Deviation
TG	Triacylglyceride
VLDL	Very Low Density Lipoprotein
β-CFI	β-cell Function Index
β-ME	2-mercaptoethanol

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CHAPTER I

INTRODUCTION

Cardiovascular disease (CVD) is the highest cause of mortality in the United States, killing almost 17 million individuals each year (1). Atherosclerotic cardiovascular disease (ASCVD) plays an important role in the development of CVD; it has a much higher rating as a causal factor than other independent risk factors such as cigarette use and hypertension (2). Currently, we know that ASCVD is a chronic disease that is characterized by the formation of atheroma (plaque) as the mature lesion. Lesion formation reflects progressive lipid accumulation in the large- and medium-sized arteries and is the fundamental pathological process in this disease. Chronic inflammation underlies ASCVD (2, 3). During chronic inflammation, the endothelial cells lining the arteries become injured and respond by inappropriate cell-signaling that propagates damage to the surrounding cell types and macromolecules, thereby leading to plaque formation in the intima and media of the artery wall (4). For example, pathological studies have revealed that oxidized low-density lipoprotein (ox-LDL) and inflammatory mediators such as monocyte chemotactic protein (MCP-1) can be found in the injured artery wall (2, 5). Further, studies showed that oxidative modification of LDL, either in the circulation or in the artery wall, plays a critical role in the development of ASCVD (6, 7). Thus, oxidative stress within artery, the formation of ox- LDL and their

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This thesis follows the style of the American Journal of Clinical Nutrition.

accumulated ions within the subendothelium alter endothelial function and impair flow-mediated vasodilatation, an index of vascular wall compliance and health (2, 8).

Two potent lipoproteins, LDL and high-density lipoprotein (HDL) play important albeit usually opposite, roles in the development of ASCVD. In this regard, LDL can promote endothelial dysfunction, arterial injury and plaque formation (9, 10) and is commonly known as "bad cholesterol." In contrast, HDL is commonly known as "good cholesterol," as it mediates cholesterol efflux from the arterial wall and contains antioxidant enzymes such as PON1. In general, both increasing LDL concentrations and decreasing HDL concentrations increase the ASCVD risk (9, 10). Indeed, changes in circulating concentrations of both types of lipoproteins are independent risk factors for ASCVD. As an adverse risk factor, LDL-cholesterol (LDL-C) has been estimated to increase ASCVD risk by 4% for each 1.0 mg/dL increase (9). In contrast, every 1.0 mg/dL decrease in HDL-cholesterol (HDL-C) increases ASCVD risk by 2% in men and 3% in women (10, 11). The National Cholesterol Education Program (NCEP) in the Adult Treatment Panel III now lists low HDL-C concentration (Table 3) as an independent risk factor for ASCVD (12). Moreover, a high circulating concentration (>16 μM/L) of the amino acid homocysteine is also considered as a risk for ASCVD (13-15). Homocysteine is an oxidative stressor able to cause endothelial cell dysfunction and provoke chronic inflammation within arterial walls and, as a result, smooth muscle cell proliferation (16-18). C-reactive protein (CRP) is an acute phase reactant, and a concentration > 1.5 mg/dL indicates chronic inflammation within the vascular wall (19-22), and CRP is a biomarker for increased ASCVD risk (23, 24). To assess the CRP

inflammation marker, the American Heart Association and the Centers for Disease Control and Prevention (AHA/CDC) panel defined the cut-off CRP value for ASCVD risk as 1.0 mg/dL for "low risk," 1.0-3.0 mg/dL for "average risk" and >3.0 mg/dL for "high risk" (25).

Central obesity is a characteristic associated with risk for metabolic syndrome, i.e. diet-induced insulin resistance (normal glucose, high insulin) and altered lipoprotein metabolism that increase the risk for ASCVD (14). Epidemiological studies suggest that MUFA may stimulate β-cells in the pancreas to secrete insulin (26, 27).

Studies showed that oxidized LDL-C could be recovered from the artery wall (28). The presence of oxidized lipids in LDL-ox particles—so called peroxide "seeding," —can initiate inflammation in the artery wall. This inflammatory process can be inhibited by normal HDL (5, 29, 30). Hence, HDL possesses antioxidant activity and serves an anti-inflammatory function. How HDL mediates these activities is not fully understood. HDL is a heterogeneous class of lipoprotein particles. HDL can be classified by particle size, density and apolipoprotein content. In general, HDL is a cholesteryl ester (CE)-rich lipoprotein. A primary function of HDL is to carry out reverse cholesterol transport from peripheral tissues to the liver for reuse or disposal in bile. This process limits the inflammation in the vascular wall (29-31). Recent reviews illustrated how the antiatherogenic function of HDL is related to its apolipoprotein composition and intrinsic enzyme activity (31, 32). HDL particles contain as many as 48 proteins that can be grouped according to functions such as "lipid metabolism" and "acute phase response" (32). Variation in the apolipoprotein content can cause a variety of effects

depending on the HDL subclasses. Different subclasses of HDL participate differentially in ASCVD mitigation and progression.

One scheme classifies HDL subclasses by particle size (diameter), density and apolipoprotein composition (33-35). Based on this scheme, small (5.5 to 9.5 nm) HDL that are more dense (1.125 g/mL < d < 1.21 g/mL) are called HDL_3. If the particles are larger (9.5 to 12.0 nm) and less dense (1.063g/mL < d < 1.125 g/mL), they are termed HDL_2 (33). Some studies concluded that HDL_3 are more responsible for HDL's anti-atherogenic function (31, 32). The amount of HDL_2 and HDL_3 or the ratio of these two HDL subclasses is considered as a diagnostic test for ASCVD risk (36).

That dietary fat influences plasma lipoprotein profiles and that certain profiles are linked to risk of ASCVD is well known. Saturated fatty acids (SFA), especially palmitic acid (16:0) and myristic acid (14:0), elevate total and LDL-C, whereas the SFA stearic acid (18:0) had no effect on LDL-C concentrations (37). The MUFA oleic acid (18:1) reduces total and LDL-C concentrations (38). Therefore, substitution of MUFA for SFA is expected to produce lower cholesterol concentrations (39). However, HDL-C concentration reduction by dietary factors can adversely affect ASCVD risk. High carbohydrate and low fat intake increases the concentration of triacylglycerol (TG), whereas it decreases that of HDL-C in blood (40-42). This pattern of dyslipidemia is found in individuals with insulin resistance (metabolic syndrome X) (43). Compared to carbohydrate, oleic acid has the effect of lowering TG and increasing HDL-C (44). The ratio of total cholesterol (TC) to HDL-C is considered by many to be a better index of ASCVD risk than LDL-C or HDL-C because it considers relative changes in HDL-C in

its risk assessment (45). Stearic acid, which has a neutral or modest LDL-C lowering effect, has a modest HDL-C elevating effect, and as a result, stearic acid reduces total-C:HDL-C (46). Beef fat consists mostly of the SFA stearic acid and palmitate (25-30%) and MUFA (47). Oleic acid-enriched beef consumed by mildly hypercholesterolemic men significantly increased the concentration of the primary HDL apolipoprotein, apoA1 (48).

In humans, the effects of a protein-rich diet for the development of atherosclerosis have not been clearly demonstrated. Soybean protein had a beneficial hypocholesterolemic effect as shown by a decreased serum concentration of LDL and an increased concentration of HDL (49). Casein is a protein that is found in milk that has a similar effect on plasma lipids as soybean protein (50). However, animal protein consumption increases TG compared with plant protein consumption, and thus increases ASCVD risk (51, 52). The amount of protein intake influences blood cholesterol. Infants with severe protein malnutrition show significant weight loss associated with a decrease in serum fatty acid (53). Chronic renal failure patient without a protein restrict diet exhibited a hypertriglyceridemia and an increase risk for development of atherosclerosis (54). Methionine is a sulfur amino acid, which have been demonstrated has atherogenic effects (55, 56). A good diet source of methionine is animal protein, thus, these investigations suggest that a higher intake animal protein could increase ASCVD risk.

CHAPTER II

LITERATURE REVIEW

Numerous risk factors contribute to the development of atherosclerosis and CVD. LDL oxidation plays a central role in the initiation and progression of atherosclerosis. In order to study the effect of consuming ground beef with different MUFA content on risk factors of ASCVD in healthy men, it is important to understand what atherosclerosis is, how it happens and what process components risk factors estimate within the disease process. This literature review gives a brief description of the development of atherosclerosis and the basis for the putative roles of several risk factors in the development of atherosclerosis.

The development of atherosclerosis

The development of atherosclerosis is a slow and continuous process that is associated with the accumulation of lipids and fibrous elements to form plaque in the large arteries causing them to narrow. Impaired blood flow through narrowed arteries reduces the oxygen-rich blood supply to the body, especially the heart and brain. Atherosclerosis is the main contributor to CVD, which leads to heart attacks, strokes or even death in western countries. Previous evidence revealed that chronic inflammation and immune response contribute to ASCVD development (3, 57). The mechanism of atherosclerosis is complex. The pathological development of atherosclerosis (Figure 1) has been revealed as a series of protective responses to injuries of endothelial cells in the artery wall (5). Oxidatively modified lipids formed in LDL (Ox-LDL) while present in

the intima initiate atherosclerosis by damaging endothelial cells that signal inflammatory cells (monocyte and/or macrophage) migration through cell-derived adhesion and chemotactic factors. Conversion of monocytes into macrophages that subsequently engulf Ox-LDL and so become lipid-laden foam cell that constitutes a key element of the fatty steak. Endothelial cells response to Ox-LDL injury that stimulates inflammatory cells also stimulates smooth muscle cell migration and differentiation, which contribute to the fatty steak more maturation. This sequence of events can be verified by *in vivo* and *in vitro* experiments (4, 21). The lipid core of fatty steak is surrounded by a thin fibrous cap that contains reduced collagen, smooth muscle cells and macrophages that make the complex plaque vulnerable to erosion or rupture causing a series of events (58). Necropsy studies demonstrated that 60-80% of ASCVD deaths occurs as a result of fibrous-cap rupture (59). With time, the fibrous fatty plaque elevates the artery wall and intrudes into the arterial lumen reducing its diameter and affecting blood flow, oxygen and nutrient transport. During this inflammation process, non-laminar shear flow and ox-LDL reduced the production of nitric oxide (NO)—a chemical with vasodilatation function—from endothelium cell, the result of reduced NO also was shown to have atherogenic activity (60). Foam cell death forms a necrotic core of lesion, while smooth muscle cell proliferation and differentiation causes the development of the fibrous fatty streak plaque in artery wall (58). Epidemiological studies have identified numerous risk factors associated with this injury and response; these risk factors can be group into genetic factors (high LDL, low HDL, high blood pressure, high level homocysteine, high CRP) and environmental factors (high-fat diet,

smoking and lack of exercise), and these risk factor can combine to modulate inflammation process (2).

Lipoprotein

Lipoproteins have been related to disease such as atherosclerosis over the last several decades. Lipoproteins are macromolecules that contains both lipids and proteins at variable ratios, densities and sizes (61). The common nonpolar components of lipoprotein are cholesterol ester (CE) and TG. Because the CE and TG are hydrophobic, they need to be transported within the amphipathic structures of lipoprotein to make them miscible with the aqueous fluid components of blood plasma (62, 63). Their major role in the body is transport of lipid and lipophilic nutrients in the body. Apolipoproteins are also associated with lipoprotein, and serve both structural and regulatory functions within the particles. There are at least nine types of apolipoproteins. Five major classes of lipoproteins are commonly discussed, with individual classed based on particle density, component content and diameter (large to small): (Table 1) chylomicron (CM), very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), LDL and HDL (62).

Cholesterol is involved in three pathways in body (Figure 2). These are the exogenous pathway (CM transfer cholesterol from intestine to liver), the endogenous pathway (VLDL and LDL transfer cholesterol from liver to the body) (62, 63) and reverse cholesterol transport (HDL transfer excess cholesterol from body back to the liver for disposal). Dietary cholesterol and fat are assembled into CM within the enterocytes and transported through the lymph prior to release into the general

circulation (63). CM are TG-rich, large and the least dense of the lipoproteins that functions to transport dietary lipids to peripheral tissues and ultimately liver. In liver, cholesterol and TG are assembled into VLDL and serve to transport lipid nutrients to muscle and adipose tissue. Their five different major apolipoprotein ($ApoB_{100}$, ApoCI, ApoCII, ApoCIII and ApoE) (64). After VLDL transport TG and other lipids to muscle and adipose, they lose part of apolipoprotein and become denser LDL (62). LDL contains a high percentage cholesterol and CE with apolipoprotein ($ApoB_{100}$, ApoE, ApoC). $ApoB_{100}$ can bind to LDL receptor—which is located on cell surface—to allow LDL carry cholesterol in the blood stream (65). Once apolipoprotein binds to the LDL receptor, LDL enters into the cell and then is endocytosed, apolipoprotein is degrade to its amino acid, and CE is converted to cholesterol, these components can then be used by the cell (62, 65, 66). HDL contains 50% protein; therefore, it has a much higher density than other lipoproteins; it functions to transport excess cholesterol from tissue and returning it to liver.

Apolipoprotein

Apolipoproteins are associated with lipoproteins and act as structural elements, receptor ligands, enzymatic and transfer protein activities that mediate lipoprotein metabolism. There are at least nine types of apolipoprotein. $ApoB_{48}$ is a short form of $ApoB_{100}$. In humans, $ApoB_{48}$ can be found in chylomicron; it can not bind to its receptor without another apolipoprotein. In contrast, $ApoB_{100}$ is the long form of ApoB and, is not only found in VLDL, but is also found in IDL and LDL which are the end products

of VLDL metabolism.

LDL and LDL oxidation

LDL is a major transporter of cholesterol to peripheral tissue through the circulatory system. Hence, an elevated concentration of LDL causes its entry rate through the endothelium cell junction into the intima to increase, leading to endothelial dysfunction. This dysfunction alters vascular vasomotion tone, which contributes to ASCVD with increasing age and elevated LDL cholesterol concentration (67, 68). Some evidence indicates that oxidatively modified LDL in the artery wall is likely to have a key role in the development of atherosclerosis (69). All lipoproteins are susceptible to oxidation because of their fatty acid composition; however, LDL is most susceptible to lipid oxidation (70). The structural and biological properties of LDL are altered by the oxidation reaction (71, 72). LDL is CE-rich, of variable particle size, and consists of apolipoprotein of $ApoB_{100}$ and a smaller amount of ApoCIII and ApoE. ApoB is a receptor for LDL binding in the pathway of metabolism (62). Some research indicates that small, dense LDL are associated with atherosclerosis vascular disease (73).

Phospholipids are a major lipid of LDL. Oxidized phospholipids can be atherogenic and are formed when LDL undergoes oxidation. LDL lipid peroxide content is an indicator of the balance of pro- and anti-oxidative balance in the body, including the ability of HDL to exert sufficient antioxidation activity to protect LDL from oxidation *in vivo.* The conjugated dienes formed during the oxidative reaction can be used to measure the LDL lipid peroxide content (74). Conjugated dienes are measured spectrophotometrically, with the oxidative stability of LDL described by lag time to

initial conjugated diene formation, and propagation rate of that free radical reaction per hour under standardized assay conditions (6, 75, 76). In biochemical terms, LDL oxidation is a free radical-driven chain reaction where polyunsaturated fatty acids are converted to lipid peroxides that easily decompose to many products, including biologically active aldehydes. The *in vitro* assay of LDL oxidation detects a product of LDL oxidation, conjugated diene (CD), at 234nm and the time to a measurable increase in CD (lag time) and the rate of CD accumulation (propagation rate) gives an indication of LDL susceptibility to oxidation.

Insulin resistance

As known for several decades (Himsworth, 1936), insulin resistance is associated with the increasing risk of ASCVD (77). Insulin resistance, is a key component of what has been come to be known as metabolic syndrome X. Metabolic syndrome X consists of a characteristic constellation of risk factors for heart disease that include hyperglycemia (high triglyceride > 150 mg/dL), low HDL-C (< 40 mg/dL for man and 50 mg/dL for woman), insulin resistance (high fasting blood glucose > 110 mg/dL), central obesity and hypertension (130/85 mmHg or higher) (14, 78, 79), NCEP defined metabolism syndrome as presence any three of these five risk factors. Insulin resistance can be affected by both genetic and environmental factors. environmental factors are modifiable and include physical activity, dietary intake, and most especially carbohydrate and fat content and composition (14). Epidemiological studies indicate that dietary MUFA stimulate β-cell insulin secrete (26, 27), suggesting that increased MUFA intake can delay the onset of frank diabetes in insulin resistant individuals.

Homocysteine

Hyperhomocysteinemia is characterized by a high plasma concentration of homocysteine and is an independent risk factor for ASCVD including cerebral and peripheral vascular disease. Early evidence of the pathological development of atherosclerosis in hyperhomocysteinemia was demonstrated in 1969 in three children with a genetic defect in homocysteine metabolism (56). Many epidemiological studies in populations lacking genetic defects in homocysteine metabolism demonstrated that elevated blood concentrations of homocysteine have a relationship with the development of atherosclerosis. The mechanism by which homocysteine induces the development of atherosclerosis is not fully understood. The probable mechanism involves homocysteine-induced endothelial cell dysfunction and promotion of blood clotting. Cell culture studies indicate that homocysteine, a sulfur-containing amino acid, has a role in endothelial injury and stimulates smooth muscle cell proliferation (16-18). A high homocysteine concentration was suggested to cause endothelial injury as a consequence of hydrogen peroxide generation (80). Further study indicated that normal endothelial cells function to modulate the adverse effects of homocysteine by releasing NO and forming S-No-homocysteine (81, 82). Thus, homocysteine's adverse effect in vascular tissue may cause the production of NO, leading in turn to dysfunction endothelial cells due to the formation of the S-NO-homocysteine.

Homocysteine is an endogenous amino acid that can be converted by dietary methionine to cysteine or produced in body via remethylation and transsulfuration pathway (83) (Figure 3). Thus, elevated homocysteine concentrations result from the

effects of many factors, including diseases such as chronic renal failure, cancer and genetic defects in relevant enzymes, physiological factors such as age and sex and environmental factors such as smoking, coffee consumption and dietary adequacy of folic acid, vitaminB_6 and B_{12} (84, 85). Thus, dietary and lifestyle factors can modify the concentration of homocysteine in plasma.

C - reactive protein

CRP is an acute phase reactant as its concentration rises during inflammatory episodes that occur in the body (21). Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments (23, 24). CRP is therefore a marker of inflammation. It was recently discovered that CRP also plays a role in heart disease (19, 20). Research suggests that an elevated basal concentration of CRP is closely related to risk of hypertension and cardiovascular disease (21); therefore, measurement of CRP may be a good way to determine the risk of ASCVD.

The amount of CRP produced by the body varies in each individual, and its concentration can be increased by factors such as smoking and high blood pressure, whereas weight loss and exercise are associated with reductions in CRP concentrations (24, 86). An epidemiological study indicated that CRP can be used as an independent risk index for ASCVD and also for the possible future development of ASCVD (24). CRP can be used as an inflammation marker to test ASCVD risk, even in healthy people, because it is a stable molecule. Clinical evidence indicates that the concentration of CRP is more stable for over a five-year period than other risk factors (87-89).

HDL

Epidemiologic and clinical studies have identified a multitude of risk factors for ASCVD, and low HDL concentrations have emerged as the strongest predictors for the disease (2, 90, 91). Acceleration of ASCVD development in individuals with low plasma HDL concentrations is the result of its biological role in circulation (92). The well known anti-atherogenic property of HDL is a result of its role as a lipid transporter in the reverse cholesterol transport pathway—HDL transports excess cholesterol from peripheral tissues to the liver, and its associated enzymatic and transfer protein activities, including the enzyme paraoxonase, that are able to destroy oxidized lipids (93, 94). In addition, HDL is believed to protect against atherosclerosis by inhibiting the oxidative modification of LDL (29, 30, 93). The induction of adhesion molecules and monocyte chemoattractants that facilitate the formation of foam cells was shown to both protect LDL against oxidation and to attenuate the biological activity of oxidized LDL (95). HDL has the ability to retard LDL oxidation *in vitro* as was confirmed in HDL gene knockout mice (96). However the mechanism by which HDL protects against atherosclerosis is not fully known. HDL is a diverse particle class in which individual HDL particles contain varying amounts of apolipoproteins and enzymes (97). The antioxidant and anti-atherogenic properties of HDL have been attributed to the various proteins associated with HDL (93, 95). The protective effect of HDL against the development of ASCVD appears to be complex. Most research has focused on identifying and characterizing the enzymes and proteins responsible for HDL's anti-

atherogenic propensities. PON1 is one such enzyme that is believed to contribute to the protective effects of HDL (98).

Paraoxonase

Paraoxonase is a family of enzymes, and PON1 is primarily expressed in liver and found in the circulatory system where its association with HDL-C and $ApoA_1$ has been documented to be related to its anti-atherogenic ability (99-101) through its ability to protect of lipoproteins and arterial cells against oxidation (102) (Figure 4). *In vivo* studies of PON1 knockout mice and transgenic mice suggest that this protective effect may contribute to PON1's ability to attenuate the oxidative modification of lipoprotein (96, 103, 104). Moreover, low PON1 activity is found in diseases known to provoke oxidative stress disease such as systemic lupus erythematosus, chronic liver diseases, renal dysfunction and diabetes (99, 105-108). Clinical evidence confirmed PON1's protective effect by showing that low serum PON1 enzyme can occur in subjects with ASCVD and immunostaining for PON1 confirmed that PON1 has the anti-atherogenetic activity (109, 110). The statistics study of the relationship between PON1 activity in ASCVD and non-ASCVD patient is a remarkable (111). The mechanism of PON1 retardation of atherosclerosis development is unclear, although previous study has documented that the mechanism of PON action by hydrolysis of LDL-associated phospholipids and CE hydroperoxide contributes to the development of atherosclerosis (112).

PON1 concentrations can be altered by environmental or lifestyle factors. Diet fatty acid composition alters serum PON1 activity (113). For example, used cooking oil

or *trans* fat lowers serum PON1 activity, and oleic acid can elevate serum PON1 (114-116). Moreover, fat alteration of PON1 activity was verified in mice model by showing that is a high-fat diet reduced PON activity and the anti-atherogenic effect compared to a low-fat diet (117, 118). However, studies of PON1 activity in healthy men consuming ground beef rich in oleic acid are lacking.

Dietary composition and lipid profile

Of the environmental risk factors that contribute to ASCVD, dietary factors can be controlled. Previous studies (42, 119-122) have shown that the composition and amount of dietary carbohydrates, fatty acids, proteins, amino acids, and minerals (iron) and vitamin affect the blood lipoprotein profile in humans and experimental animals. The effects of changes in dietary fatty acid (SFA and MUFA) composition and non-fat nutrient (protein, methionine and B-vitamins) on serum lipid profile changes is discussed in the following sections.

