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

A Dissertation

by

GHAZAL GHAHRAMANY

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

DOCTOR OF PHILOSOPHY

May 2012

Major Subject: Nutrition

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

A Dissertation

by

GHAZAL GHAHRAMANY

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

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	Stephen B. Smith
Committee Members,	Steven E. Riechman
	Alice Villalobos
	Chaodong Wu
Intercollegiate Faculty Chair,	Stephen B. Smith

May 2012

Major Subject: Nutrition

ABSTRACT

High-Oleic Ground Beef and Risk Factors for Cardiovascular Disease in Men and Postmenopausal Women. (May 2012)

Ghazal Ghahramany, B.S., Shiraz University, Iran

Chair of Advisory Committee: Dr. Stephen B. Smith

About half of all deaths in developed countries are caused by cardiovascular disease. It is well known that cardiovascular disease (CVD) risk can be influenced by diet, but optimal dietary content of fatty acids continues to be debated. The effect of fatty acid composition of ground beef on selected cardiovascular disease risk indicators was evaluated with two primary goals. The first goal was to document effects of ground beef fatty acid composition on plasma lipoprotein concentrations, whereas the second goal was to determine the effects of ground beef fatty acid composition on gene expression in peripheral blood mononuclear cells (PBMC). In both studies the results were compared between men and women.

Twelve men and women over age of 45 out of initially 15 completed a two-way crossover design. Subjects consumed five, 114-g ground beef patties per week for 5-wk periods separated by a 3-wk washout period. Patties contained on average 20% fat and monounsaturated fatty acid (MUFA): saturated fatty acid (SFA) of 0.8 and 1.1 for low-MUFA (conventional) ground beef high-MUFA (premium) ground beef patties, respectively. Blood was collected from each subject before and at the end of each diet

period. Overall, the ground beef interventions decreased total plasma cholesterol, triacylglycerol, and very low density lipoprotein (VLDL) cholesterol. Plasma concentrations of high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol decreased and increased, respectively with premium ground beef consumption. The change in HDL cholesterol was significant in women but not in men suggesting that premium ground beef consumption had a greater impact on women than in men.

For the second goal PBMC were isolated and the expression of selected genes was quantified by real-time PCR. ATP-binding cassette A1, ATP-binding cassette G1, and low-density lipoprotein receptor relative expression was increased with premium ground beef consumption. A significant increase was seen in stearoyl-Coenzyme-A desaturase 1 expression after premium ground beef treatment. With the exception of stearoyl-Coenzyme-A desaturase 1, all these genes were down-regulated with conventional ground beef consumption. Both sterol regulatory element binding transcription factor 1 and mediator complex subunit 1 were down-regulated after each beef patty treatment, but the effect was significant after consuming conventional ground beef. This suggests that genes involved in cholesterol metabolism were down-regulated with conventional ground beef consumption; whereas genes related to lipogenesis were up-regulated with premium ground beef consumption. From these data we concluded that different ground beef dietary interventions have different impacts on the PBMC gene expression that is related to cholesterol metabolism, inflammation and liver X receptor pathways.

DEDICATION

This work is dedicated to my beloved parents, Nader Ghahramany and Behnaz Baghbanbashi, for their unconditional love, continuous support and encouragement at this time and always; and, to my wonderful husband, Amin Zollanvari for his love, patience and understanding throughout the duration of this project.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Stephen Smith, for offering invaluable assistance, support and guidance throughout this project. I also wish to express my gratitude to my committee members, Dr. Riechman, Dr. Villalobos and Dr. Wu, for their guidance, support and encouragement throughout the course of this research.

Thank you also to my colleagues and the department faculty and staff for giving me the opportunity to complete my doctoral studies at Texas A&M University and experience a great time. Also, thanks to the men and women who made this project possible by participating in the study and by being patient with all the work involved.

I would like to express my love and gratitude to my precious family, my parents, my brothers and sister for their understanding and endless love. Thanks also to all my friends for being supportive and caring. Special thanks to my dearest Amin, who has always been there for me. He is the love of my life and without him this work could have not been accomplished. Finally and foremost, I would like to thank my God for his unending love and grace, for always being there and for listening to my prayers in my good times and bad times.

TABLE OF CONTENTS

Page

ABSTRACT iii

DEDICATION v

ACKNOWLEDGEMENTS vi

TABLE OF CONTENTS vii

LIST OF FIGURES ix

LIST OF TABLES xiii

CHAPTER

I INTRODUCTION: THE IMPORTANCE OF RESEARCH 1

CVD & Atherosclerosis 1
Disease Prevalence 2
Mortality 3
Lipoprotein, CVD and Atherosclerosis 3
Inflammation and CVD 8
Genes Involved in Atherosclerosis and Inflammation 10
Dietary Intervention and Atherogenic Dyslipidemia 16
Beef Consumption and its Effect on Serum Cholesterol 18
Benefits of Red Meat Consumption 19
Present Study 24

II HIGH-OLEIC GROUND BEEF AND RISK FACTORS FOR CARDIOVASCULAR DISEASE IN MEN AND POSTMENOPAUSAL WOMEN 26

Introduction 26
Methods 28
Results 32
Discussion 73

CHAPTER Page

III THE EFFECTS OF GROUND BEEF DIETS CONTAINING DIFFERENT MUFA:SFA RATIOS ON GENE EXPRESSION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS 79

Introduction 79
Methods 84
Results 85
Discussion 97

IV THE EFFECTS OF GROUND BEEF DIETS CONTAINING DIFFERENT MUFA:SFA RATIOS ON GENE EXPRESSION AND CVD MARKERS IN WOMEN WITH A COMPARISON TO MEN 103

Introduction 103
Methods 106
Results 106
Discussion 107

V CONCLUSIONS 123

REFERENCES 126

VITA 146

LIST OF FIGURES

FIGURE		Page
1	Changes from baseline in total cholesterol, LDL, and HDL cholesterol concentration (mmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	36
2	Changes from baseline in triglycerides (TAG), intermediate lipoprotein (IDL), very low density lipoprotein (VLDL) and remnant lipoprotein (RLP) cholesterol concentration (mmol/L) of women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	38
3	Changes from baseline in LDL III, LDL IV, and Lp(a) cholesterol concentration (mmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	40
4	Changes from baseline in LDL particle size (nm) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	41
5	Changes from baseline in HDL subfractions cholesterol concentration (mmol/L) for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	43
6	Changes from baseline in VLDL and RLP particle concentration (nmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	45
7	Changes from baseline in LDL subfraction particle concentration (nmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	47
8	Changes from baseline in HDL particle concentration (nmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	49

FIGURE Page

9 Changes from baseline in HDL particle concentration (nmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) 51

10 Changes from baseline in major plasma fatty acid of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) 53

11 Changes from baseline in total cholesterol concentration (mmol/L) of men and women after beef treatment in each phase..... 61

12 Changes from baseline in total cholesterol concentration (mmol/L) of men and women in each phase including wash out period 62

13 Changes from baseline in HDL concentration (mmol/L) of men and women after beef treatment in each phase 63

14 Changes from baseline in HDL concentration (mmol/L) of men and women in each phase including wash out period 64

15 Changes from baseline in LDL concentration (mmol/L) of men and women after beef treatment in each phase 65

16 Changes from baseline in LDL concentration (mmol/L) of men and women in each phase including wash out period 66

17 Changes from baseline in Linoleic acid concentration (g/100g fatty acid) of men and women after beef treatment in each phase 69

18 Changes from baseline in Oleic acid concentration (g/100g fatty acid) of men and women after beef treatment in each phase 70

19 Changes from baseline in Palmitic acid concentration (g/100g fatty acid) of men and women after beef treatment in each phase 71

20 Changes from baseline in Stearic acid concentration (g/100g fatty acid) of men and women after beef treatment in each phase 72

FIGURE Page

21 Changes from baseline in SCD1, LDLR and MED1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) 87

22 Changes from baseline in ABCA1, ABCG1 and SREBF1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) 88

23 Changes from baseline in SCD1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase 91

24 Changes from baseline in ABCA1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase 92

25 Changes from baseline in ABCG1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase 93

26 Changes from baseline in LDLR relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase 94

27 Changes from baseline in MED1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase 95

28 Changes from baseline in SREBF1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase 96

FIGURE Page

29 Changes from baseline in plasma total cholesterol concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women 109

30 Changes from baseline in plasma total LDL concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women..... 110

31 Changes from baseline in plasma total HDL concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women..... 111

32 Changes from baseline in plasma total VLDL concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women 113

33 Changes from baseline in plasma total HDL-2b concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women 114

34 Changes from baseline in plasma total HDL particle concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women 116

35 Changes from baseline in plasma total HDL-2b particle concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women 117