Saturated fatty acid

Epidemiologic studies indicated that a high intake of SFA is associated with a high risk of ASCVD (123). The mechanism by which an increase in SFA intake may provoke atherosclerotic progression, included elevations in the concentration of total and LDL-C (124, 125). Palmitic acid (16:0) and myristic acid (14:0) were more atherogenic and increased total cholesterol and LDL-C, whereas the SFA stearic acid (18:0) had no effect on LDL-C concentrations (37). The increase in LDL concentration by high SFA intake is caused by retardation of the activity of LDL receptor in liver (126), delaying particle clearance and increasing the oxidative susceptibility of the lipoproteins (6).

Furthermore, a high intake SFA may stimulate atherosclerosis by adhesion molecules. Importantly, individual SFA have different effects on the HDL-C. For example, lauric acid (12:0) decreases total-C: HDL-C, whereas myristic acid and palmitic acid increase its value (37).

Monounsaturated fatty acid

MUFA have the beneficial effect on lowering atherogenic risk (127). A diet with high MUFA content decreases total cholesterol by 10% and LDL-C by 14% compared to the "step II" diet containing a low, 25% dietary energy (DEI), fat diet designed to provide 7% DEI as SFAs, 12% DEI of MUFAs) (128). Furthermore, replacing a SFA diet with a high MUFA diet has a positive dose-dependent effect on lowing plasma cholesterol especially LDL-C concentration without changing the HDL-C concentration (129). Many studies have investigated mechanisms of MUFA's proposed mechanisms for anti-atherogenic effects. These studies indicated that a high MUFA diet decreases ASCVD risk by attenuating LDL oxidation which has an atherogenic effect (130). Oxidation of LDL promotes atherogenesis in several ways: 1) it stimulates adhesion and chemotactic factors that cause the accumulation of inflammatory molecules (131), 2) it stimulates platelet activating factor accumulation thus promoting a thrombogenic effect (132) and 3) it stimulates the macrophage scavenger receptor that increases foam cell formation (71).

Protein

Epidemiological study has revealed that dietary protein intake may correlate with atherosclerosis. The effect of dietary protein on the development of atherosclerosis is

dependent on its source. Soy protein and lean fish were shown to have a beneficial association, lowering risk of atherosclerosis (133, 134) as a result of protective changes the lipoprotein profile (135). In contrast, casein appeared to elevate ASCVD risk. Animals protein have more positive effect for raising ASCVD risk compared to soybean protein (135, 136). Although animal protein increased affected atherosclerosis by several studies, there was no association between dietary protein and heart disease in other studies (137). Also, a study demonstrated that the relationship between nutrients of carbohydrate, fat and cholesterol was more important than the amount or nature of protein (138). A recent review indicated that there are still many areas of diet and heart disease that need to be addressed (119).

Methionine and vitamin B

The effect of methionine on the development of atherosclerosis is well illustrated (55, 56). Methionine increases plasma lipid concentrations, which may contribute to endothelial cell injury or dysfunction as confirmed in a rabbit study in which increases in dietary methionine and cholesterol led to myocardial fibrosis (139). Methionine is enriched in animal protein and is the only dietary source of homocysteine, which is partly responsible for atherogenic effects. Methionine metabolism in the body involves several B vitamins. Previous studies indicated that deficiencies of vitamins B_6, B_{12} and folate are associated with risk of atherosclerosis by increasing plasma homocysteine (140, 141). Although other studies showed little evidence for this relationship (142, 143). Vitamin B_6, B_{12} and folate acid are essential cofactors for homocysteine metabolism via the remethylation or transsulfuration pathway (144). Folate can decrease

the hyperhomocysteinemia that causes endothelial cell dysfunction by induction of adhesion molecules (145, 146). In dietary intervention studies, elevated plasma homocysteine concentration was lowered by a daily supplement of 1 mg of folic acid and 5–100 mg vitamin B_6 (147).

CHAPTER III

MATERIAL AND METHOD

Subjects

28 healthy men (Texas A&M University faculty and staff) were recruited for this study. Originally, 30 men were recruited but 2 men were excluded for personal and health reasons. Subjects were eligible to participate if they had a fasting serum cholesterol concentration < 260 mg/dL; were between the ages of 30 and 60 yr, had no restriction of diet and no current medication, and had no history of ASCVD, diabetes, liver disease, kidney disease or cancer. All subjects were given a blood test to exclude diseases indicated. These blood tests included plasma lipid profile (total cholesterol, LDL-C, HDL-C and triglycerides), glucose, liver function test (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, albumin) and kidney function test (blood urea nitrogen, protein, major minerals). All subjects were required to be nonsmokers. Subjects provided written informed consent and were instructed to maintain regular activities and body weight (initial bodyweight were 91.3 ± 2.2kg). All 28 subjects finished three dietary phases in a crossover design. This study sample size was determined by statistics power calculations based on LDL-C and CRP and LDL diameter results as reported by Smith et al (48). The power calculation indicated that based on serum cholesterol concentration, at least 21 subjects would need to complete the treatment period (power = 0.79 at $\alpha = 0.05$).

Overall experiment design

The overall experimental design was a randomized complete block (RCB), crossover and repeated measured (Figure 5), single-blinded design. Briefly, the initial 30 healthy men were randomly and evenly divided into two groups (block). Then three ground beefs (treatment) of low MUFA, mid MUFA and high MUFA content were randomly assigned within each group for 5-wk durations, followed by crossover to a different diet after consuming the habitual diets for a 3-wk washout period following each of the three treatments. For each subject, the habitual diet (baseline) was used as control for comparison with the treatment diet (final). Finally, two subjects (subjects 3 and 23) were excluded for as personal and health reason to give a final number of 28 subjects. As these two subjects were from two separate groups, the study still met the RCB design.

The total duration of this experimental design was 24-week (Figure 6). Each test ground beef was consumed for 5 weeks, followed by a 3 week habitual diet washout period prior to crossover to the next test ground beef diet phase. Prior to each diet phase, and once during each phase, participants completed a four-day record that included one weekend day of their habitual diets. All blood samples were taken before and after consumption of each test ground beef diet.

Each subject involved in this study was blinded to diet assignments. Body weight and diet were recorded at baseline and final. DEXA Body fat was determined at pre-study (28.2%) and post-study (27.8%). Energy change was calculated during the course of treatment to provide more information of the effect of diet as a factor in measured

variable. All subjects were instructed to maintain their regular activity and to restrict exercise and physical activities and to avoid making any special efforts toward changing their weight. Prior to each diet phase, and once during each phase, participants completed a 4-day record that included one weekend day of their customary or habitual diets (Table 2). The dietary record provided additional information about energy source and was analyzed by a registered dietitian to determine the quantity of calories being consumed from protein, carbohydrates and fat, and amount of other components in the diet, including cholesterol, vitamins, and mineral. The fatty acid compositions of diet and plasma at each control and treatment phase were determined. Two kinds of contact method were used in this study, E-mail and telephone, to encourage participation to complete this study.

Diet

In each dietary phase, the participants consumed five 114 g (4oz) ground beef patties with a final fat content of 24% by weight. Test ground beefs differed in their ratio of MUFA: SFA. The “low MUFA” test ground beef MUFA: SFA was 0.71, of the “mid MUFA” test ground beef MUFA: SFA was 0.83 and the “high MUFA” test ground beef MUFA: SFA was 1.10.

The experimental diet was provided by Texas A&M Rosenthal Meat Science & Technology Center from carcasses of Angus cattle fed either a corn- or a hay-based diet. The tested ground beef lean and fat trim were mixed to make the MUFA: SFA ratio of 0.71 (lean and fat trim from 20 mo of age, hay-fed steers,), 0.83 (lean from hay-fed steers, fat from 500-kg corn-fed steers) and 1.10 (lean and fat trim from a 24 mo of age,

600-kg corn-fed steers). The tested ground beef patties were prepared with appropriate ratios of MUFA: SFA and stored frozen. A single patty in a single meal was considered as replacement of regular meat. On the day of initial blood sampling during each phase, participants received an unlabeled box containing all of the patties for each phase. The participants cooked patties themselves. The diet fatty acid composition of the participants habitual diet was determined by calculation from daily record and tested ground beef in this study is shown in Table 2.

Blood draw and storage

All blood samples were collected from the brachial vein at each phase. Plasma was harvested from blood collected into Vacutainer® tubes containing EDTA to prevent blood clotting and held on ice prior to centrifugation within two hours. Serum was harvested from blood collected into Vacutainer® tubes with clot-activating inserts. Blood was allowed to clot at room temperature for 1h prior to placement on ice until centrifuged within 3 h of collection. Both plasma and serum were separated from the cellular elements of blood by centrifugation at 2,000 × g for 15 min at 4 ºC. Resulting plasma or serum supernatants fractions were transferred to a clean, labeled storage vial and held at –80ºC prior to use. Prior to initiation of the test period, and at end of each treatment phase, blood was drawn from participants on two non-consecutive days.

Laboratory assay method

Plasma total lipoproteins were isolated as the d < 1.2 g/mL fraction of plasma. The plasma total cholesterol was used with value for relative cholesterol distribution to calculated VLDL, LDL and HDL-C. The quantitative assay of homocysteine, CRP,

glucose, insulin and paraoxonase was determined using serum. Homocysteine was assayed by using HPLC, insulin and CRP by ELISA kit and glucose assay by an enzymatic assay kit (Sigma Chemical Co., St. Louis, MO). Paraoxonase assay was assayed by spectrophotometry. Paraoxonase was assayed in serum, not plasma because paraoxonase requires Ca^{2+} for activity and to stabilize the enzyme, Because EDTA inhibits blood clotting by Ca^{2+} chelation, EDTA-plasma samples can inhibit its activity (148).

Glucose assay

Glucose was measured using an enzymatic assay kit (Sigma Chemical Co., St. Louis, MO).

Insulin assay

Serum insulin was assayed using a commercially available ELISA kit according to the manufacturer's instructions (Millipore, Cat. # EZHI-14K). This method was based on the sandwich ELISA principle (Figure 9). The amount of each serum sample was measured in duplicate and quantified by measurement of absorbance at 450 nm using spectrophotometer. The insulin concentration was calculated using a standard curve that was prepared in the same assay using manufacturer supplied insulin standards.

HOMA calculation method

The homeostasis model assessment (HOMA) method evaluates both insulin resistance and the likelihood of pancreas dysfunction (insulin secretion) to determine glucose regulation in an individual (149). These values were used to test the effect of the consumption of different test ground beefs on insulin resistance and insulin secretion.

Insulin resistance index (IRI) = (FI (U/ml) × FG (mmol/L)/22.5

β-cell function index (β-CFI) = (20× FI (μU/ml))/ (FG (mmol/L)-3.5)

Where: fasting insulin is FI and fasting glucose is FG

Homocysteine assay

Chromatography conditions

All HPLC solvents filtered through a 5-μm pore size filter (Sigma-Alcorich, 58230-U Col: 86651-05 SUPELCOSIL™ LC-18 HPLC Column) and sparged with helium before use. To prepare solvent A, 5.443 g sodium acetate trihydrate (NaAc·3 H_2O) and 0.8 g triethanolamine was dissolved in 2 L HPLC H_2O. This solution was adjusted to pH 7.2 ± .01 at room temperature with acetic acid or sodium hydroxide. Then 8 ml tetrahydrofuran was added with mixing. Solvent B was prepared by mixing 200 mL of 100 mM NaAc·3 H_2O with 2L HPLC H_2O. The pH was adjusted to 7.2 ± .01 at room temperature, and 400 mL methanol and 400mL methyl cyanide (CH3CN). Solvent C was H_2O: methanol (1:1). Due to the "lag" in the gradient system, the washout procedure required that the gradient be commenced for 2 h with solvent C at flow rate 0.8 mL/min. Re-equilibration required use of a gradient for 30 min with solvent of A and B (86%:14%) at flow rate 0.8 mL/min. Temperature were set at 4º C and excitation and emission were set at 340 nm and 450 nm, respectively.

Procedure

The concentration of homocysteine was measured by fluorescence detection using o-phthalaldehyde and 2-mercaptoethanol (OPA/β-ME) derivatives of analytes, following separation using HPLC (150). All analyte standards and samples were

derivatized prior to analysis. The sample preparation procedure was based on a published method (151, 152) with modification. A reduction step was used to convert the bound homocysteine form to free thiol form, which was maintained in the reduced state by 100 μL of 1% β-ME in 0.04 M sodium borate solution, pH 9.5, at 37ºC for 30 min. The remaining protein was then precipitated using 100μL of 1.5 M $HClO_4$ as the precipitating reagent and 50 μL of 2 M K_2CO_3 as the neutralizing agent. Before derivatization with OPA/β-ME solution, 200 μL of mixture comprised of 25mg OPA, 625 μL methanol, 6.25 mL, 0.04 M pH 9.5 sodium borate solution, 25μL β-ME, 200μL brij® 35 solution 30%, and 50 μL of an iodoacetic acid solution were used to bind at the thiol moiety of homocysteine to the fluorophore for fluorescence detection. The iodoacetic acid solution consisted of 52 mg iodoacetate in 5 mL 0.04 M, pH 9.5, sodium borate solution. Homocysteine concentration in the samples was identified by the retention time as compared to elution time of authentic homocysteine standards (Figure 7). Homocysteine concentration was quantified using an external standard by comparing sample peak areas with areas of diluted standards of known concentration (Figure 8).

C - reactive protein assay

The CRP concentration was measured by immunonephelometry within the clinical laboratory at Scott and White Hospital, Temple, Texas. The method used serum, and employed particle-enhanced immumonephelometry (Dade Behring) (153) to test antigen-antibody complex formed by human serum CRP and monoclonal anti-CRP antibody. In this assay system, the concentration of CRP is proportional to the intensity

of the scattered light. Therefore, the CRP concentration was determined by comparison with dilutions of a standard of known concentration.

Serum paraoxonase activity assay

The activity of PON1 was assayed by using freshly prepared phenylacetate (Sigma, St. Louis Mo) as substrate (154) and determined by spectrophotometric assay. Materials were held on ice until assayed at 25 ºC in a 20 mM Tris·HCl buffer, pH 8, containing 1 mM $CaCl_2$ and 1 mM phenylacetate. Enzymatic activities were calculated based on the conversion of phenylacetate into phenyl in diluted serum as measured kinetically by the increase velocities from initial velocities at 270 nm. The enzyme activity was calculated by subtracting the increase in absorbance observed in a separately incubated blank in order to control for phenylacetate self hydrolysis. The slope of the linear portion of kinetic curve was normalized to total protein in the sample (Figure 10). An extinction coefficient for the reaction was 1310 $M^{-1}cm^{-1}$. One unit of PON1 activity was equal to 1μmol of phenylacetate hydrolysis/mL/min.

Statistical analysis

Data were analyzed using SPSS 14.0 software for Windows. Analyzed data were expressed as mean ± SD of appropriate unit notations unless otherwise noted. The effects of consumption of the different test ground beefs meals was estimated by ANOVA using a general linear model (GLM) for repeated measures ($P < 0.05$) with test ground beef and study phase as classifying variables. The treatment block effect and phase effect were estimated by ANOVA using GLM for univariate ($P < 0.05$). The

model was simplified if study block and phase was found to be non-significant. A boxplot among each three diet treatments was used in analysis.

CHAPTER IV

RESULTS

Twenty-eight of the thirty recruited subjects successfully completed this study. Two subjects (3 and 23) were excluded for personal and health reasons. The average age of all subjects was 35.6 ± 11.2 (mean ± SD); the pre-study body weight (91.3 ± 2.2 kg) and post-study body weight (90.7 kg) were not different. Similarly, pre-study DEXA body fat (28.2%) and post-study DEXA body fat (27.8%) were not different. Serum concentrations of TC, LDL-C and HDL- C were determined by another student as a component of their thesis and are presented in Table 4. Overall mean baseline values for measured parameters were calculated using data from all three phases of the study. As shown in reference normal range (Table 3), all baseline mean values (Table 5) and final mean value fell within normal ranges. All final mean values of each measured parameter (Table 6) were similar to three test ground beefs as well as being similar to baseline values.

Plasma glucose concentration, serum insulin, IRI and β-CFI, homocysteine, CRP and PON1 were analyzed for all subjects at the end of washout and diet treatment periods (Table 5 and 6) as mean value. Median values are not affected by outliers and are a better indication of central tendency. From median on all diet treatments, glucose, insulin, IRI, β-CFI and PON1 were decreased (Table 7, Figure 11-15, 17), whereas homocysteine concentration increased (Table 7, Figure16) by the test ground beefs. CRP concentration was increased when subjects consumed the low- and high-MUFA test

ground beefs and decreased when subjects consumed the mid-MUFA test ground beef (Table 7, Figure 15).

This study design was a RCB design. The block effect in each measurement was tested. The average age for group 1 (block 1) and group 2 (block 2) was 34.7 ± 11.2 and 36.5 ±11.3 yr, respectively, and there was no significant different between these two groups ($p = 0.303$). The p-value for block effect of glucose was 0.441, of insulin, 0.470, of IRI, 0.440, of β-CFI, 0.425, of homocysteine, 0.245; and of PON1 activity, 0.538 (Table 8). These p-values were not < 0.05; therefore, treatment block 1 and treatment block 2 were similar, block effect for these measurements can be removed. The p-value of the block effect for CRP was 0.020, meaning that the treatment block affected the test results. In advance to know where is block effect in three treatments. Test treatment block effect for each of three treatments based on baseline and final is shown in Table 9. The p value for the low-MUFA diet for final was 0.02. This indicated that there was a treatment block effect for the final, low-MUFA diet. The boxplot of low-MUFA diet treatment for the treatment block data was showed that there were two outliers (Figure 19) this was also shown in Q-Q plot (Figure 20).

This study was of a crossover, repeated measurement design. Each subject finished treatments in all studied periods at a different time period, and each subject served as his own control. Each subject was randomly assigned to each treatment order (Phase). In order to exclude a phase effect, the phase effect was tested based on the baseline value. The significance level of the phase effect for baseline value of glucose was $p = 0.271$, for insulin $p = 0.432$, for IRI $p = 0.391$, for β-CFI $p = 0.984$, for CRP $p =$

0.822 and for paraoxonase p = 0.892 (Table 10). These p values were not < 0.05; hence there was no significant effect of phases. Thus the phase effects on glucose, insulin, IRI, β-CFI, CRP and paraoxonase were removed, and then the effect of treatment on these values were tested. The p value for homocysteine baseline was 0.000, meaning that the three phases did not have a similar effect. The p value of the phase effect for low-MUFA beef was 0.168, for mid-MUFA beef it was 0.109 and for the high-MUFA beef it was 0.000 (Table 11). In high-MUFA diet, the mean value of homocysteine for phase 1 is 15.140 ± 0.929 μmol/L, for phase 2 is 9.600 ± 0.747 μmol/L and for phase 3 is 10.567 ± 0.828 μmol/L (Table 12). Hi MUFA diet has a greater mean value of homocysteine compare to other two diet treatment (Figure 21). In the normal Q-Q plot (Figure 22), three outliers—subject of 7 (6.1μmol/L), 24 (19.9 μmol/L) and 27(19.7 μmol/L)—for the high-MUFA diet appeared. These greatly affected the mean value of baseline value in the high-MUFA diet treatment. Habitual diet data for each subject were not completely recorded, and then there is no enough information to determine the cause of the different phase effects on the high-MUFA diet. Hence, the mean values of other two phases at baseline were used for these three subjects in place of the outlier. The overall result for all measurement of MUFA content effect among MUFA diet treatments is show in Table 13.

CHAPTER V

DISCUSSION

The overall purpose of this study was to investigate whether provision of ground beef with a fractionally higher MUFA content could lower or improve several ASCVD risk factors. Previous studies using non-whole food source of fatty acids found that SFA, especially myristic acid and palmitic acid, can increase total and LDL-C, while MUFA had the opposite result. Diets low in total fat, and therefore high in carbohydrate, increased blood TAG and lowered HDL-C (37, 42). Therefore, substitution of MUFA for SFA was expected to lower the cholesterol concentration, and hence lower ASCVD. However there is a lack of information about the effects of increasing dietary MUFA using the whole food of ground beef ASCVD risk factors.

The results of this study are in accord with the original studies by Smith et al. (48) in mildly hypercholesterolemic men in which increasing beef intake significantly increased the concentration of the primary HDL apolipoprotein, $ApoA_1$. A portion of the present study completed by another student determined that there was an increase diameter of HDL-C when the subjects consumed the additional ground beef. The boxplot shown in Figure 23-25 showed the central tendency of median, 25% and 75% percentile for cholesterol values. The central tendency of LDL-C was to increase when the low-MUFA test ground beef was consumed and to decrease with consumption of the mid- and high-MUFA test ground beefs. Consumption of the high-MUFA test ground beef produced a greater decrease in LDL-C than the mid-MUFA test ground beef.

The fat composition of test ground beefs influenced HDL-C in an expected way. While, the central tendency for HDL-C was to increase with all diet treatments, the high-MUFA test ground beef increased HDL-C more than the mid- and low-MUFA test ground beefs. Lastly, the central tendency of TC was to decrease with low-MUFA test ground beef consumption, while slightly increasing with consumption of the mid-MUFA test ground beef, and to be unchanged by the consumption of the high-MUFA test ground beef. Overall, addition of the high-MUFA test ground beef increased HDL-C and decreased LDL, while the central tendency of TC was to be similar before and after addition of ground beef to the diet. In previous studies (37, 39, 44) that compared diets enriched in either SFA or MUFA decreasing SFA or increasing MUFA in the diet decreased LDL-C and increased HDL-C as compared with a reduced SFA with different proportion of MUFA diet (155). The earlier study by Smith et al. (48), as well as the present study used healthy men with an average age of 35.6 and who underwent regular physical activity.

Insulin resistance has been determined to be a risk for ASCVD. Insulin resistance is characterized by increased TAG and decreased plasma HDL-C. Obesity and physical inactivity aggravate insulin resistance. Previous studies indicates that high carbohydrate and low fat intake aggravate hyperglycemia as evidenced by increases in plasma TAG and reductions in plasma HDL-C (40-42). Compared with carbohydrate, oleic acid has no such effect (44, 156). However, a diet rich in MUFA has an effect on individual components of the HOMA score, specifically β-CFI, that SFA and PUFA rich diets do not (26). The findings in the present study showed that consumption of additional

ground beef, regardless of fat composition, decreased glucose, insulin and HOMA score components of IRI and β-CFI when compared to values obtained at baseline. All measured plasma TAG concentrations were in the normal range and were similar among the three dietary treatments. Lowering results indicate that MUFA improved the insulin resistance and lowered fasting glucose and insulin concentration. Our experiment subjects are normal-lipidemic with weight maintenance men consumed different ratio of MUFA content ground beef, and decreased metabolic indexes was shown in this study, it can be conclude that MUFA enriched ground beef has beneficial effect to lower insulin resistance.