36 Changes from baseline in plasma total LDL particle size for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women..... 118

LIST OF TABLES

TABLE		Page
1	Baseline characteristics for subjects	29
2	Fatty acid composition Conventional (Low MUFA) and Premium (High MUFA) ground beef patty	33
3	Total daily energy intake per day from major nutrients for habitual diets (Baseline) and for test diets of women rotated through test ground beefs low in monounsaturated fatty acids (Conventional) or high in monounsaturated fatty acids (Premium)	34
4	Major lipoprotein cholesterol concentrations (mmol/L) for men and women rotated through test ground beefs low in monounsaturated fatty acids (Conventional) or high in monounsaturated fatty acids (Premium)	35
5	TAG, VLDL, RLP, and IDL cholesterol concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	37
6	LDL III, LDL IV, and Lp(a) lipoprotein cholesterol concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	39
7	HDL subfraction cholesterol concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	42
8	VLDL and RLP particle concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	44
9	LDL and LDL subfraction particle concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	46
10	HDL particle concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)	48

TABLE Page

11 C-reactive protein, homocysteine, and insulin levels pre- and post-test patty consumption 50

12 Particle density and size pre- and post-test patty consumption 52

13 Plasma fatty acid concentrations (g/100 g fatty acids) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) 54

14 Simple correlations between plasma fatty acids and cholesterol fractions, triglycerides, hs-CRP, insulin and homocysteine 57

15 Simple correlations between plasma fatty acids and cholesterol subfractions, HDL mean density, LDL particle size and density 58

16 Major lipoprotein plasma concentration of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase and the interaction between diet and phase 60

17 CRP, HCY and insulin concentration of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase and the interaction between diet and phase 67

18 Major plasma fatty acid concentration of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase and the interaction between diet and phase 68

19 Relative gene expression for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) 86

20 Relative gene expression for men and women consumed test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase and the interaction of phase and diet 89

TABLE Page

21 Simple correlations between gene expression, plasma major fatty acids, cholesterol subfractions and plasma insulin 98

22 Major lipoprotein cholesterol concentrations (mmol/L) for men rotated through test ground beefs low in monounsaturated fatty acids (Conventional) or high in monounsaturated fatty acids (Premium)..... 108

23 Major lipoprotein cholesterol concentrations (mmol/L) for women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)..... 108

24 VLDL and HDL2b concentrations for men rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) 112

25 VLDL and HDL2b concentrations for women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) 112

26 HDL and HDL2b particle concentrations and LDL particle size for men rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) 115

27 HDL and HDL2b particle concentrations and LDL particle size for women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium).. 115

28 Overall effects of conventional (low-MUFA) and premium (high-MUFA) ground beef on major cholesterol fractions, triglycerides and gene expression 124

CHAPTER I

INTRODUCTION: THE IMPORTANCE OF RESEARCH

CVD & Atherosclerosis

Cardiovascular disease (CVD) remains the single leading cause of death in both men and women. CVD risk assessment begins with a close examination of genetic modifiers such as age, sex and family history, as well as non-genetic environmental modifiers for instance smoking, alcohol and diet. It is well known that CVD risk can be influenced by diet, but optimal dietary content of fatty acids continues to be debated. Atherosclerosis, a type of arteriosclerosis (often referred to as hardening of the arteries) is responsible for the majority of CVD events; therefore considered to be the major contributor to CVD. Complications of atherosclerosis are the most common causes of death in Western societies. Atherosclerosis is defined as a form of chronic inflammation which begins with the initiation of endothelial dysfunction and involves interaction between monocyte-derived macrophages, T cells, the normal cellular elements of the arterial wall and modified lipoproteins. Some of the factors that lead to endothelial dysfunction can be mentioned as mechanical injury, toxins and oxygen radicals (1). This inflammatory process can ultimately result in the development of complex lesions or

---

This dissertation follows the style of The Journal of Nutrition.

plaques inside the arterial lumen. Plaque rupture and thrombosis lead to the acute clinical complications of myocardial infarction and stroke (2). According to epidemiologic studies many genetic and environmental risk factors have been involved in the development of atherosclerosis; however elevated levels of serum cholesterol have been shown to have the major impact even in the absence of other known risk factors (2, 3). Molecular mechanisms that control cholesterol biosynthesis and serum cholesterol levels were elucidated by Goldstein and Brown in 1977. Statins, a potent class of cholesterol lowering drugs were then developed and the development of these drugs significantly reduces cardiovascular mortality in hypercholesterolemic patients (4, 5).

Disease Prevalence

According to 2011 updates from Heart Disease and Stroke Statistics report by the American Heart Association, an estimated 82, 600, 000 American adults (more than 1 in 3) have or more types of CVD. Of these, 40, 400, 000 are estimated to be over 60 years of age. Total CVD includes diseases listed in the bullet points below (6).

● High blood pressure (HBP)—76, 400, 000 (defined as systolic pressure >140 mm Hg and/or diastolic pressure> 90 mm Hg, use of antihypertensive medication, or being told at least twice by a physician or other health professional that one has HBP)

● Coronary heart disease (CHD)—16, 300, 000

— Heart attack— 7, 900, 000

— Chest pain— 9, 000, 000

- Heart failure—5, 700, 000
- Stroke— 7, 000, 000

Mortality

Mortality data show that CVD as the underlying cause of death, including congenital cardiovascular defects, accounted for 33.6% of all 2,423, 712 deaths in 2007, or one of every three deaths in the United States. On average, 2,200 Americans die of CVD each day, an average of one death every 39 seconds. CVD claims more lives each year than cancer, chronic lower respiratory diseases (CLRD), and accidents combined (6).

Lipoprotein, CVD and Atherosclerosis

Cardiovascular risk is not only related to total plasma cholesterol concentration but also to the relative proportion of cholesterol carried in individual ipoprotein fractions. A triad of increased blood concentrations of small, dense low density lipoprotein (LDL) particles, decreased high-density lipoprotein (HDL) particles, and increased triglycerides lead to Atherogenic dyslipidemia. (SFA) Obesity, insulin resistance, and type 2 diabetes mellitus can all lead to atherogenic dyslipidemia which has emerged as an important risk factor for myocardial infarction and cardiovascular disease (7). While a positive relationship exists between LDL cholesterol and CVD risk, HDL cholesterol has an inverse relationship and is therefore considered as protective.

The liver is a major site of synthesis of both cholesterol and triacylglycerol and plays a key role in regulating the availability of lipid to the other tissues of the body by modulating lipid synthesis and the uptake and secretion of lipoproteins (8, 9). When lipid supply exceeds the ability to mobilize lipid the liver has the capacity to store relatively large amounts of both cholesterol ester and triacylglycerol. In terms of lipoprotein metabolism, the liver is the site of assembly and secretion of VLDL and the major site of removal of chylomicron remnants, intermediate-density lipoprotein (IDL) and LDL from the circulation. It synthesizes and secretes apolipoprotein (apo) A1, the major protein component of HDL, and actively removes cholesterol from the circulating HDL particles following interaction with cell surface receptors (10).

High Density Lipoproteins

Clinical and epidemiological studies have consistently shown that plasma concentration of HDL is one of the most reliable negative risk factor for CVD meaning low plasma levels of HDL cholesterol (HDL-C) are strongly and independently associated with an elevated risk of coronary heart disease (CHD) (11). Furthermore, the concept of HDL quality rather than quantity, in other words HDL functionality versus HDL concentration in terms of its protective capability from atherosclerosis also has been the focus of increasing attention. The central role of HDL is reverse cholesterol transport (RCT), which is a pathway for removing excess cholesterol from cells and peripheral tissues, including the arterial wall, and transporting it to liver, the major organ for degrading and excreting cholesterol through the bile. RCT is widely considered the

major mechanism responsible for HDL-mediated atheroprotection (12). However, differences in the relative content of apolipoproteins and lipids in HDL makes plasma HDL particles highly heterogeneous in structure, composition and biological function. The main protein component of HDL is apolipoprotein A-I (apoA-I), a protein that contains eight amphipathic α-helical domains of 22 amino acids each. Lecithin:cholesterol transferase (LCAT) is the enzyme that generates most of the cholesteryl esters present in plasma and is activated by apoA-I. Cholesteryl esters are extremely hydrophobic and sequester into the core of the particles as they are formed; this effect converts discoid HDL into the large spherical HDL particles which predominate in normal human plasma. Spherical HDL particles are larger in size and in addition to a hydrophobic core of cholesteryl esters they contain triacylglycerols (TAG). There are two main subfractions of HDL particles which differ in density: large, light, lipid-rich HDL2 (density 1.063–1.125 g/mL) and small, dense, protein-rich HDL3 (density 1.125–1.21 g/mL) (13). HDL2 and HDL3 can be further subfractionated into five distinct subpopulations of decreasing size: HDL2b, HDL2a, HDL3a, HDL3b and HDL3c (13); Moreover, HDL particles possess other potent biological activities, including anti-thrombotic, antioxidative, anti-inflammatory, anti-infectious and vasodilatory actions. Other atheroprotective properties of HDL include protection of endothelial function by stimulation of eNOS, stimulation of fatty acid oxidation, glucose uptake and insulin secretion (13, 14).