Homocysteinemia is associated with an increase risk for ASCVD even though it was not listed in NCEP. A high plasma homocysteine concentration is associated with several traditional risk factors. Dietary deficiency of the vitamins folic acid, vitamin B6 and vitamin B12 have been considered as factors that play key roles in plasma homocysteine concentration. Methionine, an essential amino acid, greatly affects homocysteinemia. Ground beef furnishes the greatest percentage of animal protein with a high amount of methionine, thus, the dietary methionine status and amount of protein intake seemingly influence the plasma homocysteine concentration. In the present study, each of the three dietary treatments increased plasma homocysteine. The ground beef used in the present study was 110g beef patties with a total fat content of 24% by weight. Based on the USDA database, 110g raw ground beef with 24% fat contains 16.04 g protein, 24 g lipid, 0.305 g vitamin B6, 0 g folic acid, 2.11 g vitamin B12 and 1.82 g iron. There is a lack of folic acid in ground beef. Folic acid is a key cofactor in the

remethylation pathway for conversion of homocysteine to methionine and it lowers the plasma concentration of homocysteine. Thus increasing folic acid intake results in decreased plasma homocysteine. However, a recent large and long term intervention trial in women (157) found that reductions in plasma homocysteine concentration by folic acid supplementation had no effect on ASCVD events.

Many studies have documented that CRP can be used as a marker for chronic vascular inflammation and that elevations in CRP can indicate the degree of ongoing atherosclerosis. However, CRP values can be changed by environmental, lifestyle and mild infection (e.g. a cold). Thus, a single increased CRP value cannot be a specific indicator for a particular disease. In the present study, CRP increased slightly from 1.56 to 1.94 (+0.38) mg/L when the low-MUFA diet was consumed, from 1.92 to 2.03 (+0.11) mg/L when the high-MUFA was consumed and decreased form 2.36 to 1.45 (–0.91) mg/L when the mid-MUFA was consumed. These CRP values were not significantly different among the three treatments. CRP value did not follow a trend from low-, mid- to high-MUFA test ground beef as did the cholesterol values discussed earlier. Based on AHA/CDC panel cutoff point of CRP value for ASCVD risk (1.0 mg/dL for low risk, 1.0-3.0 mg/dL for average risk and >3.0 mg/dL for high risk), mean values remained in the average risk category following consumption of any of the test ground beefs. However, among individuals were some different responses. Following consumption of the low-MUFA test ground beef three individuals converted from an average risk classification to high risk classification. In the mid-MUFA test ground beef group, these individuals converted from average to high risk while one individual

converted from average to low risk. In the high-MUFA group there were no changes in the number of individuals in each risk category (Table 14).

Paraoxonase has been reported to play an antioxidative function that lowers the risk of ASCVD. Its activity is affected by genetic, environmental and developmental factors such as age. Dietary fatty acid intake is known to alter serum PON1 activity (28). The present study result showed that consumption of any of the test ground beefs decreased PON1 activity. In order to more fully evaluate these results, the ratio of PON1 activity: HDL concentration was calculated. A decreased ratio of PON1 activity: HDL-C was observed after consumption of the test ground beefs (Figure 18). Also, most of PON1 values fell in the normal range (Table 15). The number of individuals with PON1 activity in the lower normal range was increased after each dietary treatment. Following consumption of the high-MUFA test ground beef more subjects tended to have PON1 activity that was in the lower than normal range. This reduced activity was unexpected. A previous study indicated that diet affects PON1 activity, being reduced by consumption of used-cooking fat containing more oxidized fat (114). Furthermore, another study showed reduced PON1 activities in women consuming a diet high in vegetable enriched with PUFA (158). Interestingly, reduced PON1 activity was found in transgenic rabbits over-expressing human $ApoA_1$ and possessing a high concentration of HDL-C (159). A recent study found that a negative relationship of homocysteine and PON1 activity (160). Furthermore, a clinical study indicated that reduced PON1 activity was associated with normal HDL concentration in acute infarction patients (161, 162).

Therefore, it is a reasonable to suggest that PON1 activity can be reduced with oleic acid provided in ground beef.

CHAPTER VI

CONCLUSION

Measurement of plasma glucose, insulin, insulin resistance and β-cell function index showed that these metabolic indexes were decreased by consumption of an additional 114g of 24% fat ground beef five times per week regardless of fat composition. Thus increased beef consumption improved insulin sensitivity in normal-lipemic, weight stable men. Together with previous study results obtained in this laboratory of reducing LDL-C, increasing the ratio of HDL-C/LDL-C and decreasing TAG it can be conclude that dietary MUFA have the beneficial effect to lower the risk of metabolic index for ASCVD. Improved insulin sensitivity is often accompanied by reduced inflammation; however, measurement of homocysteine and CRP concentrations, and PON1 activity in HDL suggested that this was not the case. Increased ground beef consumption increased the concentration of pro-oxidative amino acid homocysteine and reduced the activity of the antioxidative enzyme PON1 that is associated with HDL. This latter effect was best shown by the reductions in the PON1: HDL-C observed following 5 weeks of ground beef consumption. Interestingly, there was a progressively greater reduction in PON1: HDL-C with increasing fractional MUFA content in the test ground beefs. This is a new finding that is greatly different from those of previous studies. While these results need to be confirmed by others, these new findings suggest that whole food dietary fatty acid sources have a more complex relationship to vascular health than previously thought and require further study.

REFERENCES

1. Callow AD. Cardiovascular disease 2005--the global picture. Vascul Pharmacol 2006;45:302-7.

2. Lusis AJ. Atherosclerosis. Nature 2000;407:233-41.

3. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868-74.

4. Ross R, Glomset J, Harker L. Response to injury and atherogenesis. Am J Pathol 1977;86:675-84.

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

6. Walzem RL, Watkins S, Frankel EN, Hansen RJ, German JB. 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.

7. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol 1995;15:551-61.

8. Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. Free Radic Biol Med 2000;28:1815-26.

9. Liu J, Sempos C, Donahue RP, Dorn J, Trevisan M, Grundy SM. Joint distribution of non-HDL and LDL cholesterol and coronary heart disease risk prediction among individuals with and without diabetes. Diabetes Care 2005;28:1916-21.

10. Wilson PW. High-density lipoprotein, low-density lipoprotein and coronary artery disease. Am J Cardiol 1990;66:7A-10A.

11. Chapman MJ, Assmann G, Fruchart JC, Shepherd J, Sirtori C. Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid--a position paper developed by the European Consensus Panel on HDL-C. Curr Med Res Opin 2004;20:1253-68.

12. James T, Hein J, W, Jay N, David R. Management of Cholesterol Disorders Cardiovascular Medicine, Third Edition 2007:2667-91.

13. Rifai N, Ridker PM. Inflammatory markers and coronary heart disease. Curr Opin Lipidol 2002;13:383-9.

14. Hornstra G, Barth CA, Galli C, et al. Functional food science and the cardiovascular system. Br J Nutr 1998;80 Suppl 1:S113-46.

15. Eikelboom JW, Lonn E, Genest J, Jr., Hankey G, Yusuf S. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999;131:363-75.

16. Wall RT, Harlan JM, Harker LA, Striker GE. Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. Thromb Res 1980;18:113-21.

17. Woo KS, Sanderson JE, Sun YY, et al. Hyperhomocyst(e)inemia is a risk factor for arterial endothelial dysfunction in humans. Circulation 2000;101:E116.

18. Chen C, Halkos ME, Surowiec SM, Conklin BS, Lin PH, Lumsden AB. Effects of homocysteine on smooth muscle cell proliferation in both cell culture and artery perfusion culture models. J Surg Res 2000;88:26-33.

19. Morrow DA, Ridker PM. C-reactive protein, inflammation, and coronary risk. Med Clin North Am 2000;84:149-61, ix.

20. Mosca L. C-reactive protein--to screen or not to screen? N Engl J Med 2002;347:1615-7.

21. Ridker PM, Morrow DA. C-reactive protein, inflammation, and coronary risk. Cardiol Clin 2003;21:315-25.

22. Rifai N. C-reactive protein and coronary heart disease: diagnostic and therapeutic implications for primary prevention. Cardiovasc Toxicol 2001;1:153-7.

23. Abrams J. C-reactive protein, inflammation, and coronary risk: an update. Cardiol Clin 2003;21:327-31.

24. Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation 2001;103:1813-8.

25. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511.

26. Rojo-Martinez G, Esteva I, Ruiz de Adana MS, et al. Dietary fatty acids and insulin secretion: a population-based study. Eur J Clin Nutr 2006;60:1195-200.

27. Salas J, Lopez Miranda J, Jansen S, et al. [The diet rich in monounsaturated fat modifies in a beneficial way carbohydrate metabolism and arterial pressure]. Med Clin (Barc) 1999;113:765-9.

28. Navab M, Berliner JA, Watson AD, et al. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. Arterioscler Thromb Vasc Biol 1996;16:831-42.

29. Navab M, Hama SY, Cooke CJ, et al. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. J Lipid Res 2000;41:1481-94.

30. Navab M, Hama SY, Anantharamaiah GM, et al. 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.

31. Kontush A, Chapman MJ. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. Pharmacol Rev 2006;58:342-74.

32. Vaisar T, Pennathur S, Green PS, et al. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. J Clin Invest 2007;117:746-56.

33. Berglund L, Oliver EH, Fontanez N, et al. HDL-subpopulation patterns in response to reductions in dietary total and saturated fat intakes in healthy subjects. Am J Clin Nutr 1999;70:992-1000.

34. Patsch JR, Gotto AJ. Metabolism of high density lipoproteins. New Comprehensive Biochemistry 1987;14:221-259.

35. 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.

36. Fellin R, Baroni L, Baiocchi MR, Baldo Enzi G, Grego F, Valerio G. Selective determination of cholesterol in high density lipoprotein subfractions (HDL2 and HDL3) in patients with cerebral and peripheral arteriosclerosis. Clin Chim Acta 1985;147:233-40.

37. Grundy SM. Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. Am J Clin Nutr 1994;60:986S-990S.

38. Allman-Farinelli MA, Gomes K, Favaloro EJ, Petocz P. A diet rich in high-oleic-acid sunflower oil favorably alters low-density lipoprotein cholesterol, triglycerides, and factor VII coagulant activity. J Am Diet Assoc 2005;105:1071-9.

39. Grundy SM, Denke MA. Dietary influences on serum lipids and lipoproteins. J Lipid Res 1990;31:1149-72.

40. Starc TJ, Shea S, Cohn LC, Mosca L, Gersony WM, Deckelbaum RJ. Greater dietary intake of simple carbohydrate is associated with lower concentrations of high-density-lipoprotein cholesterol in hypercholesterolemic children. Am J Clin Nutr 1998;67:1147-54.

41. Jeppesen J, Schaaf P, Jones C, Zhou MY, Chen YD, Reaven GM. Effects of low-fat, high-carbohydrate diets on risk factors for ischemic heart disease in postmenopausal women. Am J Clin Nutr 1997;65:1027-33.

42. Abbasi F, McLaughlin T, Lamendola C, et al. High carbohydrate diets, triglyceride-rich lipoproteins, and coronary heart disease risk. Am J Cardiol 2000;85:45-8.

43. Taskinen MR. Quantitative and qualitative lipoprotein abnormalities in diabetes mellitus. Diabetes 1992;41 Suppl 2:12-7.

44. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. Lancet 1987;1:122-5.

45. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation 1998;97:2007-11.

46. Mensink RP, Zock PL, Kester AD, Katan MB. 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.

47. Sturdivant CA, Lunt DK, Smith GC, Smith SB. Fatty acid composition of subcutaneous and intramuscular adipose tissues and M. longissimus dorsi of Wagyu cattle. Meat Science 1992;32:449-458.

48. Smith DR, Wood R, Tseng S, Smith SB. Increased beef consumption increases apolipoprotein A-I but not serum cholesterol of mildly hypercholesterolemic men with different levels of habitual beef intake. Exp Biol Med (Maywood) 2002;227:266-75.

49. Wang MF, Yamamoto S, Chung HM, et al. Antihypercholesterolemic effect of undigested fraction of soybean protein in young female volunteers. J Nutr Sci Vitaminol (Tokyo) 1995;41:187-95.

50. Grundy SM, Abrams JJ. Comparison of actions of soy protein and casein on metabolism of plasma lipoproteins and cholesterol in humans. Am J Clin Nutr 1983;38:245-52.

51. Williams PT, Krauss RM, Kindel-Joyce S, Dreon DM, Vranizan KM, Wood PD. Relationship of dietary fat, protein, cholesterol, and fiber intake to atherogenic lipoproteins in men. Am J Clin Nutr 1986;44:788-97.

52. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333:276-82.

53. Schendel HE, Hansen JD. Studies on fat metabolism in kwashiorkor. I. Total serum cholesterol. Metabolism 1958;7:731-41.

54. Loschiavo C, Ferrari S, Panebianco R, et al. Effect of protein-restricted diet on serum lipids and atherosclerosis risk factors in patients with chronic renal failure. Clin Nephrol 1988;29:113-8.

55. Murphy-Chutorian DR, Wexman MP, Grieco AJ, et al. Methionine intolerance: a possible risk factor for coronary artery disease. J Am Coll Cardiol 1985;6:725-30.

56. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111-28.

57. Licastro F, Candore G, Lio D, et al. Innate immunity and inflammation in ageing: a key for understanding age-related diseases. Immun Ageing 2005;2:8.

58. Shah PK. Pathophysiology of plaque rupture and the concept of plaque stabilization. Cardiol Clin 2003;21:303-14, v.

59. Constantinides P. Atherosclerosis--a general survey and synthesis. Surv Synth Pathol Res 1984;3:477-98.

60. Harrison DG, Cai H. Endothelial control of vasomotion and nitric oxide production. Cardiol Clin 2003;21:289-302.

61. Walzem RL. Chronic disease: long-term outcomes of metabolic dysfunction. Trends in Food Science & Technology 2004;15:519-527.

62. Michael S. Brown JLG. How LDL Receptors Influence Cholesterol and Atherosclerosis.58-66.

63. Kroon PA, Powell EE. Liver, lipoproteins and disease: I. Biochemistry of lipoprotein metabolism. J Gastroenterol Hepatol 1992;7:214-24.

64. Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radical Biology and Medicine 1992;13:341-390.

65. Michael S. Brown JLG. A receptor-mediated pathway for cholesterol homeostasis. Nobel lecture, Physiology or Medicine, 1985:284-324.

66. Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radic Biol Med 1992;13:341-90.

67. Felix C Tanner MRT, Thomas F Lüscher Endothelium, lipoproteins and atherosclerotic vascular disease. Vascular Medicine 1991;2:161-176.

68. Kruth HS. Lipoprotein cholesterol and atherosclerosis. Curr Mol Med 2001;1:633-53.

69. Yla-Herttuala S, Palinski W, Rosenfeld ME, et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest 1989;84:1086-95.

70. Esterbauer H, Jurgens G, Quehenberger O, Koller E. Autoxidation of human low density lipoprotein: loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes. J Lipid Res 1987;28:495-509.

71. Hoff HF, O'Neil JA. Oxidation of LDL: role in atherogenesis. Klin Wochenschr 1991;69:1032-8.

72. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320:915-24.

73. Rizzo M, Berneis K. Low-density lipoprotein size and cardiovascular prevention. Eur J Intern Med 2006;17:77-80.

74. Auerbach BJ, Kiely JS, Cornicelli JA. A spectrophotometric microtiter-based assay for the detection of hydroperoxy derivatives of linoleic acid. Analytical Biochemistry 1992;201:375-380.

75. Yu J, Wang L, Walzem RL, Miller EG, Pike LM, Patil BS. Antioxidant activity of citrus limonoids, favonoids, and coumarins. J. Agric. Food Chem. 2005;53:2009-2014.

76. Yu J, Smith G, Gross HB, Hansen RJ, Levenberg J, Walzem RL. Enzymatic O-methylation of flavanols changes lag time, propagation rate, and total oxidation during in vitro model triacylglycerol-rich lipoprotein oxidation. J. Agric. Food Chem. 2006;54:8403-8408.

77. Himsworth H. Diabetes mellitus: a differentiation into insulin-sensitive and insulin-insensitive types. Lancet 1936;1:127-30

78. Grundy SM. Metabolic syndrome: therapeutic considerations. Handb Exp Pharmacol 2005:107-33.

79. Gotto AM. NCEP ATP III guidelines incorporate global risk assessment. Am J Manag Care 2003;Suppl:1, 3.

80. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest 1986;77:1370-6.

81. Radomski MW, Salas E. Nitric oxide--biological mediator, modulator and factor of injury: its role in the pathogenesis of atherosclerosis. Atherosclerosis 1995;118 Suppl:S69-80.

82. Stamler JS, Osborne JA, Jaraki O, et al. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. J Clin Invest 1993;91:308-18.

83. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338:1042-50.

84. Gerhard GT, Duell PB. Homocysteine and atherosclerosis. Curr Opin Lipidol 1999;10:417-28.

85. Vrentzos GE, Papadakis JA, Malliaraki N, et al. Diet, serum homocysteine levels and ischaemic heart disease in a Mediterranean population. Br J Nutr 2004;91:1013-9.

86. Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces C-reactive protein levels in obese postmenopausal women. Circulation 2002;105:564-9.

87. Rifai N, Ridker PM. Proposed cardiovascular risk assessment algorithm using high-sensitivity C-reactive protein and lipid screening. Clin Chem 2001;47:28-30.

88. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002;347:1557-65.

89. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation 1999;100:230-5.

90. Superko HR, King S, 3rd. Lipid management to reduce cardiovascular risk: a new strategy is required. Circulation 2008;117:560-8; discussion 568.

91. Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. Jama 1986;256:2835-8.

92. Hughes SD, Verstuyft J, Rubin EM. HDL deficiency in genetically engineered mice requires elevated LDL to accelerate atherogenesis. Arterioscler Thromb Vasc Biol 1997;17:1725-9.

93. Davidson MH, Toth PP. High-density lipoprotein metabolism: potential therapeutic targets. Am J Cardiol 2007;100:n32-40.

94. Rader DJ. High-density lipoproteins and atherosclerosis. Am J Cardiol 2002;90:62i-70i.

95. McPherson PA, Young IS, McKibben B, McEneny J. High density lipoprotein subfractions: isolation, composition, and their duplicitous role in oxidation. J Lipid Res 2007;48:86-95.

96. Rozenberg O, Rosenblat M, Coleman R, Shih DM, Aviram M. Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knockout mice. Free Radic Biol Med 2003;34:774-84.

97. Asztalos BF, Schaefer EJ. High-density lipoprotein subpopulations in pathologic conditions. Am J Cardiol 2003;91:12E-17E.

98. Moren X, Deakin S, Liu ML, Taskinen MR, James RW. HDL subfraction distribution of paraoxonase-1 and its relevance to enzyme activity and resistance to oxidative stress. J Lipid Res 2008;49:1246-53.

99. Camps J, Marsillach J, Joven J. Measurement of serum paraoxonase-1 activity as a potential biomarker for chronic liver impairment. Clin Chim Acta 2007;386:114-5.

100. Cabana VG, Reardon CA, Feng N, Neath S, Lukens J, Getz GS. Serum paraoxonase: effect of the apolipoprotein composition of HDL and the acute phase response. J Lipid Res 2003;44:780-92.

101. Bradshaw G, Gutierrez A, Miyake JH, et al. Facilitated replacement of Kupffer cells expressing a paraoxonase-1 transgene is essential for ameliorating atherosclerosis in mice. Proc Natl Acad Sci U S A 2005;102:11029-34.

102. Van Lenten BJ, Hama SY, de Beer FC, et al. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. J Clin Invest 1995;96:2758-67.

103. Shih DM, Gu L, Xia Y-R, et al. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature 1998;394:284-287.

104. Shih DM, Xia YR, Wang XP, et al. Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. J Biol Chem 2000;275:17527-35.

105. Kiss E, Seres I, Tarr T, Kocsis Z, Szegedi G, Paragh G. Reduced paraoxonase1 activity is a risk for atherosclerosis in patients with systemic lupus erythematosus. Ann N Y Acad Sci 2007;1108:83-91.

106. Ferre N, Marsillach J, Camps J, et al. Paraoxonase-1 is associated with oxidative stress, fibrosis and FAS expression in chronic liver diseases. J Hepatol 2006;45:51-9.

107. Tartan Z, Orhan G, Kasikcioglu H, et al. The role of paraoxonase (PON) enzyme in the extent and severity of the coronary artery disease in type-2 diabetic patients. Heart Vessels 2007;22:158-64.

108. Dirican M, Sarandol E, Serdar Z, Ocak N, Dilek K. Oxidative status and prevalent cardiovascular disease in patients with chronic renal failure treated by hemodialysis. Clin Nephrol 2007;68:144-50.

109. Mackness B, Hunt R, Durrington PN, Mackness MI. Increased immunolocalization of paraoxonase, clusterin, and apolipoprotein A-I in the human artery wall with the progression of atherosclerosis. Arterioscler Thromb Vasc Biol 1997;17:1233-8.

110. Mackness MI, Durrington PN, Ayub A, Mackness B. Low serum paraoxonase: a risk factor for atherosclerotic disease? Chem Biol Interact 1999;119-120:389-97.

111. Kotur-Stevuljevic J, Spasic S, Jelic-Ivanovic Z, et al. PON1 status is influenced by oxidative stress and inflammation in coronary heart disease patients. Clin Biochem 2008.

112. Kriska T, Marathe GK, Schmidt JC, McIntyre TM, Girotti AW. Phospholipase action of platelet-activating factor acetylhydrolase, but not paraoxonase-1, on long fatty acyl chain phospholipid hydroperoxides. J Biol Chem 2007;282:100-8.

113. Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. Biochem Pharmacol 2005;69:541-50.

114. Sutherland WH, Walker RJ, de Jong SA, van Rij AM, Phillips V, Walker HL. Reduced postprandial serum paraoxonase activity after a meal rich in used cooking fat. Arterioscler Thromb Vasc Biol 1999;19:1340-7.

115. de Roos NM, Schouten EG, Scheek LM, van Tol A, Katan MB. Replacement of dietary saturated fat with trans fat reduces serum paraoxonase activity in healthy men and women. Metabolism 2002;51:1534-7.

116. Nguyen SD, Sok DE. Beneficial effect of oleoylated lipids on paraoxonase 1: protection against oxidative inactivation and stabilization. Biochem J 2003;375:275-85.

117. Shih DM, Gu L, Hama S, et al. Genetic-dietary regulation of serum paraoxonase expression and its role in atherogenesis in a mouse model. J Clin Invest 1996;97:1630-9.

118. Mackness M, Boullier A, Hennuyer N, et al. Paraoxonase activity is reduced by a pro-atherosclerotic diet in rabbits. Biochem Biophys Res Commun 2000;269:232-6.

119. Kritchevsky D. Diet and atherosclerosis. Am Heart J 1999;138:S426-30.

120. Coombes JS, Powers SK, Demirel HA, et al. Vitamin E deficiency fails to affect myocardial performance during in vivo ischemia-reperfusion. Int J Vitam Nutr Res 2000;70:293-300.

121. Szponar L, Respondek W. [The role of nutrition in the development of circulatory system diseases]. Wiad Lek 1994;47:771-8.

122. Rein D, Yokoyama WH, Xu R, Walzem RL, German JB. Dietary vitamin e in an atherogenic hamster model. Nutrition Research 1998;18:567-579.

123. Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: a critical review. J Am Coll Nutr 2001;20:5-19.

124. Caggiula AW, Mustad VA. Effects of dietary fat and fatty acids on coronary artery disease risk and total and lipoprotein cholesterol concentrations: epidemiologic studies. Am J Clin Nutr 1997;65:1597S-1610S.

125. Dreon DM, Fernstrom HA, Campos H, Blanche P, Williams PT, Krauss RM. Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men. Am J Clin Nutr 1998;67:828-36.

126. Bucci C, Seru R, Annella T, et al. Free fatty acids modulate LDL receptor activity in BHK-21 cells. Atherosclerosis 1998;137:329-40.

127. Ruiz-Gutierrez V, Morgado N, Prada JL, Perez-Jimenez F, Muriana FJ. Composition of human VLDL triacylglycerols after ingestion of olive oil and high oleic sunflower oil. J Nutr 1998;128:570-6.

128. Kris-Etherton PM, Pearson TA, Wan Y, et al. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am J Clin Nutr 1999;70:1009-15.

129. Gill JM, Brown JC, Caslake MJ, et al. Effects of dietary monounsaturated fatty acids on lipoprotein concentrations, compositions, and subfraction distributions and on VLDL apolipoprotein B kinetics: dose-dependent effects on LDL. Am J Clin Nutr 2003;78:47-56.

130. Grundy SM. Role of low-density lipoproteins in atherogenesis and development of coronary heart disease. Clin Chem 1995;41:139-46.

131. Cushing SD, Berliner JA, Valente AJ, et al. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. Proc Natl Acad Sci U S A 1990;87:5134-8.

132. Drake TA, Hannani K, Fei HH, Lavi S, Berliner JA. Minimally oxidized low-density lipoprotein induces tissue factor expression in cultured human endothelial cells. Am J Pathol 1991;138:601-7.

133. Oomen CM, Feskens EJ, Rasanen L, et al. Fish consumption and coronary heart disease mortality in Finland, Italy, and The Netherlands. Am J Epidemiol 2000;151:999-1006.

134. Anderson RL, Wolf WJ. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. J Nutr 1995;125:581S-588S.

135. Goldberg AP, Lim A, Kolar JB, Grundhauser JJ, Steinke FH, Schonfeld G. Soybean protein independently lowers plasma cholesterol levels in primary hypercholesterolemia. Atherosclerosis 1982;43:355-68.

136. RMG CKaH. Effects of dietary protein and carbohydrate on plasma cholesterol levels in relation to atherosclerosis. Journal of Food Science 1975;40:18-23.

137. Tzonou A, Kalandidi A, Trichopoulou A, et al. Diet and coronary heart disease: a case-control study in Athens, Greece. Epidemiology 1993;4:511-6.

138. Rifkind BM. Nutrient--high-density lipoprotein relationships: an overview. Prog Biochem Pharmacol 1983;19:89-109.

139. Zulli A, Hare DL, Buxton BF, Black MJ. The combination of high dietary methionine plus cholesterol induces myocardial fibrosis in rabbits. Atherosclerosis 2006;185:278-81.

140. Verhoef P, Stampfer MJ, Buring JE, et al. Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12, and folate. Am J Epidemiol 1996;143:845-59.

141. Kok FJ, Schrijver J, Hofman A, et al. Low vitamin B6 status in patients with acute myocardial infarction. Am J Cardiol 1989;63:513-6.

142. Vermaak WJ, Barnard HC, Potgieter GM, Theron HD. Vitamin B6 and coronary artery disease. Epidemiological observations and case studies. Atherosclerosis 1987;63:235-8.

143. Brattstrom L, Israelsson B, Norrving B, et al. Impaired homocysteine metabolism in early-onset cerebral and peripheral occlusive arterial disease. Effects of pyridoxine and folic acid treatment. Atherosclerosis 1990;81:51-60.

144. Castro R, Rivera I, Blom HJ, Jakobs C, Tavares de Almeida I. Homocysteine metabolism, hyperhomocysteinaemia and vascular disease: an overview. J Inherit Metab Dis 2006;29:3-20.

145. Brown KS, Huang Y, Lu ZY, Jian W, Blair IA, Whitehead AS. Mild folate deficiency induces a proatherosclerotic phenotype in endothelial cells. Atherosclerosis 2006;189:133-41.

146. Li M, Chen J, Li YS, Feng YB, Gu X, Shi CZ. Folic acid reduces adhesion molecules VCAM-1 expession in aortic of rats with hyperhomocysteinemia. Int J Cardiol 2006;106:285-8.

147. Mojtahedzadeh F, Kosaryan M, Mahdavi MR, Akbari J. The effect of folic acid supplementation in beta-thalassemia major: a randomized placebo-controlled clinical trial. Arch Iran Med 2006;9:266-8.

148. Mackness MI. Why plasma should not be used to study paraoxonase. Atherosclerosis 1998;136:195-6.

149. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

150. Nekrassova O, Lawrence NS, Compton RG. Analytical determination of homocysteine: a review. Talanta 2003;60:1085-1095.

151. Tcherkas YV, Denisenko AD. Simultaneous determination of several amino acids, including homocysteine, cysteine and glutamic acid, in human plasma by isocratic reversed-phase high-performance liquid chromatography with fluorimetric detection. J Chromatogr A 2001;913:309-13.

152. Turnell DC, Cooper JD. Rapid assay for amino acids in serum or urine by pre-column derivatization and reversed-phase liquid chromatography. Clin Chem 1982;28:527-31.

153. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem 1999;45:2136-41.

154. Gan KN, Smolen A, Eckerson HW, La Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. Drug Metab Dispos 1991;19:100-6.

155. Aro A, Pietinen P, Valsta LM, et al. Effects of reduced-fat diets with different fatty acid compositions on serum lipoprotein lipids and apolipoproteins. Public Health Nutr 1998;1:109-16.

156. Paniagua JA, de la Sacristana AG, Sanchez E, et al. A MUFA-rich diet improves posprandial glucose, lipid and GLP-1 responses in insulin-resistant subjects. J Am Coll Nutr 2007;26:434-44.

157. Albert CM, Cook NR, Gaziano JM, et al. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. Jama 2008;299:2027-36.

158. Rantala M, Silaste ML, Tuominen A, et al. Dietary modifications and gene polymorphisms alter serum paraoxonase activity in healthy women. J Nutr 2002;132:3012-7.

159. Boullier A, Hennuyer N, Tailleux A, et al. Increased levels of high-density lipoprotein cholesterol are ineffective in inhibiting the development of immune responses to oxidized low-density lipoprotein and atherosclerosis in transgenic rabbits expressing human apolipoprotein (apo) A-I with severe hypercholesterolaemia. Clin Sci (Lond) 2001;100:343-55.

160. Ferretti G, Bacchetti T, Marotti E, Curatola G. Effect of homocysteinylation on human high-density lipoproteins: a correlation with paraoxonase activity. Metabolism 2003;52:146-51.

161. Lakshman MR, Gottipati CS, Narasimhan SJ, Munoz J, Marmillot P, Nylen ES. Inverse correlation of serum paraoxonase and homocysteine thiolactonase activities and antioxidant capacity of high-density lipoprotein with the severity of cardiovascular disease in persons with type 2 diabetes mellitus. Metabolism 2006;55:1201-6.

162. Ayub A, Mackness MI, Arrol S, Mackness B, Patel J, Durrington PN. Serum paraoxonase after myocardial infarction. Arterioscler Thromb Vasc Biol 1999;19:330-5.

163. Grundy SM, Cleeman JI, Merz CN, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. J Am Coll Cardiol 2004;44:720-32.

164. Scott M. Grundy e. Third Report of the Expert Panel on Detection, Evaluation, and Treatment of the High Blood Cholesterol in Adults (Adult Treatment Panel III): Executive Summary 2001.

APPENDIX A

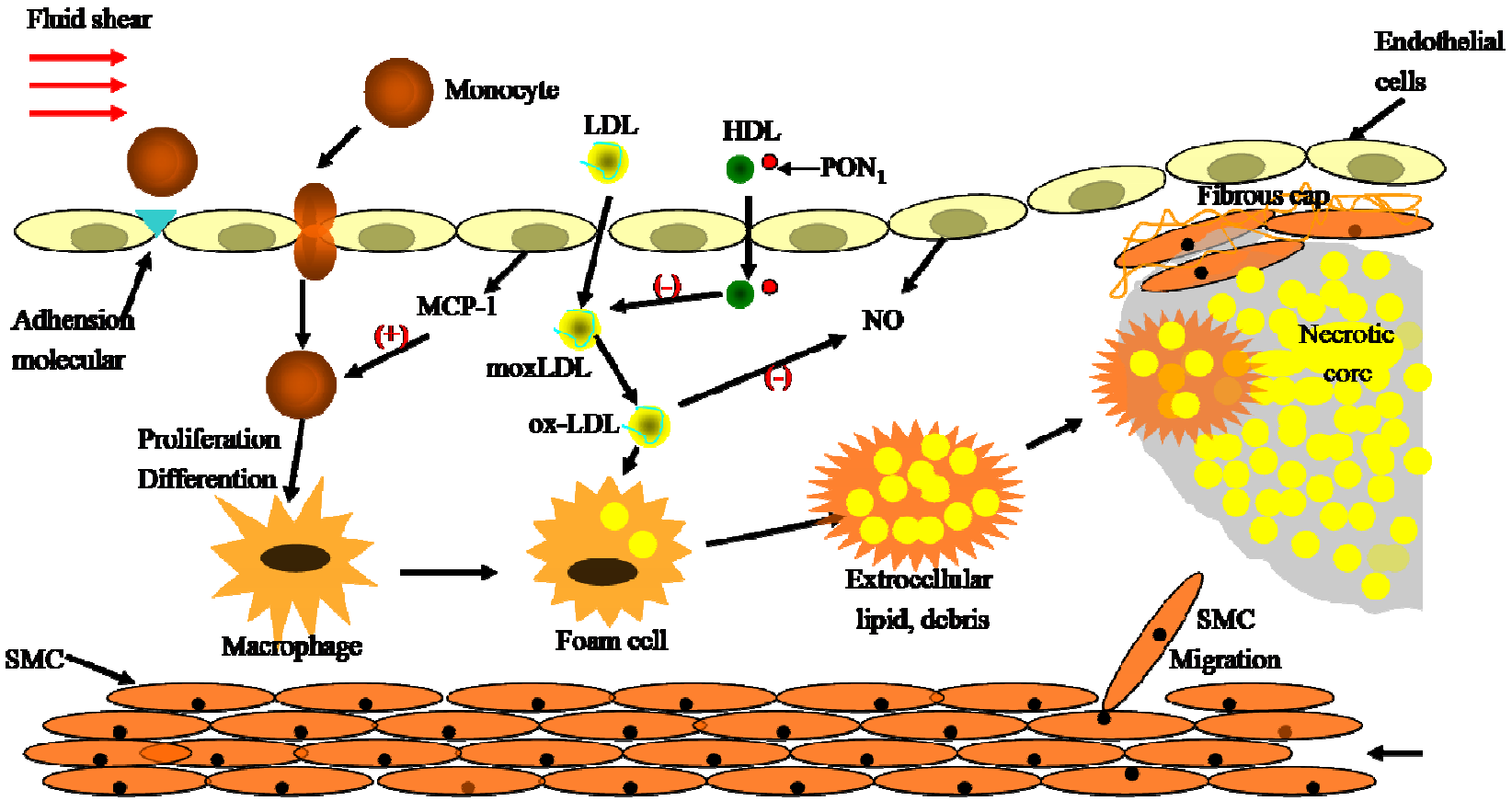

FIGURE 1. The pathological development of atherosclerotic and plaque formation.
The figure represents the monocyte and macrophage that participate in the inflammation process. This is initiated by LDL accumulation and progression by macrophage cell uptakes Ox-LDL and continues to proliferate and differentiate to form foam cell in the artery wall. HDL has the anti-atherogenetic property and caused by reverse cholesterol transport and its enzyme.

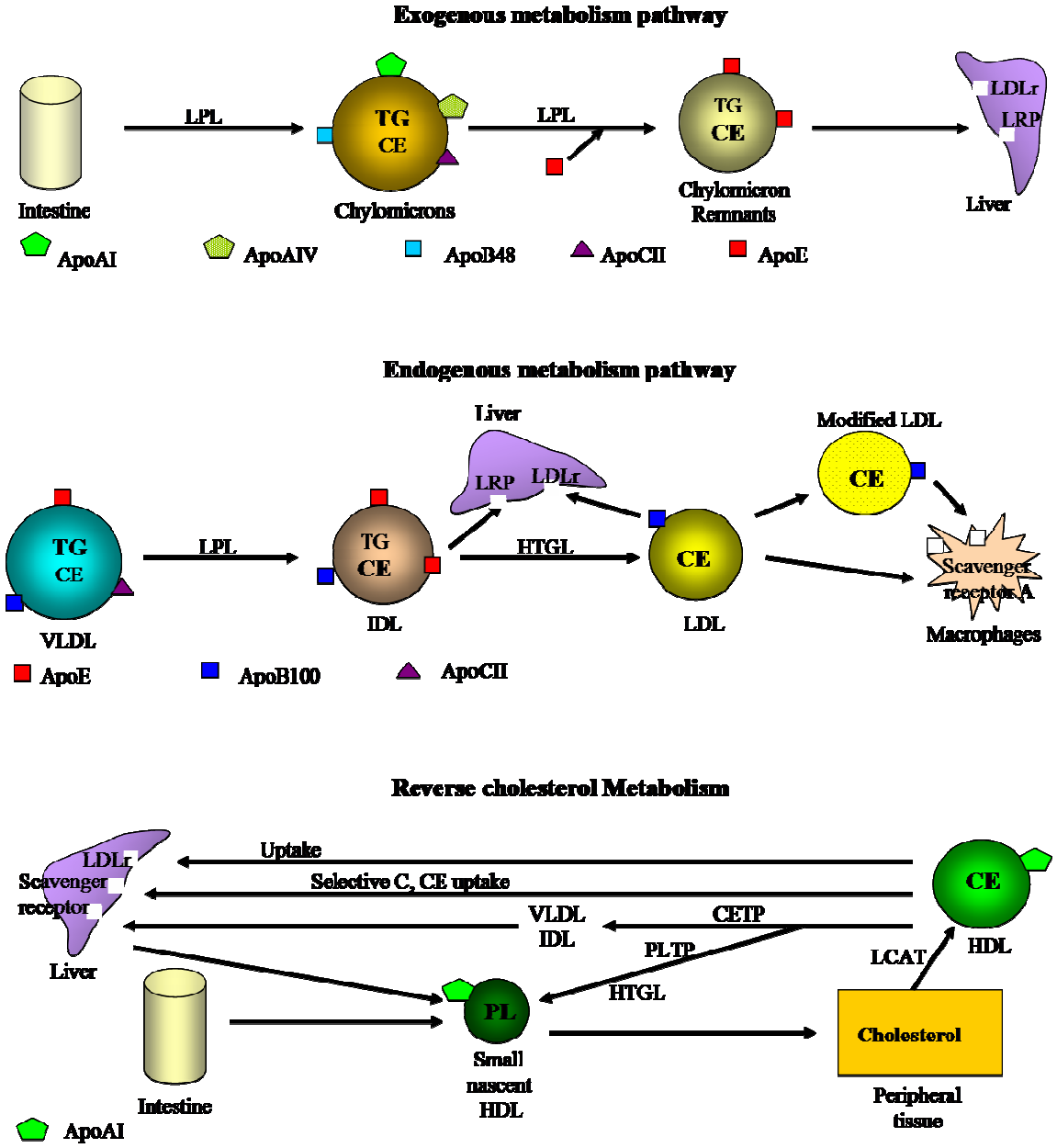

FIGURE 2. Lipoprotein metabolism.
Up: CM transfer cholesterol from intestine to liver;
Middle: VLDL and LDL transfer cholesterol from liver to the body;
Bottom: HDL transfer excess cholesterol from body back to the liver. CE: Cholesterol Ester; TG: Triacylglycerol; LPL: Lipoprotein Lipase; LDLr: LDL Receptor; LRP: Receptor-Related Protein; CETP: Cholesteryl Ester Transfer Protein; LCAT: Lecithin: Cholesterol Acyltransferase; HTGL: Hepatic Triglyceride Lipase; PLTP: Phospholipids Transfer Protein.

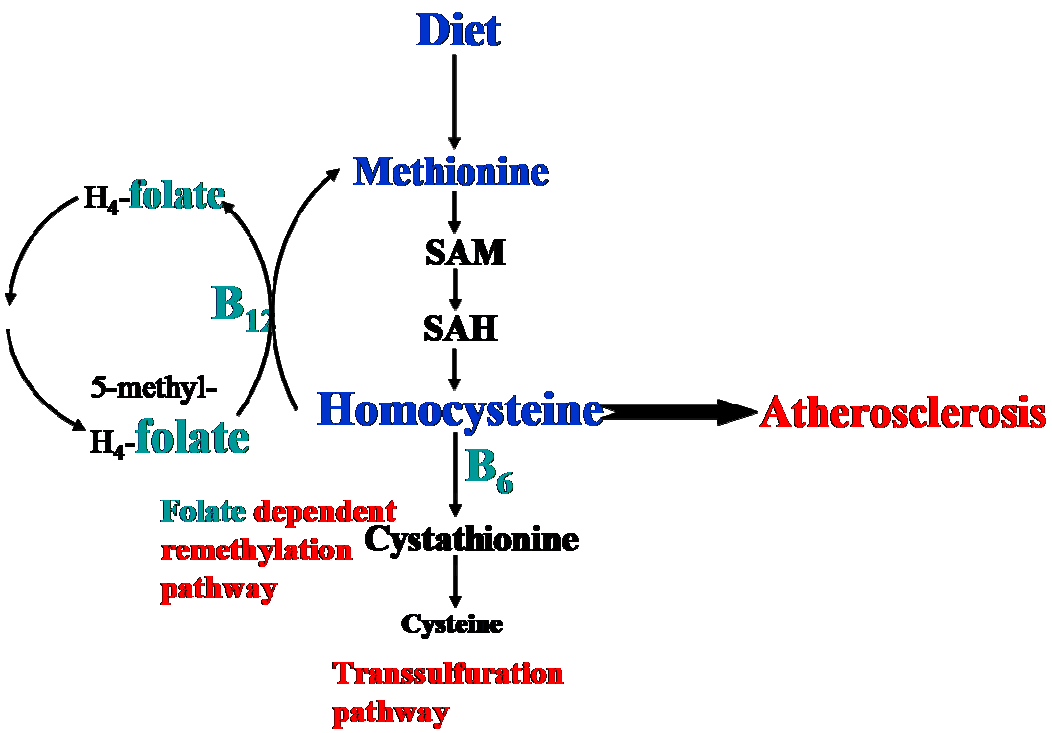

FIGURE 3. Homocysteine metabolism.
SAM: S-Adenosyl-methionine, SAH: S-Adenosyl-homocysteine, H4-folate: tetrahydrofolate, 5-methyl H4-folate. Homocysteine is a sulfur con
amino acid formed via transsulfuration and remethylation pathway.

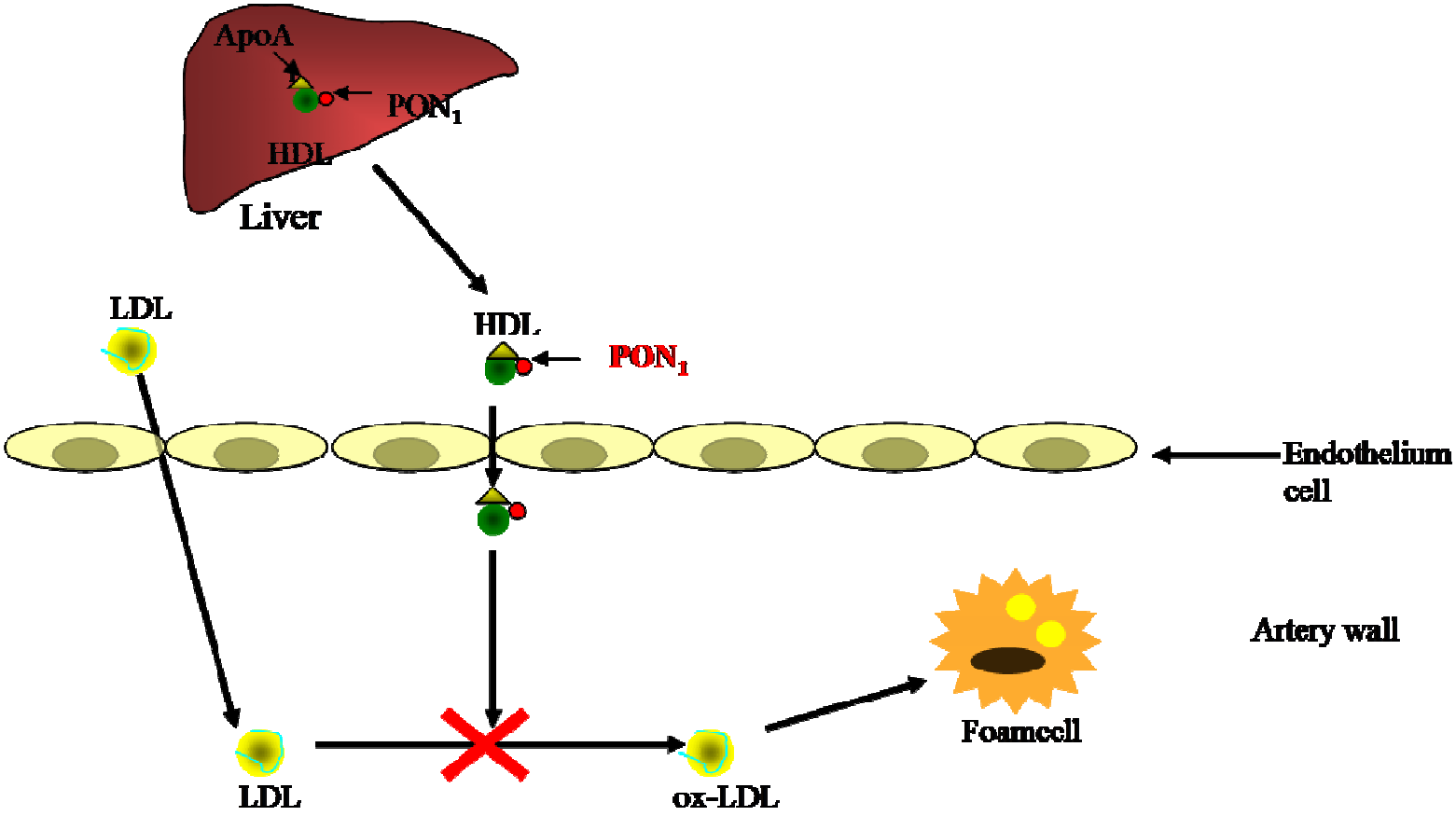

FIGURE 4. Paraoxonase (PON1) biological function.
PON1 mainly produced in liver and associated with HDL and $ApoA_1$ circulating in blood. It has the ability to inhibit LDL oxidation to form foam cell and reduce the lesion in the artery wall.