Low Density Lipoproteins

In human, the majority of serum cholesterol is carried by LDL particles. After TAG removal in peripheral tissues, a portion of the remaining LDL remnants are metabolized to LDL particles by further removal of TAG and dissociation of apo-lipoproteins except for apoB100. The major physiological role of LDL is the delivery of cholesterol to peripheral tissues; however, increased levels of LDL cholesterol are positively correlated with increased risk of CVD. LDL is taken up by cells via LDL receptors. Decreased activity and/or expression of LDL receptors leads to accumulation of LDL in plasma; for instance, in patients with homozygous familial hypercholesterolemia (4). Oxidative modifications in lipid and apoB components of LDL have been suggested to drive the initial formation of fatty streaks which result in atherosclerotic lesions. Macrophages and endothelial cells specialize in the uptake of modified LDL particles, which leads to the progression of atherogenesis. LDL particles may be modified in a number of different ways, including acetoecetylation, carbamylation, LDL-dextran sulfate complex formation, acetylation, malondialdehyde addition, oxidation, glycation and desialylation. However, not all modifications lead to increased uptake by the scavenger receptor. Oxidation of LDL particles may be the most studied mechanism in which LDL particles are modified and lead to increased uptake by the scavenger receptor. Like HDL, LDL are heterogeneous and can be defined on the basis of particle density, size, charge and chemical composition. LDL concentration, particle size and density are influenced by age, gender, hormone replacement therapy or contraceptive use, abdominal adiposity, diet and exercise. Small, dense LDL particles

with increased plasma TAG and low HDL-C are often associated with metabolic syndrome and diabetes mellitus secondary to insulin resistance (14, 15). It is still believed that small, dense LDL particles are a contributing factor to atherosclerosis based on evidence that smaller LDL are more suitable to penetrate arterial tissue (15) and are taken up less readily by LDL receptors (16). Furthermore, smaller LDL are oxidized at a greater rate than larger LDL particles (17).

Very Low Density Lipoproteins

Formation and secretion of lipoprotein particles is primarily achieved in the intestine as chylomicrons and in the liver as VLDL. The assembly process of hepatic VLDL is initiated in the endoplasmic reticulum (ER) as soon as apoB100 is translated and translocated into the lumenal side. Each VLDL is composed of one molecule of apoB100, multiple other apolipoproteins, varied amounts of TAG and cholesteryl esters, depending upon the size of resulting particles. The synthesis and secretion of VLDL from the liver is largely governed by lipid substrate supply (18, 19). The expression of apoB100 appears to be largely constitutive. When sufficient TAG is available, the apoB is lipidated and a VLDL particle is formed. In the absence of sufficient TAG, the emerging apoB is degraded. Transfer of TAG onto apoB is mediated by the microsomal triglyceride transfer protein (MTP) (20). Evidence is emerging that certain polymorphisms in MTP may result in impaired functioning, reduced VLDL production and accumulation of lipid in the liver (8). When secreted, VLDL are large TAG-rich particles and contain apoB100. Once in circulation, VLDL acquire apoE and apoC (I, II,

and II). VLDL are delipidated by lipoprotein lipase, which is found on the surface of extra-hepatic tissues. The cholesterol, phospholipids and proteins that are hydrolyzed from VLDL triglycerides, are then transferred to higher density lipoproteins such as HDL and LDL, leaving a small, dense remnant lipoprotein (RLP). RLP are further metabolized to smaller more dense lipoproteins called IDL, which are remodeled to form the endproduct LDL. Both IDL and LDL can be recognized and cleared from plasma by LDL receptor due to the retention of apoB100. Hyperinsulinemia and increased free fatty acids secondary to insulin resistance lead to an increase in plasma TAG and VLDL concentrations. Although VLDL and TAG correlate strongly to LDL density and decreasing LDL size, these characteristics are inversely related to HDL-C especially the HDL_2 subfraction (21, 22).