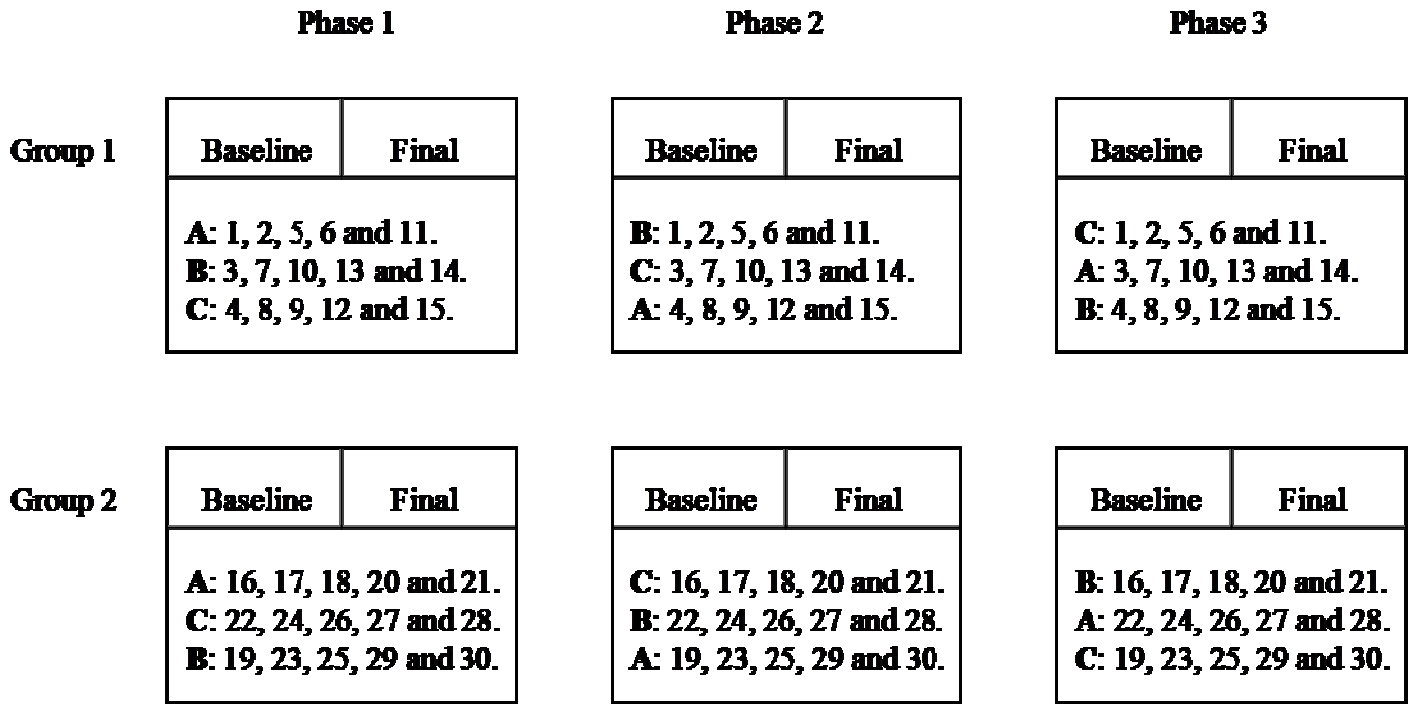

FIGURE 5. Experimental design.
The experiment design is a randomized completely block, crossover and repeated measurement study design. Group 1 and Group 2 represent treatment block 1 and treatment block 2. There are three treatments, A, B and C. "A" represents low-MUFA, "B" represents mid-MUFA and "C" represents high-MUFA ground beef. Three treatments are randomly assigned within treatment block, each treatment once in each treatment block. Each subject received all treatment (A, B and C) in randomized sequence. Each subject was randomly assigned to each treatment, and has his own control (baseline) in each treatment.

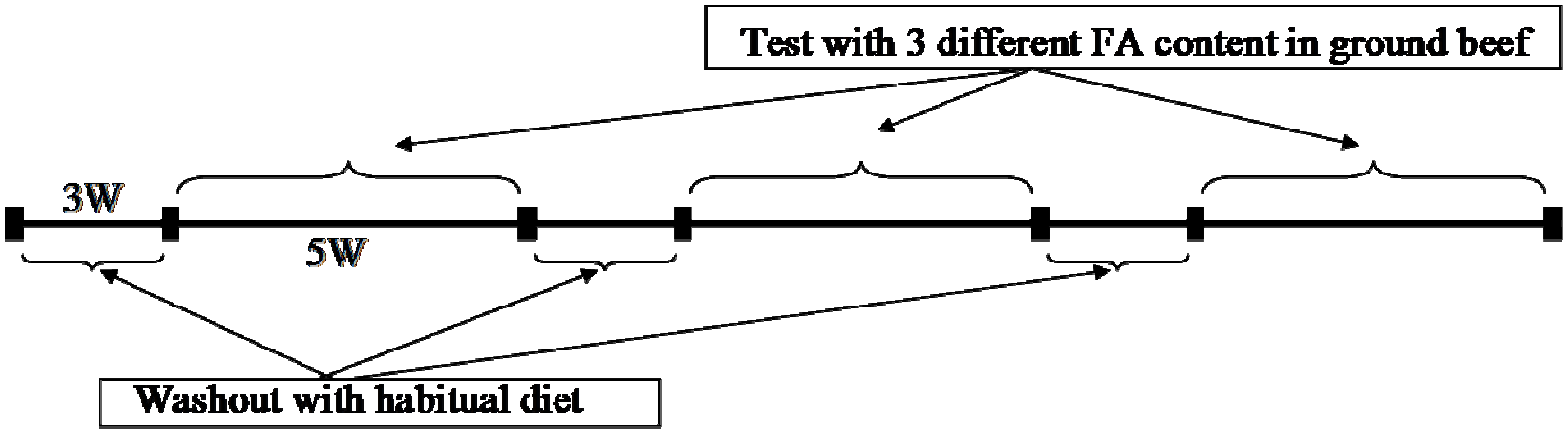

FIGURE 6. Experimental timeline.
The study was done in crossover design and was conducted in three phases. The total duration is 24 weeks. 3 weeks for washout (baseline) with habitual diet; 5 weeks for intervention (final) with three different FA compositions of ground beef. There were two blood collections at the end of baseline and final period in each phase. There were three phases with different FA composition. 3 weeks habitual diet and 5 weeks intervention diet in each phase.

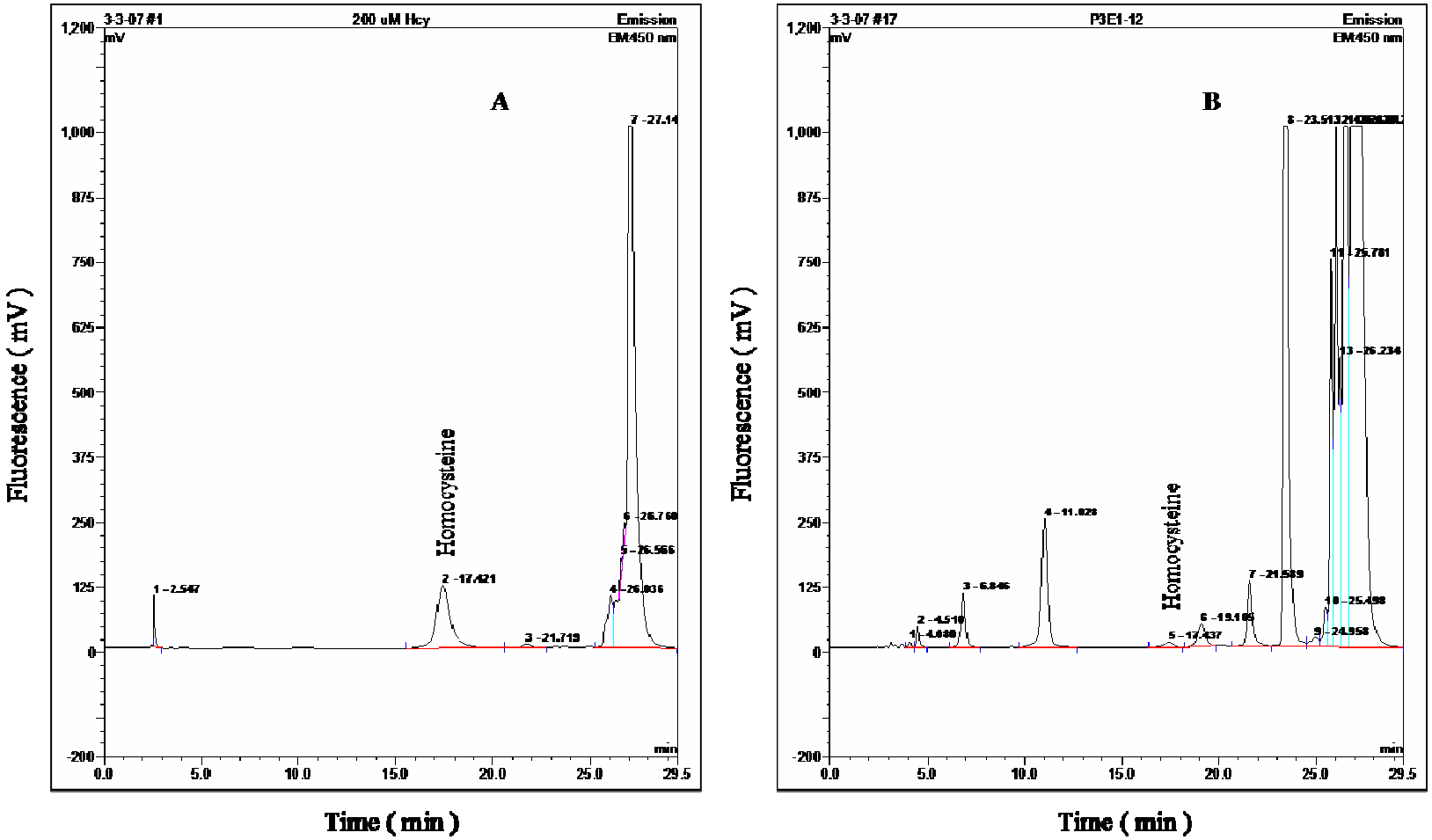

FIGURE 7-1. Chromatogram of HPLC analysis of human plasma homocysteine (Homocysteine peak).
Figure A: Homocysteine standard was analyzed after an injection of 25µL of the derivatized homocysteine standard.
Figure B: 100 µL human samples were derivatized with 200µl OPA/β-ME, injected volume 25ul.

Sample Name	Ret.Time min	Peak No	Height mV	Area mV*min	Rel.Area %	Amount nmol/ml	Type
200uM Hcy standard	17.42	2	120.003	101.243	13.94	n.a.	BM
P3E1-12	17.44	5	9.894	5.892	0.28	n.a.	BMB

FIGURE 7-2. Chromatogram of HPLC analysis of human plasma homocysteine (Retention time).
Human plasma homocysteine concentration was calculated by comparing peak areas of sample and diluted standard. Human sample peak was determined by retention time.

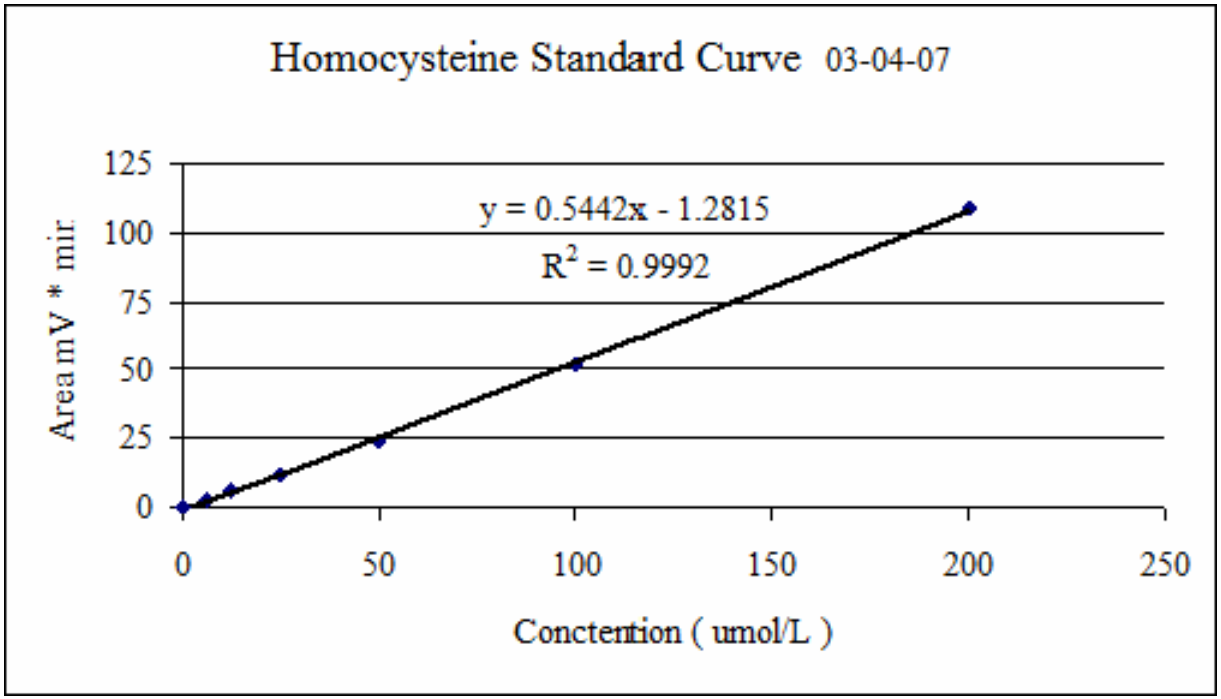

FIGURE 8. Homocysteine standard curve (one of several standard curves)**.**
Human plasma homocysteine concentration was calculated by comparing human sample with diluted homocysteine standard of known concentration. X axis indicates the concentration of standard; Y axis indicates the Area mV * min of homocysteine peak. This standard curve is the one of several standard curves.

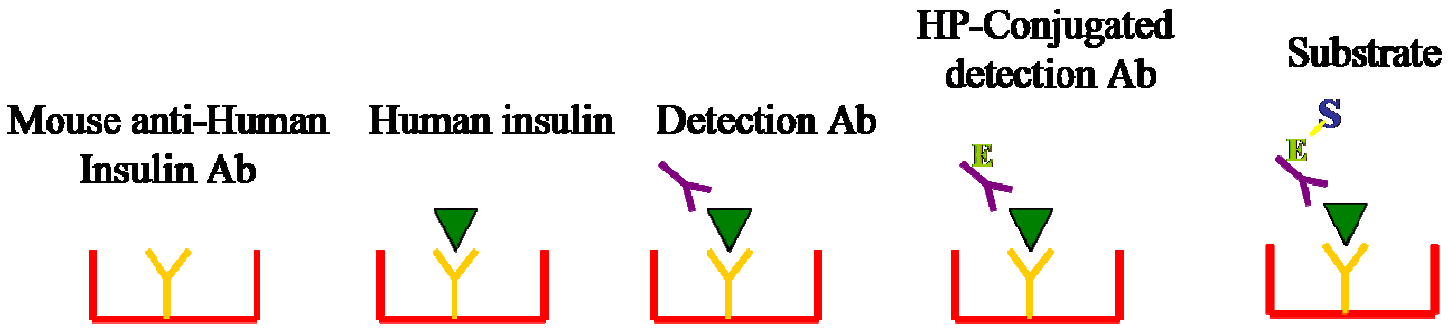

FIGURE 9. The sandwich ELISA principle for insulin assay**.**
Y represents the pre-incubate mouse anti-human insulin antibody (Ab); ▲ represents human insulin; Y represents biotinylated Ab to detect the human insulin; E represents enzyme HRP (streptavidin-horseradish peroxidase); S represents substrate (3, 3', 5, 5'-tetramethylbenzidine in Buffer-TMB).

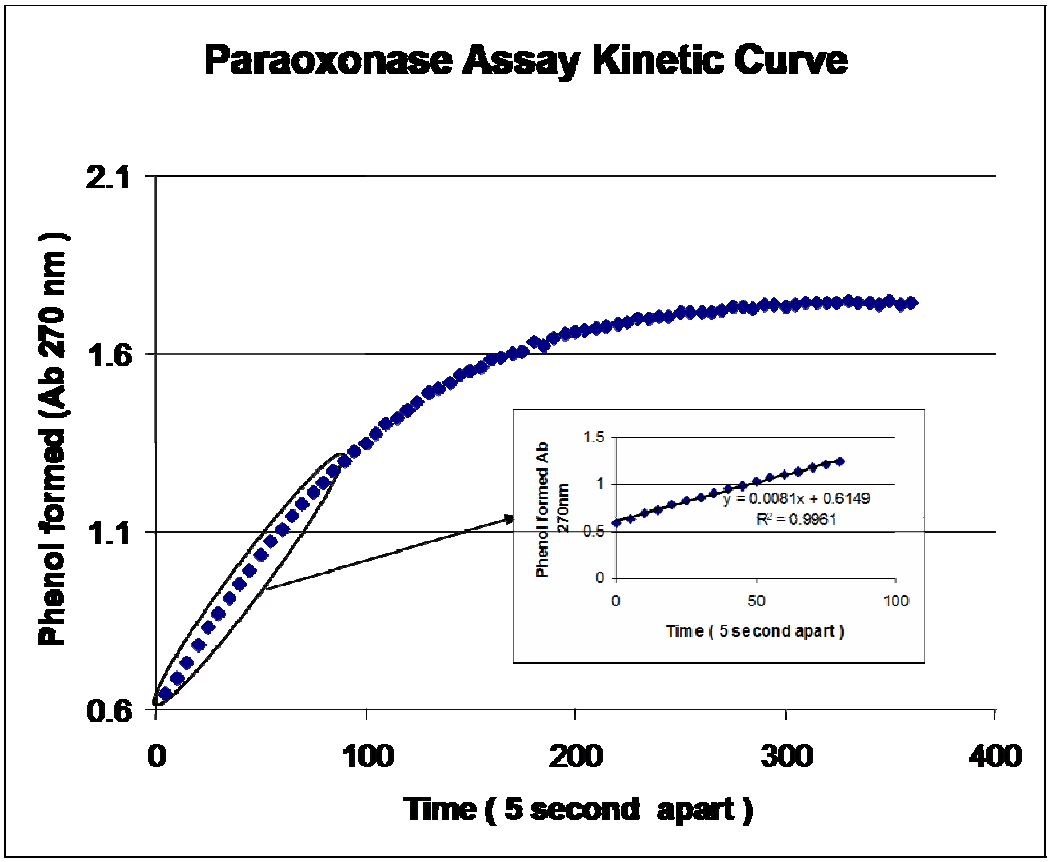

FIGURE 10. Paraoxonase assay kinetic curve.
X axis indicates the time (5 second apart) after adding paraoxonase; Y axis indicates the phenol formed based on specific absorbance at 270 nm wavelength. In the bigger figure, there is showing a kinetic curve with time changing (the product was measured apart from 5 second, total time is 360 second). The smaller insertion figure was taken the first 80 seconds of this measurement from the bigger figure and to make a linear curve (phenol formed *vs.* Time); a straight line (R^2=0.9961) indicates a linear portion is present in the paraoxonase kinetic curve at the first 80 seconds.

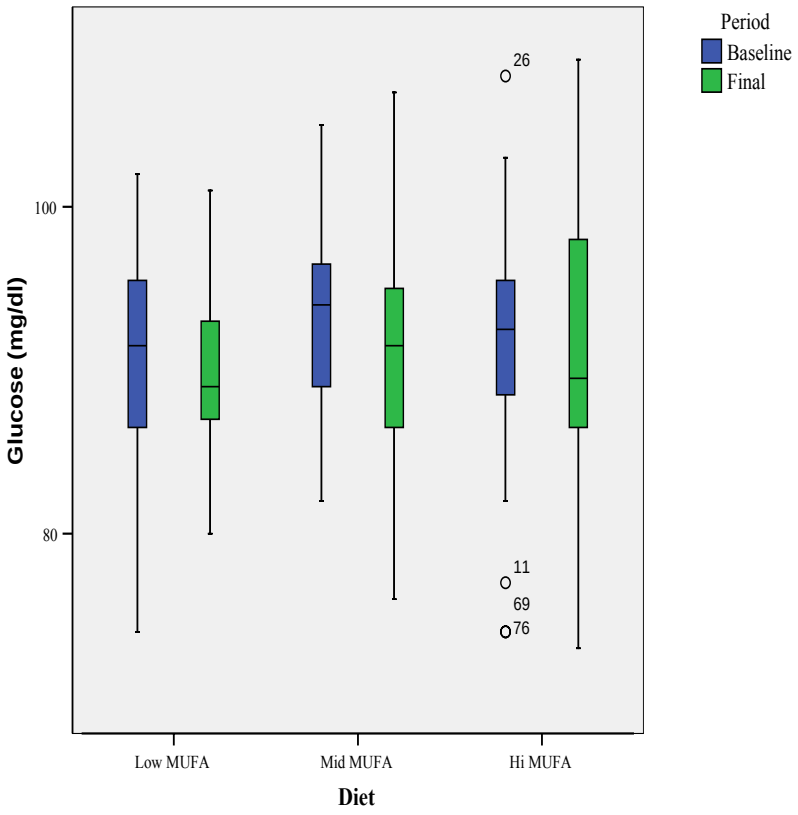

FIGURE 11. Glucose boxplot for three diet treatments.
The central line in box represents the median value for glucose concentration (mg/dl), and the bottom and upper line of box represent the lower and upper quartile. The two vertical lines outside the box extend to the smallest and largest observation 1.5 QR of the quartiles. The closed circle represents mild outlier. 26 (28-P1B1 (108)) 11 (12-P1B1 (74)) 69 (14-p2b1 (74)) 76 (21-p2b1 (74)).

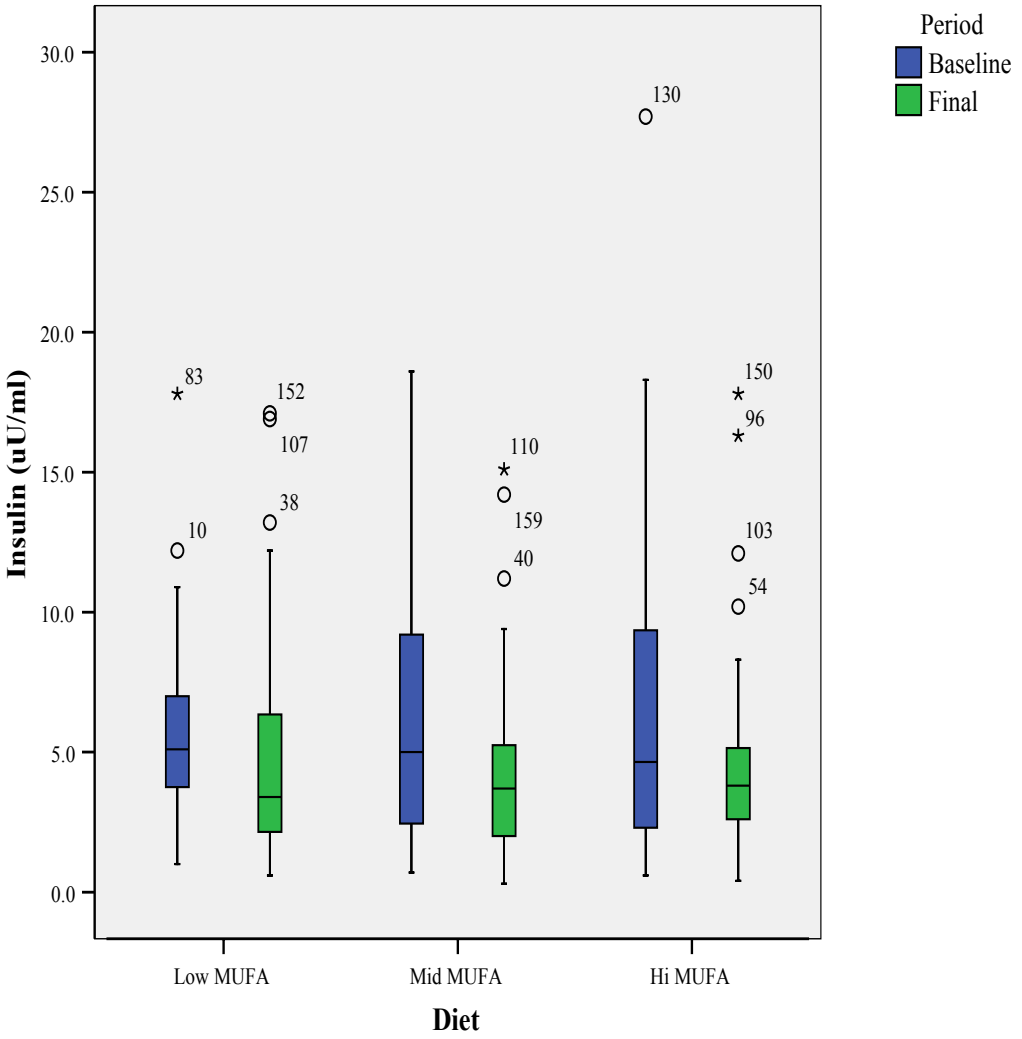

FIGURE 12. Insulin boxplot for three diet treatments.
The central line in box represents the median value for insulin concentration (μU/ml), and the bottom and upper line of box represent the lower and upper quartile. The two vertical lines outside the box extend to the smallest and largest observation 1.5 QR of the quartiles. The closed circle represents mild outlier, star presents extremely outlier.

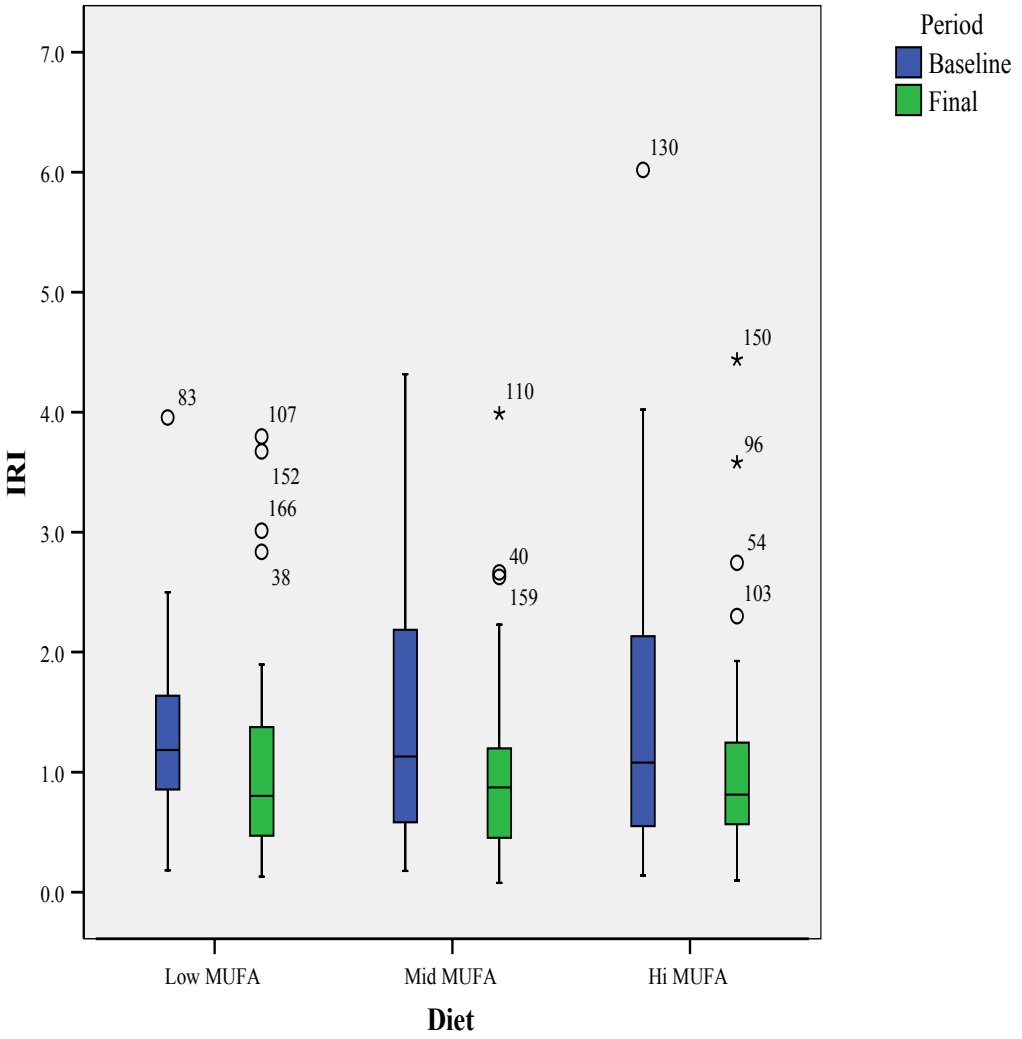

FIGURE 13. Insulin resistance index boxplot for three diet treatments.
The central line in box represents the median value of IRI, and the bottom and upper line of box represent the lower and upper quartile. The two vertical lines outside the box extend to the smallest and largest observation 1.5 QR of the quartiles. The closed circle represents mild outlier, star presents extremely outlier.

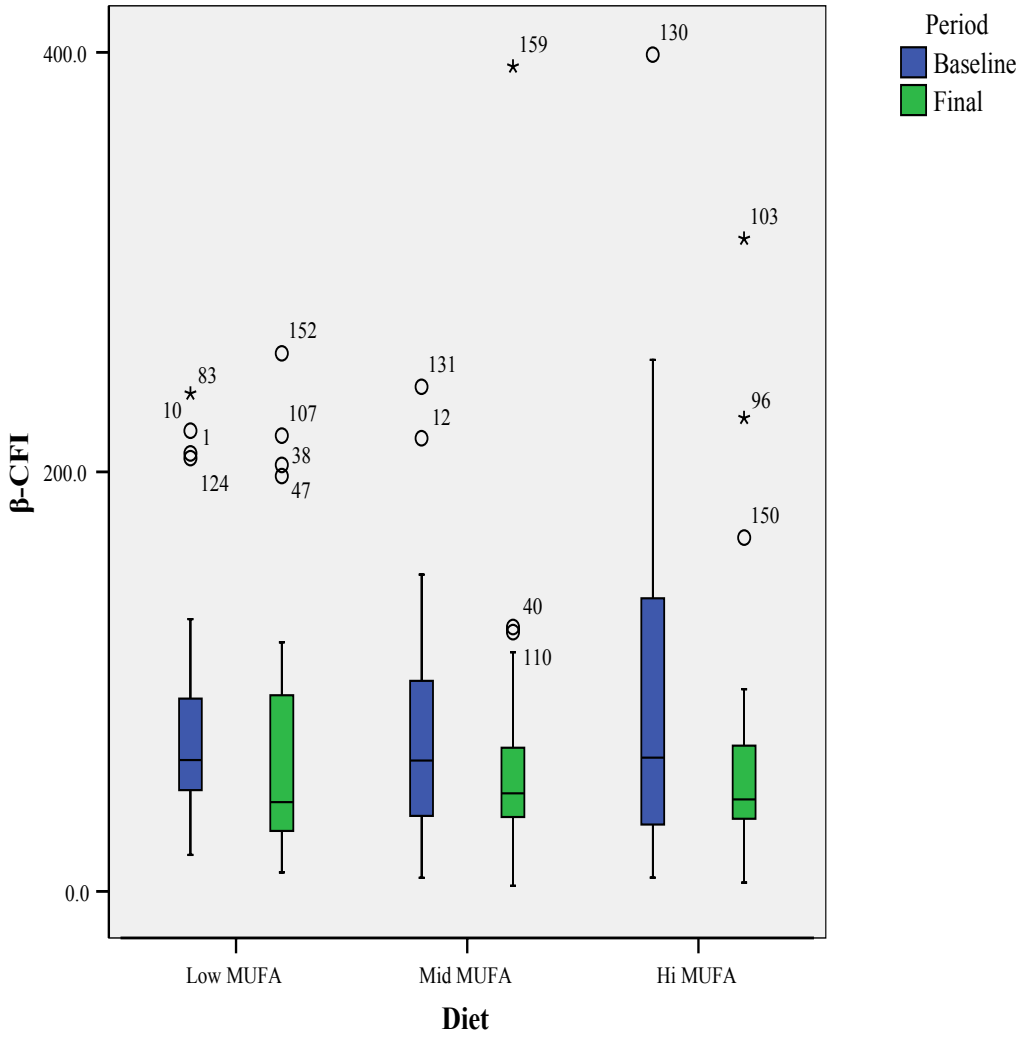

FIGURE 14. β-cell function index boxplot for three diet treatments.
The central line in box represents the median value for β-CFI, and the bottom and upper line of box represent the lower and upper quartile. The two vertical lines outside the box extend to the smallest and largest observation 1.5 QR of the quartiles. The closed circle represents mild outlier, star presents extremely outlier.

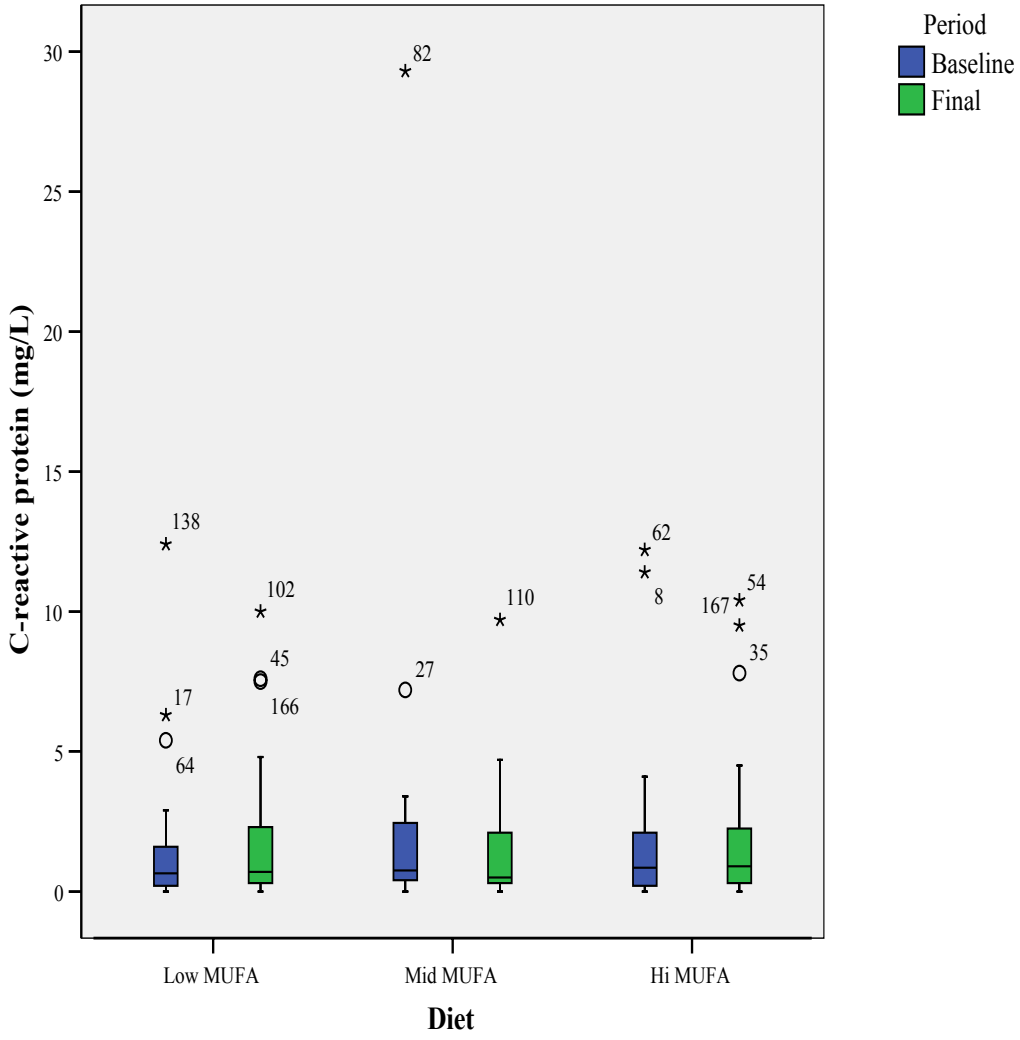

FIGURE 15. C – reactive protein boxplot for three diet treatments.
The central line in box represents the median value for CRP concentration (mg/dL), and the bottom and upper line of box represent the lower and upper quartile. The two vertical lines outside the box extend to the smallest and larges observation 1.5 QR of the quartiles. The closed circle represents outlier, star presents extremely outlier.

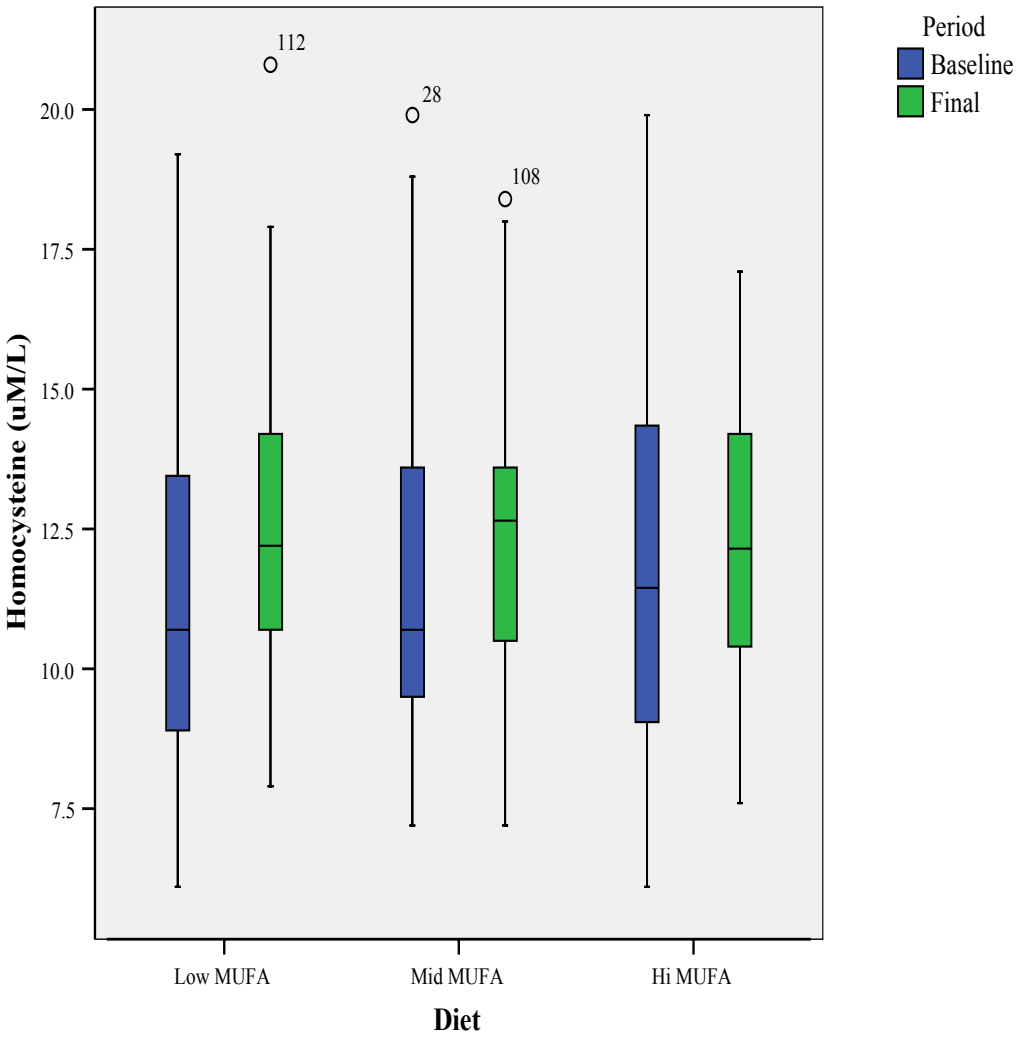

FIGURE 16. Homocysteine boxplot for three diet treatments.
The central line in box represents the median value for homocysteine concentration (μM/L), and the bottom and upper line of box represent the lower and upper quartile. The two vertical lines outside the box extend to the smallest and largest observation 1.5 QR of the quartiles. The closed circle represents outlier, star presents extremely outlier.

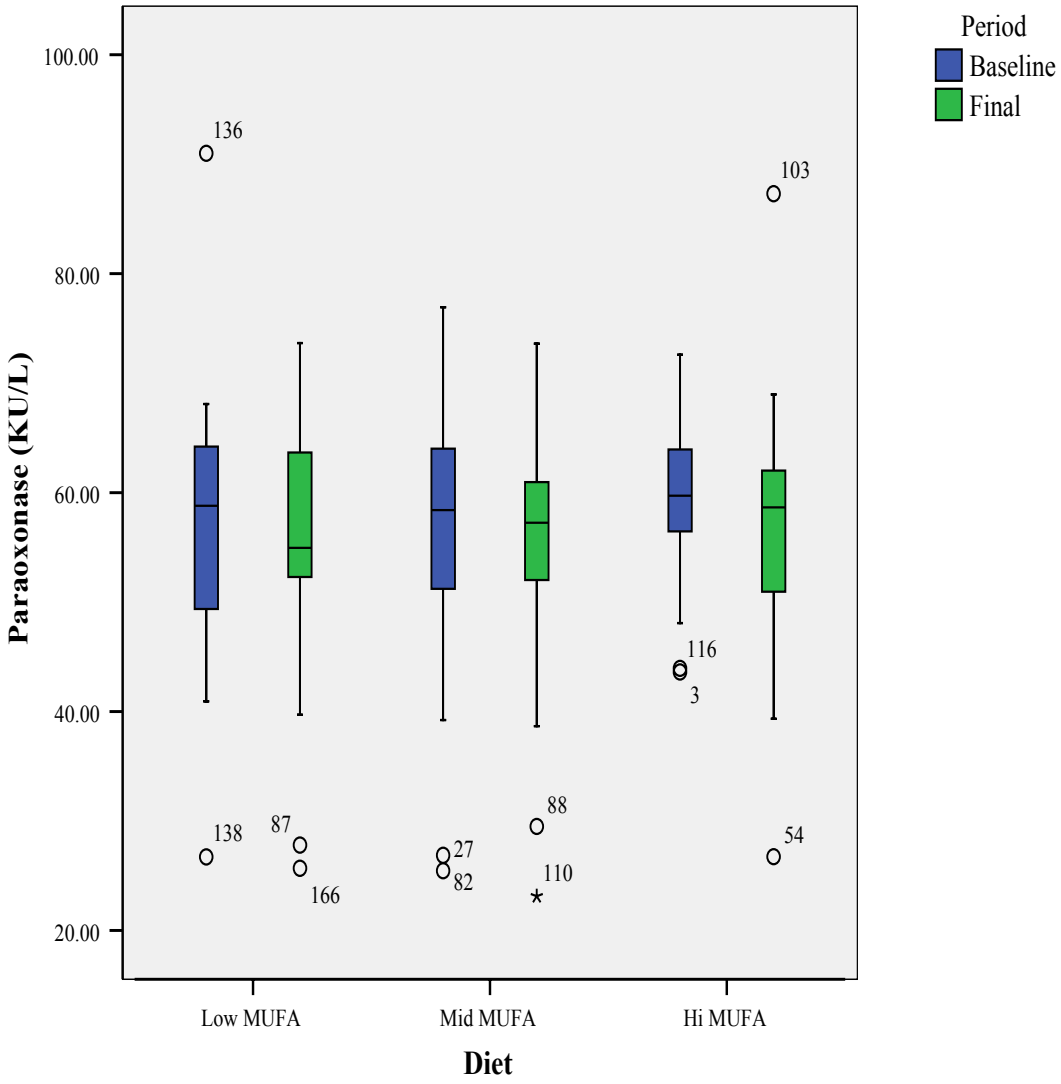

FIGURE 17. Paraoxonase boxplot for three diet treatments.
The central line in box represents the median value for glucose, and the bottom and upper line of box represent the lower and upper quartile. The two vertical lines outside the box extend to the smallest and largest observation 1.5 QR of the quartiles. The closed circle represents outlier, star presents extremely outlier.

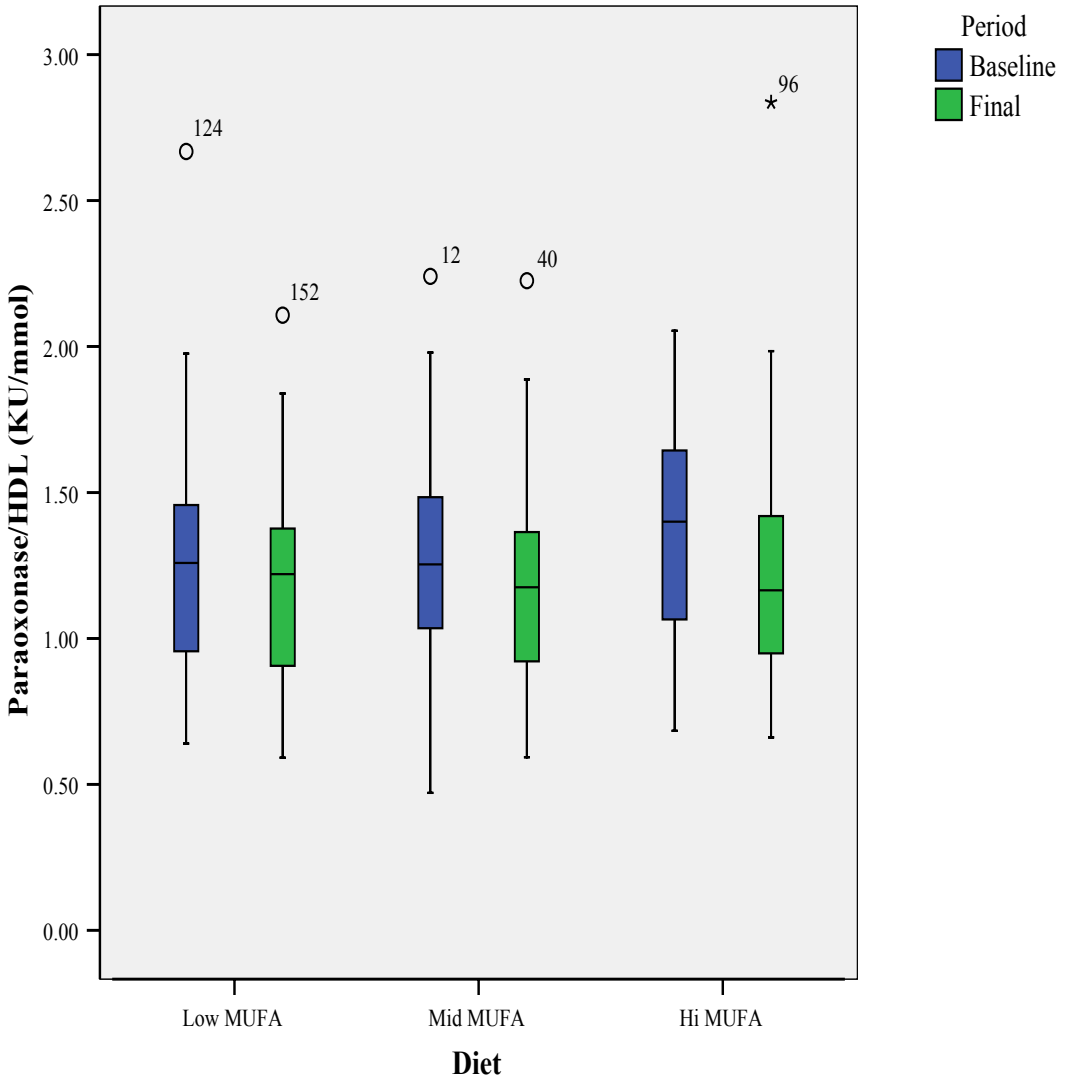

FIGURE 18. Paraoxonase/HDL boxplot for three diet treatments.
The central line in box represents median value for paraoxonase concentration (KU/mmol), and the bottom and upper line of box represent the lower and upper quartile. The two vertical lines outside the box extend to the smallest and largest observation 1.5 QR of the quartiles. The closed circle represents mild outlier, star presents extremely outlier.

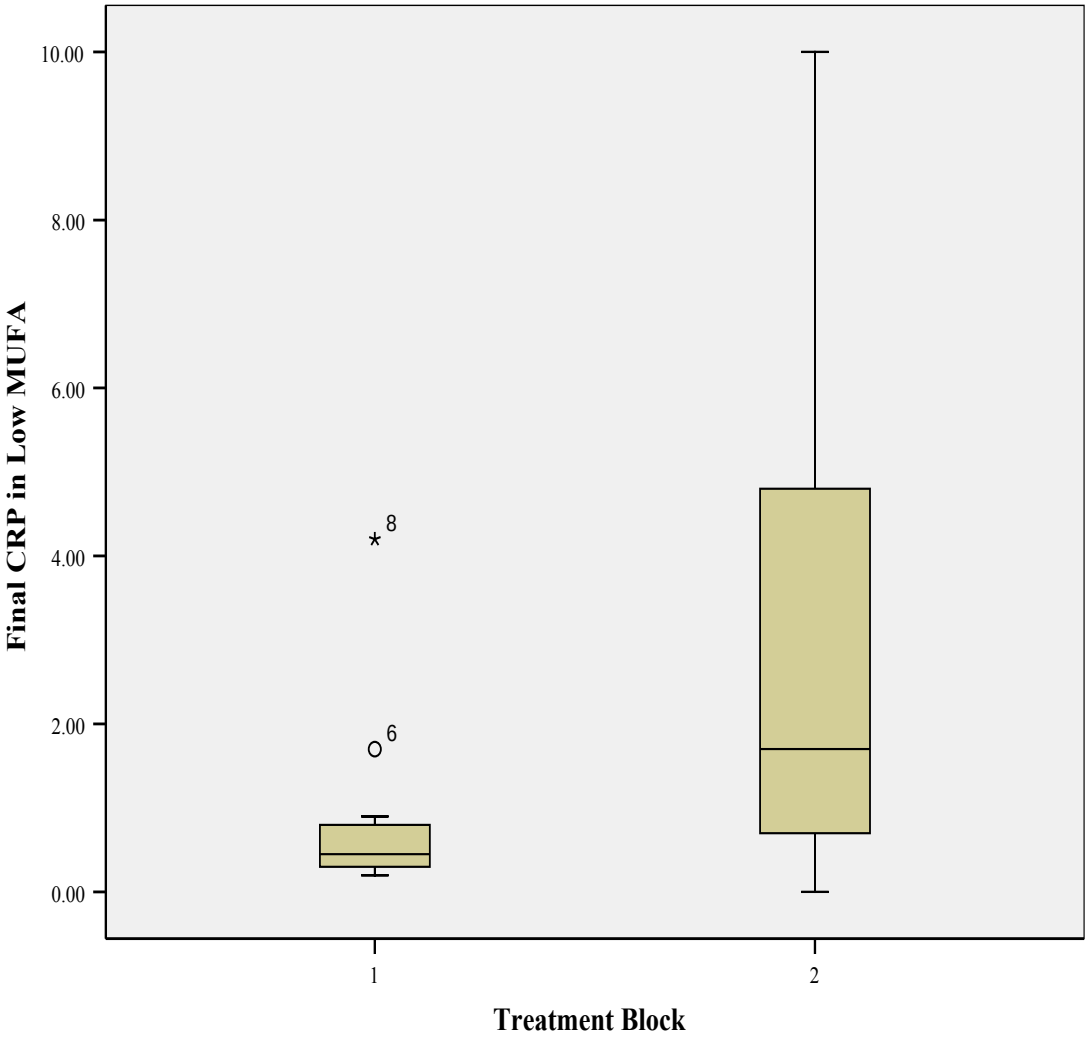

FIGURE 19. Final CRP boxplot of low-MUFA diet for treatment block.
Vertical axis indicates final CRP value in low MUFA diet treatment. The two vertical lines outside the box extend to the smallest and largest observation 1.5 QR of the quartiles. The closed circle represents mild outlier, star presents extremely outlier.

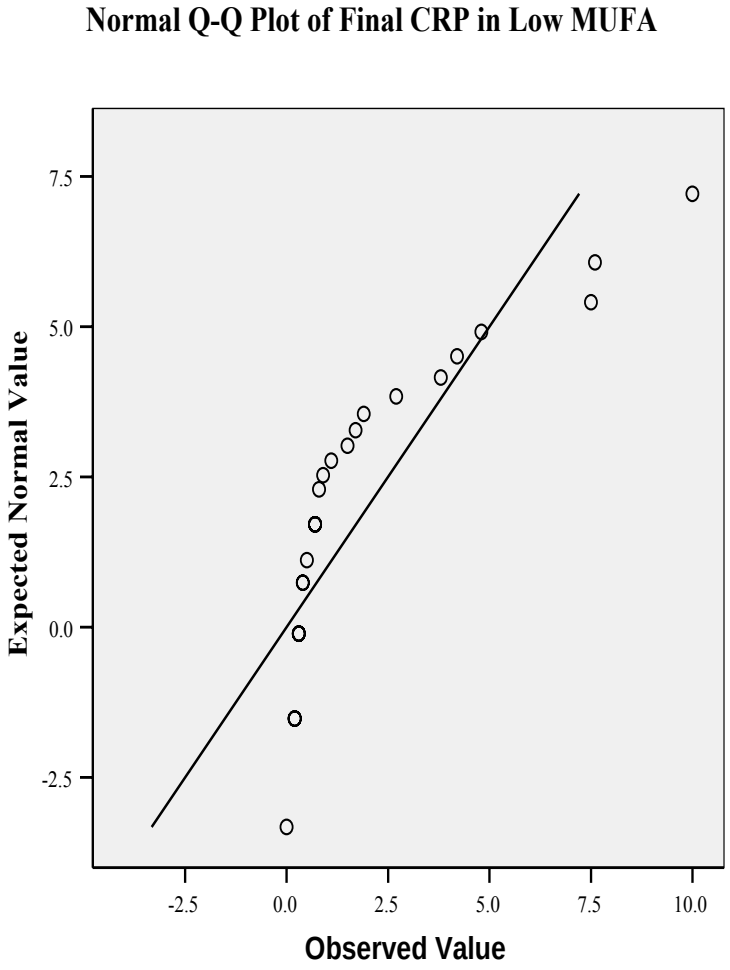

FIGURE 20. Final CRP Q-Q plot of low-MUFA diet.
Horizontal indicates observed value. Vertical axis indicates Expected normal value.

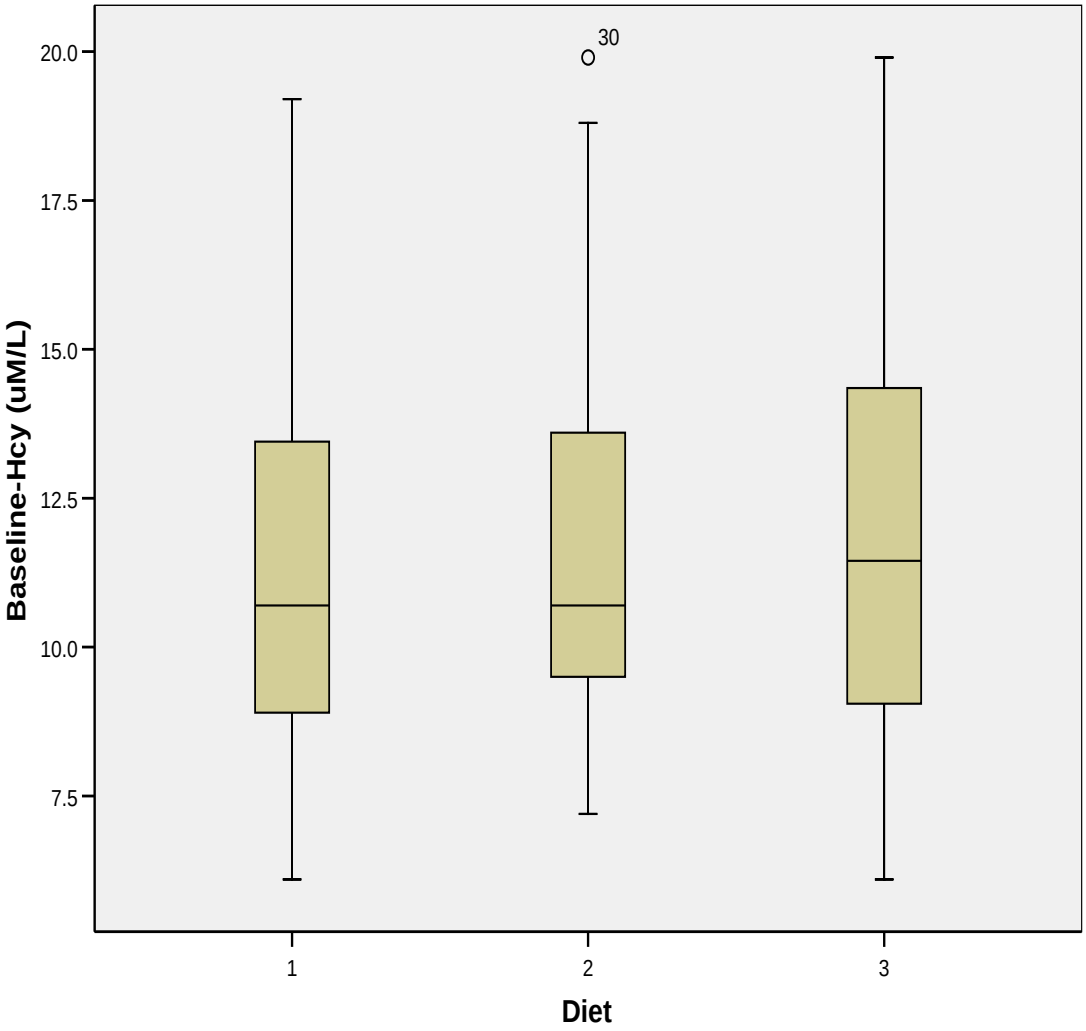

FIGURE 21. Boxplot of homocysteine baseline value for three diet treatments.
1 represents Low MUFA diet, 2 represents Mid MUFA diet and 3 presents Hi MUFA diet. Horizontal indicates 3 diet treatments. Vertical axis indicates baseline homocysteine value. The two vertical lines outside the box extend to the smallest and largest observation 1.5 QR of the quartiles. The closed circle represents mild outlier.

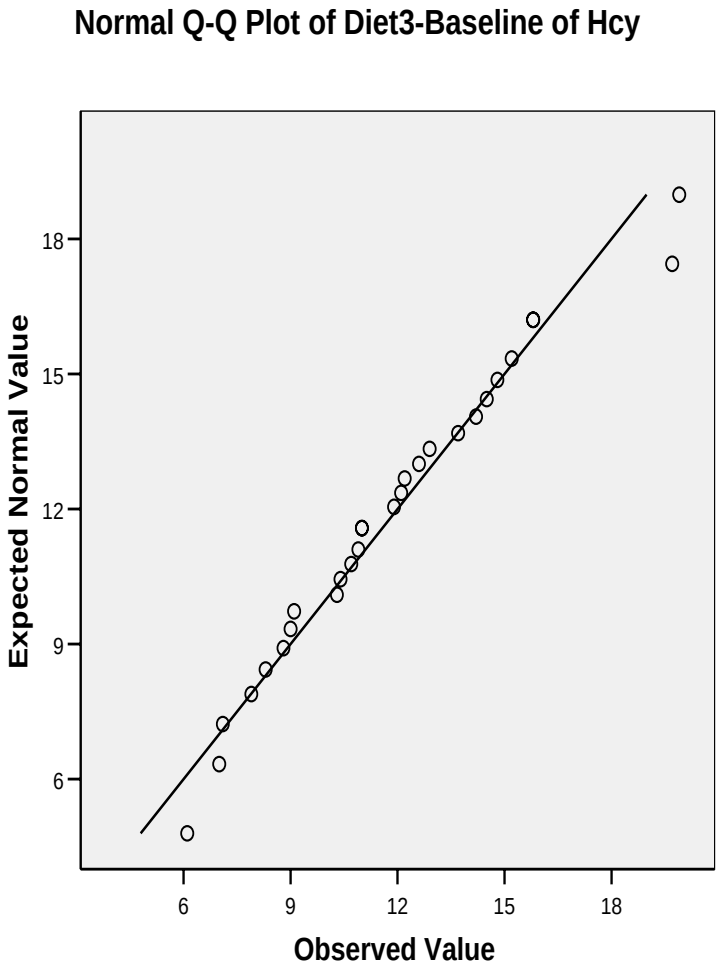

FIGURE 22. Q-Q plot of homocysteine baseline value for three diet treatments.
1 represent Low MUFA diet, 2 represent Mid MUFA diet and 3 present Hi MUFA diet.

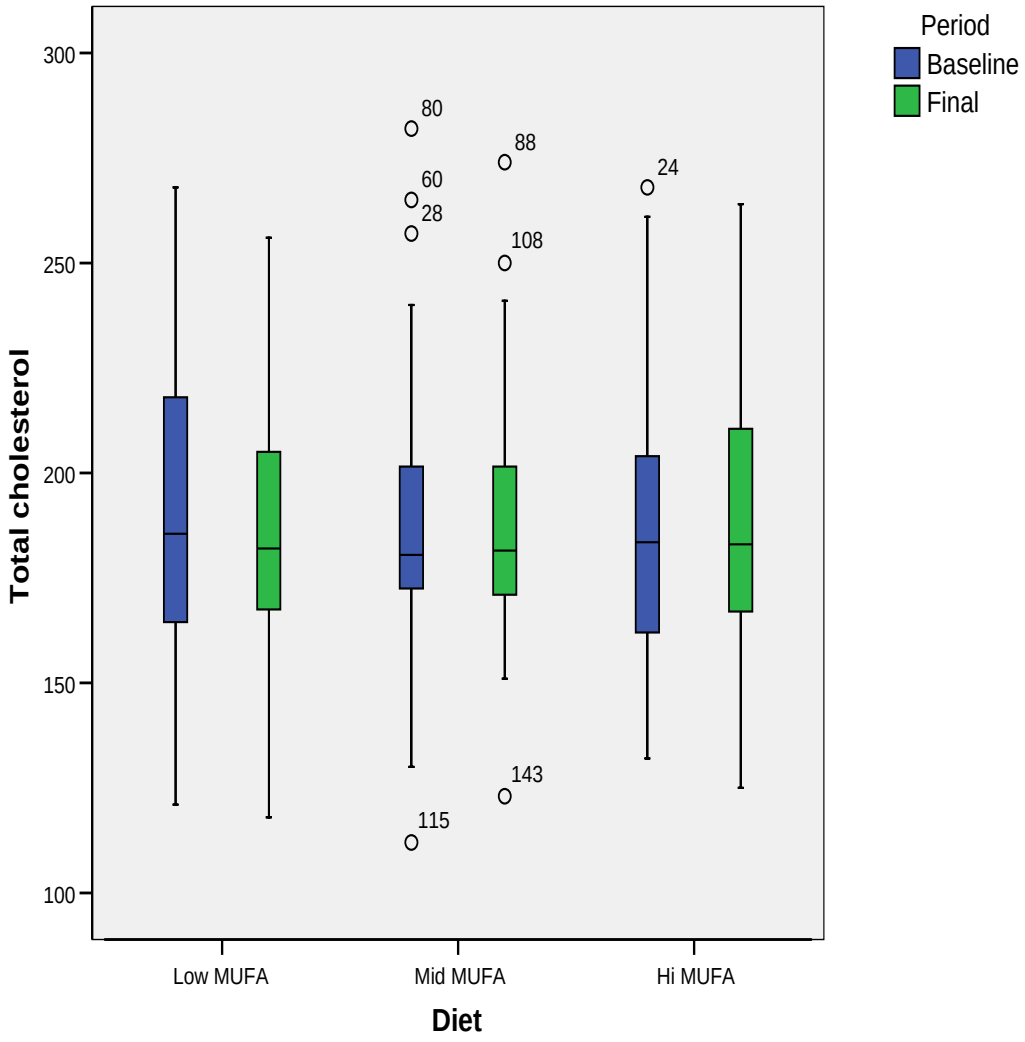

FIGURE 23. Total cholesterol boxplot for three diet treatments.

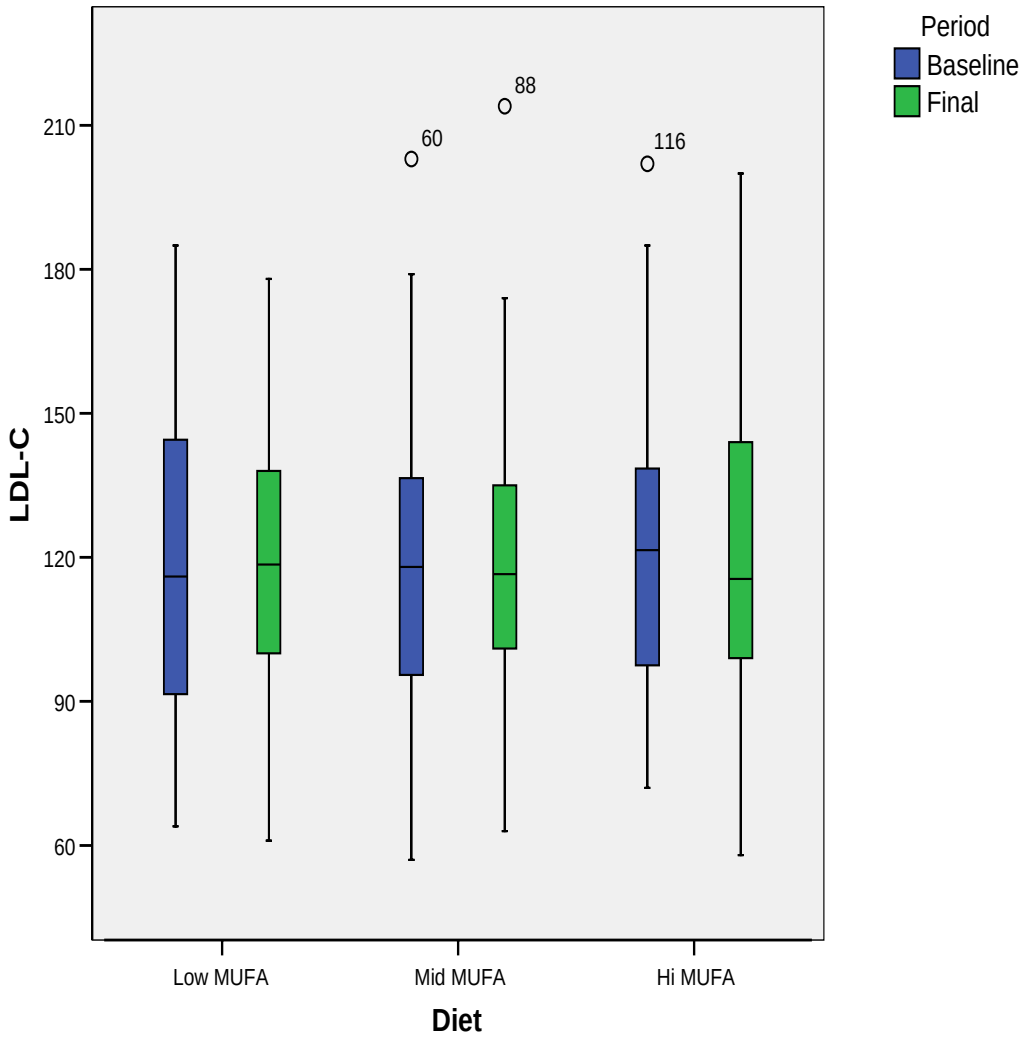

FIGURE 24. LDL-C boxplot for three diet treatments.

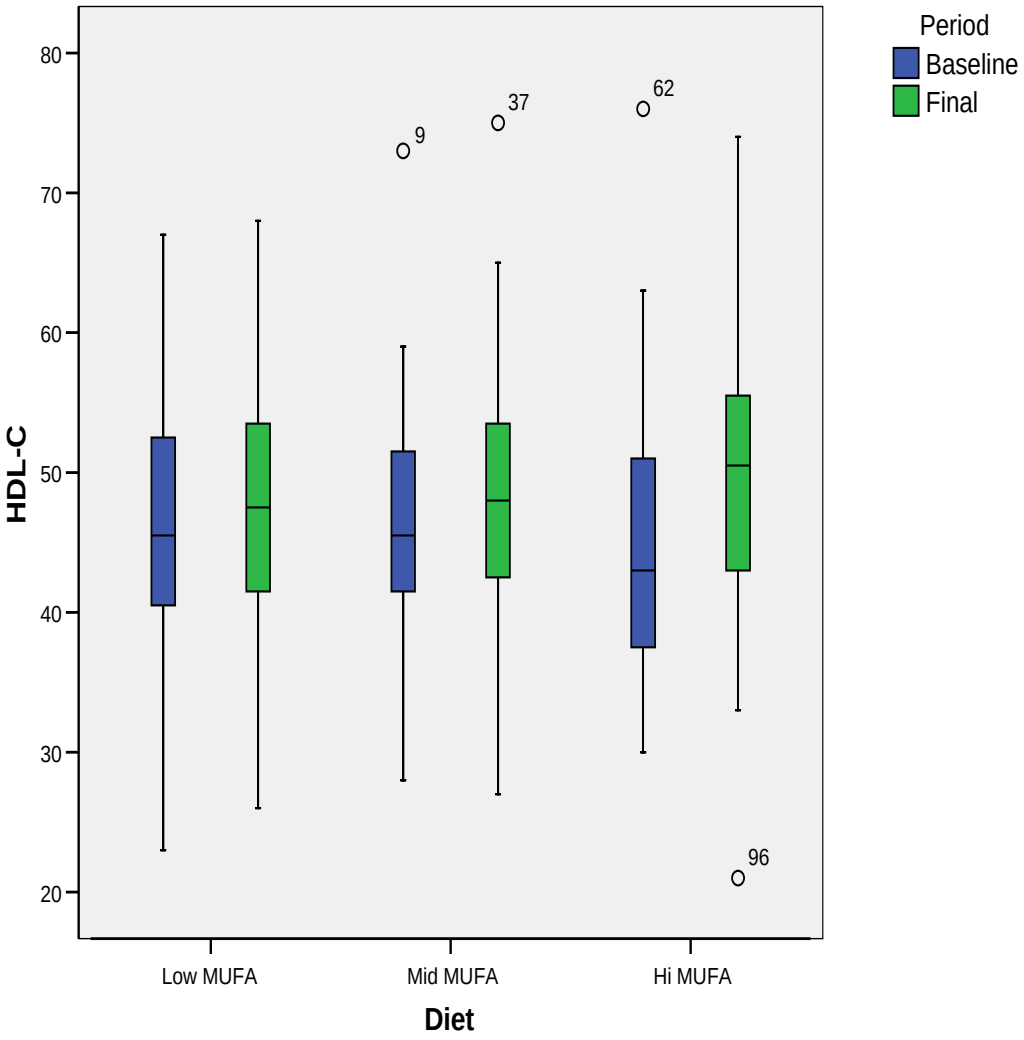

FIGURE 25. HDL-C boxplot for three diet treatments.

APPENDIX B

TABLE 1
Human plasma lipoprotein classification.

	Chylomicron	VLDL	IDL	LDL	HDL
Density (g/cm)	< 0.95	9.5-1.006	1.006-1.019	1.019-1.063	1.063-1.21
Component					
% Protein	1.5-2.5	5-10	15-20	20-25	40-55
% Phospholipids	7-9	15-20	22	15-20	20-35
% Cholesterol	1-3	5-10	8	7-10	3-4
% Triacylglycerol	84-89	50-65	22	7-10	3-5
% Cholesterol ester	3-5	10-15	30	35-40	12
Apolipoprotein	AI, AII, B_{48}, CI-CIII, E	B_{100}, CI-CIII, E	B_{100}, CIII, E	B_{100} , E	AI, AII, D, E CI-CIII,
Size (nm)	75-1200	30-80	25-35	18-25	5-12

TABLE 2
Dietary fatty acid composition.

Fatty acid	g/114-g ground beef party		
	Low MUFA	Mid MUFA	High MUFA
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 –linoleic)	0.09	0.04	0.03
18:2 (*cis*-9, *trans*-11)	0.18	0.13	0.14
18:2 (*trans*-10, *cis*-12)	0.04	0.07	0.09
MUFA:SFA	0.71	0.83	1.10

TABLE 3
Reference normal range and NCEP ATP III criteria.

Variable	Reference normal range	NCEP ATP III treatment criteria [79, 163, 164]			
		Category	Baseline LDL level	Recommended Goal (LDL)	Therapeutic Goal
Total cholesterol [164]	< 200mg/dL				
LDL-cholesterol [164]	<100 mg/dL	High risk: CHD or CHD Equivalent (10y risk > 20%)	≥100 mg/dL	<100 mg/dL optional <70mg/dL	<70mg/dL
		Moderate high risk 2+ risk factors 10 y risk 10-20%	≥130 mg/dL	<130 mg/dL optional <100mg/dL	<100mg/dL
		Moderate risk: 2+ risk factors 10 y risk >20%	≥130 mg/dL	<130 mg/dL	<100mg/dL
		Low risk: 0-1 risk factor	≥160 mg/dL	<160 mg/dL	
HDL-cholesterol [164]	≥ 60 mg/dL	Risk	<40mg/dL(man)		
			< 50mg/dL (woman)		
Triacylglycerol [164]	<150 mg/dL				
Glucose (mg/dl)	< 110 mg/dL				
Insulin (μU/ml)	5-20 μU/mL				
Homocysteine (μM/L)	5-15 μM/L				
C-reactive protein (mg/L)	0.068-8.2 mg/L				
Paraoxonase (KU/L)	53-186kU/L [118]				

TABLE 4
Cholesterol concentrations[a].

Variable (mg/dL)	Mean ± SD		
	Baseline	Final	P value
TG			0.718
Low MUFA	124.32 ± 99.11	114.96 ± 74.63	
Mid MUFA	119.57 ± 81.38	109.64 ± 68.38	
Hi MUFA	135.75 ± 113.69	149.86 ± 258.16	
Total-C			0.39
Low MUFA	189.82 ± 36.81	186.04 ± 33.17	
Mid MUFA	188.14 ± 38.85	190.18 ± 33.51	
Hi MUFA	188.79 ± 34.29	190.50 ± 37.74	
LDL-C			0.57
Low MUFA	122.00 ± 34.28	117.71± 29.58	
Mid MUFA	120.14 ± 32.76	120.43 ± 33.30	
Hi MUFA	122.18 ± 31.27	121.46 ± 35.21	
HDL			0.316
Low MUFA	46.82 ± 10.13	47.36 ± 9.99	
Mid MUFA	46.54 ± 8.90	47.89 ±10.04	
Hi MUFA	45.61 ± 10.98	48.50 ± 10.67	
LDL/HDL			0.802
Low MUFA	2.77 ± 1.15	2.67 ± 1.09	
Mid MUFA	2.68 ± 0.89	2.63± 0.93	
Hi MUFA	2.86 ± 1.06	2.71 ± 1.26	
Total-C/HDL			0.562
Low MUFA	4.26 ±1.33	4.15 ± 1.32	
Mid MUFA	4.16 ± 1.06	4.16 ± 1.21	
Hi MUFA	4.35 ± 1.25	4.18 ± 1.50	

[a]Concentrations were measured from previous study of same study. The results are presented as mean ± SD.

TABLE 5
Baseline mean value of three diet measurements. Data presented as mean ± SD.

Variable	Baseline	
	Mean ± SD	Range
Glucose (mg/dL)		
Low MUFA	90.82 ± 6.80	74-102 (28)
Mid MUFA	93.07 ± 6.24	82-105(23)
High MUFA	91.11 ± 7.88	74-108 (34)
Insulin (μU/mL)		
Low MUFA	5.94 ± 3.64	1.0-17.8 (16.8)
Mid MUFA	6.05 ± 4.55	0.7-18.6 (17.9)
Hi MUFA	6.76 ± 6.34	0.6-27.7 (27.6)
IRI		
Low MUFA	1.33 ± 0.80	0.2-4.0 (3.8)
Mid MUFA	1.40 ± 1.06	0.2-4.3(4.1)
Hi MUFA	1.53 ± 1.43	0.1-6.0 (5.9)
ß-CFI		
Low MUFA	82.85 ± 61.24	17.3-237.3 (220)
Mid MUFA	73.50 ± 56.17	6.5-240.5 (236)
Hi MUFA	92.14 ± 87.8	6.5-398.5 (392)
Homocysteine (μM/L)		
Low MUFA	11.46 ± 3.48	6.1-19.2 (13.1)
Mid MUFA	11.73 ± 3.31	7.2-19.9 (12.7)
Hi MUFA	11.89 ± 3.52	6.1-19.9 (13.8)
CRP (mg/L)		
Low MUFA	1.56 ± 2.63	0-12.4 (12.4)
Mid MUFA	2.36 ± 5.50	0-29.3 (29.3)
Hi MUFA	1.92 ± 0.76	0-12.2 (12.2)
Paraoxonase (KU/L)		
Low MUFA	57.08 ± 11.80	26.72-90.99 (64.27)
Mid MUFA	56.43 ± 11.93	25.45-76.93 (51.48)
Hi MUFA	59.05 ± 10.50	43.61-72.60 (28.99)
Paraoxonase/HDL (KU/mmol)		
Low MUFA	1.28 ± 0.41	26.72-90.99 (64.27)
Mid MUFA	1.27 ± 0.40	25.45-76.93 (51.48)
Hi MUFA	1.38 ± 0.36	43.61-72.60 (28.99)

TABLE 6
Final mean value of three diet treatments. Data presented as mean ±SD.

Variable	Final	
	Mean ± SD	Range
Glucose (mg/dL)		
Low MUFA	90.00 ± 5.83	80-101 (21)
Mid MUFA	90.86 ± 7.17	76-107 (31)
High MUFA	91.00 ± 7.73	73-109 (36)
Insulin (μU/mL)		
Low MUFA	5.10 ± 4.63	0.6-17.1 (16.5)
Mid MUFA	4.70 ± 3.76	0.3-15.1 (14.8)
Hi MUFA	4.88 ± 4.35	0.4-17.8 (17.4)
IRI		
Low MUFA	1.13 ± 1.02	0.1-3.8 (3.7)
Mid MUFA	1.06 ± 0.88	0.1-4.0 (3.9)
Hi MUFA	1.11 ± 1.03	0.1-4.4 (4.3)
ß-CFI		
Low MUFA	71.16 ± 67.97	9.0-256.5 (247.5)
Mid MUFA	65.81 ± 71.77	2.6-393.2 (390.6)
Hi MUFA	66.36 ± 66.38	4.1-311.1 (307)
Homocysteine (μM/L)		
Low MUFA	12.56 ± 3.04	7.9-20.8 (12.9)
Mid MUFA	12.31 ± 2.55	7.2-18.4 (11.2)
Hi MUFA	12.52 ± 2.61	7.6-17.1(9.5)
CRP (mg/L)		
Low MUFA	1.94 ± 2.62	0-10 (10)
Mid MUFA	1.45 ± 2.14	0-9.7 (9.7)
Hi MUFA	2.03 ± 2.88	0-10.4 (10.4)
Paraoxonase (KU/L)		
Low MUFA	55.52 ± 11.52	25.68-73.66 (47.98)
Mid MUFA	55.06 ± 11.28	23.13-73.6 (50.47)
Hi MUFA	57.05 ± 10.82	26.74-87.3 (60.56)
Paraoxonase/HDL (KU/mmol)		
Low MUFA	1.26 ± 0.38	51.05-59.99 (8.94)
Mid MUFA	1.20 ± 0.39	50.69-59.43 (8.74)
Hi MUFA	1.25 ± 0.46	52.86-61.25 (8.39)

Table 7
Median value of three diet measurements.

Variable	Median ± SD	
	Baseline	Final
Glucose (mg/dL)		
Low MUFA	91.5 ± 6.80	89.0 ± 5.83
Med MUFA	94.0 ± 6.24	91.5 ± 7.16
High MUFA	92.5 ± 6.80	89.5 ± 7.73
Insulin (μU/mL)		
Low MUFA	5.10 ± 3.64	3.40 ± 4.63
Mid MUFA	5.00 ± 4.55	3.70 ± 3.76
Hi MUFA	4.65 ± 6.34	3.8 ± 4.35
IRI		
Low MUFA	1.18 ± 0.79	0.80 ± 1.02
Mid MUFA	1.12 ± 1.06	0.87 ± 0.88
Hi MUFA	1.08 ± 1.43	0.81 ± 1. 03
ß-CFI		
Low MUFA	62.59 ± 61.24	42.51 ± 67.97
Mid MUFA	62.39 ± 56.17	46.71 ± 71.76
Hi MUFA	63.78 ± 87.81	43.81 ± 66.38
Homocysteine (μM/L)		
Low MUFA	10.70 ± 3.48	12.20 ± 3.03
Mid MUFA	10.7 ± 3.31	12.65 ± 2.55
Hi MUFA	11.45 ± 3.53	12.15 ± 2.61
CRP (mg/L)		
Low MUFA	.65 ± 2.62	.70 ± 2.61
Mid MUFA	.75 ± 5.49	.50 ± 2.14
Hi MUFA	.85 ± 3.04	.90 ± 2.88
Paraoxonase (KU/L)		
Low MUFA	58.79 ± 11.79	54.95±11.52
Mid MUFA	58.41 ± 11.93	57.25 ± 11.28
Hi MUFA	59.72 ± 7.20	58.66 ± 10.82
Paraoxonase/HDL (KU/mmol)		
Low MUFA	1.26 ± 0.41	1.22 ± 0.38
Mid MUFA	1.25 ± 0.40	1.18 ± 0.39
Hi MUFA	1.40 ± 0.36	1.17 ± 0.46

TABLE 8
Treatment block effect of three diet measurements.

Variable	Mean		P-value[a]
	Treatment block 1	Treatment block 2	
Age	34.7	36.5	0.303
Glucose (mg/dL)	91.56	90.73	0.441
Insulin (μU/mL)	5.31	5.83	0.470
IRI	1.22	1.32	0.440
β-CFI	71.03	79.58	0.425
Homocysteine (μmol/L)	11.80	12.36	0.245
C-reactive protein (mg/L)	1.28	2.47	0.020
Paraoxonase (KU/L)	56.27	57.30	0.538
PON/HDL (KU/mmol)	1.304	1.23	0.009

[a] Treatment block effect was tested by GLM at $P < .05$.

TABLE 9
Treatment block effect of C-reactive protein in three diet treatments.

Effect in	Mean ± SD		
	Treatment block 1	Treatment block	p-value[a]
Low MUFA diet			
Baseline	0.818 ± 1.40	2.314 ± 3.37	0.133
Final	0.821 ± 1.05	3.064 ± 3.23	0.02
Mid MUFA diet			
Baseline	1.171 ± 1.198	3.557 ± 7.63	0.258
Final	1.007 ± 1.504	1.900 ± 2.610	0.278
High MUFA diet			
Baseline	2.307 ± 0.818	1.393 ± 0.818	0.437
Final	1.528 ± 2.170	2.569 ± 3.507	0.359

[a] Treatment block effect was tested by GLM at $P < .05$.

TABLE 10
Phase effect for baseline values.

Baseline value		Sum of Squares	Df	Mean Square	F	Sig[a]
Glucose (mg/dL)	Between Groups	128.667	2	64.3331	0 .325	0.271
	Within Groups	3932.000	81	48.543		
	Total	4060.667	83			
Insulin (μU/mL)	Between Groups	41.355	2	20.678	0.849	0.432
	Within Groups	1972.495	81	24.352		
	Total	2013.850	83			
IRI	Between Groups	2.371	2	1.185	0.951	0.391
	Within Groups	101.001	81	1.247		
	Total	103.372	83			
β-CFI	Between Groups	5266.799	2	2633.399	0.541	0.584
	Within Groups	394220.367	81	4866.918		
	Total	399487.165	83			
Homocysteine (μM/L)	Between Groups	234.622	2	117.311	13.029	0
	Within Groups	729.305	81	9.004		
	Total	963.927	83			
C-reactive protein (mg/L)	Between Groups	6.102	2	3.051	0.197	0.822
	Within Groups	1253.761	81	15.479		
	Total	1259.862	83			
Paraoxonase (KU/L)	Between Group	25.727	2	12.863	0.114	0.892
	Within Groups	9123.533	81	112.636		
	Total	9149.260	83			
Paraoxonase (KU/mmol)	Between Groups	0.304	2	0.152	1.003	0.371
	Within Groups	12.263	81	0.153		
	Total	12.566	83			

[a] It was tested by one way ANOVA.

TABLE 11
Baseline phase effects of homocysteine for three diet treatments.

Baseline value		Sum of Squares	df	Mean Square	F	Sig[a]
Low MUFA	Between Groups	43.593	2	21.797	1.915	0.168
	Within Groups	284.553	25	11.382		
	Total	328.147	27			
Mid MUFA	Between Groups	48.282	2	24.141	2.424	0.109
	Within Groups	249.019	25	9.961		
	Total	297.301	27			
High MUFA	Between Groups	168.58	2	84.291	12.599	0
	Within Groups	167.264	25	6.691		
	Total	335.847	27			

[a] It was measured by ANOVA method.

TABLE 12
Homocysteine baseline means value for three phases.

Effect in	Mean ± SEM			
	Phase 1	Phase 2	Phase 3	Mean value for 3 phases
Low MUFA diet	13.110 ± 1.135	10.278 ± 0.807	10.811 ± 3.900	11.461 ±3.486
Mid MUFA diet	13.788 ± 1.629	10.690 ± 0.779	11.130 ± 0.713	11.732 ± 3.318
High MUFA diet	15.140 ± 0.929	9.600 ± 0.747	10.567 ± 0.828	11.889 ± 3.527

TABLE 13
Phase effect among three diet treatments for all measurements.

Measurement		Sum of Squares	df	Mean Square	F	Sig.
Glucose	Baseline-Final	46.095	1	46.095	1.912	0.171
	Baseline-Final* Diet	32.155	2	16.077	0.667	0.516
	Error(Baseline-Final)	1952.75	81	24.108		
Insulin	Baseline-Final	77.493	1	77.493	6.97	0.010
	Baseline-Final* Diet	7.512	2	3.756	0.338	0.714
	Error(Baseline-Final)	900.58	81	11.118		
IRI	Baseline-Final	4.212	1	4.212	7.404	0.008
	Baseline-Final* Diet	0.332	2	0.166	0.292	0.748
	Error(Baseline-Final)	46.076	81	0.569		
B-CFI	Baseline-Final	9517.452	1	9517.452	4.022	0.048
	Baseline-Final* Diet	2529.202	2	1264.601	0.534	0.588
	Error(Baseline-Final)	191658.214	81	2366.151		
Homocysteine	Baseline-Final	24.994	1	24.994	3.048	0.085
	Baseline-Final* Diet	2.290	2	1.145	0.140	0.870
	Error(Baseline-Final)	664.156	81	8.199		
CRP	Baseline-Final	0.817	1	0.817	0.145	0.002
	Baseline-Final* Diet	12.932	2	6.466	1.151	0.028
	Error(Baseline-Final)	449.580	81	5.620		
Paraoxonase	Baseline-Final	137.795	1	137.795	2.955	0.089
	Baseline-Final* Diet	10.186	2	5.093	0.109	0.897
	Error(Baseline-Final)	3777.449	81	46.635		
Paraoxonase	Baseline-Final	0.277	1	0.277	6.413	0.013
/HDL	Baseline-Final* Diet	0.047	2	0.023	0.542	0.584
	Error(Baseline-Final)	3.495	81	0.043		

TABLE 14
CRP risk rate among three MUFA diet treatments[a].

	Total number	low risk	average risk	high risk
Low MUFA				
Baseline line	28	17	8	3
Final	28	17	5	6
Mid MUFA				
Baseline line	28	17	8	3
Final	28	18	5	5
High MUFA				
Baseline line	28	16	7	5
Final	27	15	7	5

[a] The number of risk based on the cutoff point CRP value, < 1.0 “low risk”, 1-3 “average risk” and >3.0 “high risk”.

TABLE 15
Paraoxonase activity[a] changing rate among three MUFA diet treatments.

	Number of value Lower normal range		Paraoxonase activity Vary after treatment	
	Baseline	final	up	down
Low MUFA	8	10	10	18
Mid MUFA	9	11	12	16
High MUFA	5	9	12	16

[a] The normal activity of PON is 53-186kU/L.

VITA

Xiaojuan Cao completed her medical school and residency training at NingXia Medical College in Ning Xia, China and received her B.M. (Bachelor of Medicine) in July 1998. Then she received her license and was employed as a cardiology physician in Beijing Anzhen Hospital in Beijing, China until July 2000 when she reunited with her husband in US. Upon her arrival, she worked as a research assistant in the Cardiology Division of Medical School in University of Louisville in Louisville, KY. In January 2006, she entered the Nutrition program at Texas A&M University in College Station, TX and graduated with her M.S. degree in December 2008.

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