Several studies have shown that certain fatty acid species are preferentially utilized over others for VLDL assembly and secretion. For instance when McA-RH7777 cells were cultured in the presence of exogenous oleate (18:1 n-9), TAG-rich VLDL were secreted many fold higher as compared to no oleate supplementation (23-25). However, when the cells were treated with polyunsaturated fatty acids (PUFA), assembly and secretion of TAG-rich VLDL was decreased (26).

Inflammation and CVD

Several independent pathways of evidence now identify inflammation as a key regulatory process that links multiple risk factors for atherosclerosis and its complications with altered arterial biology. It's been three decades since atherosclerosis

has been known as a proliferative process (27, 28). According to that concept, endothelial damage led to platelet aggregation and release of platelet-derived growth factor that would trigger the proliferation of smooth muscle cells in the arterial intima, and resulted in atherosclerotic plaque formation. Among the many biomarkers of inflammation proposed for diagnostic use, myeloperoxidase, Lp-PLA2, pentraxin-3, cytokines such as IL-6, proteases such as matrix metalloproteinase-9, and C-reactive protein (CRP) have generated considerable attention. CRP is measured by a highly sensitive assay (hsCRP) and for a variety of reasons CRP has emerged as a leading biomarker of inflammation for clinical application. Many prospective cohort studies indicate that hsCRP predicts incident myocardial infarction, stroke, and cardiovascular death (29-31).

Inflammation is also seen with conditions such as obesity, insulin resistance, hypertension, metabolic syndrome, type 2 diabetes, hypertriglyceridemia, low HDL cholesterol and smoking, all of which are correlated with increased CVD risk (32). For this reason, inflammatory proteins such as high sensitivity hs-CRP and interleukin-6 (IL-6), when elevated chronically, are considered markers of CVD (33, 34). Furthermore, homocysteine is not an inflammatory protein but it is regarded as a biomarker strongly associated with CVD. A single mechanism by which homocysteine contributes to CVD is not established. Homocysteine may cause endothelial damage and dysfunction by affecting nitric oxide production and reactivity (35). Homocysteine activates platelets and promote expression of the CD40/CD40 ligand from activated platelets (36). CD40/CD40 ligand engages on the surface of endothelial cells, smooth muscle cells, or

macrophages triggers an additional inflammatory response, characterized by the release of inflammatory cytokines such as interleukins IB, and chemokines such as chemokine ligand 2 [CCL2] as well as expression of adhesion molecules such as E selectin, VCAMl and P selectin (36, 37). Elevated homocysteine levels are normally caused by either nutritional deficiencies in vitamin cofactors (folate, vitamin B12, and vitamin B6) or by genetic defects in the enzymes involved in homocysteine metabolism (37). Other factors affecting homocysteine metabolism include chronic kidney disease, hypothyroidism, psoriasis, certain cancers and several drugs such as theophylline and niacin (36).

Genes Involved in Atherosclerosis and Inflammation

A number of genes have now been linked to the pattern of lipoprotein changes. Achieving a better understanding of the genetic and dietary influences underlying atherosclerosis and inflammation may be valuable in order to improved interventions to reduce the risk of CVD in high-risk individuals (7).

It has been well established that fatty acids can regulate the expression of a variety of genes involved in lipid and lipoprotein metabolism. This occurs through a number of pathways within the liver. Peroxisome proliferator activated receptors (PPARs), sterol regulatory element binding proteins (SREBPs) and liver X receptors (LXRs) are included in these pathways (38, 39).

Three transcription factor families have been found to play a major role in the regulation of hepatic gene transcription which is stimulated by dietary fatty acids. These include PPARα, LXR, and SREBPs. The PPARs and LXR play important roles in both

lipid metabolism and inflammation. These ligand-activated intracellular transcription factors bind to specific response elements within the promoters of genes as heterodimers with the retinoid X receptor (RXR). After binding to their ligands they form conformational changes which can facilitate interaction with co-activators and through recruitment of multiple factors involved in gene expression, this leads to activation of transcription (40).

Peroxisome Proliferator-Activated Receptors

PPARs represent three isoforms, PPARα, PPARβ (also known as δ) and PPARγ, with PPARα being the predominant isoform in liver. These isoforms are encoded by different genes. PPARs are nuclear receptors that are regulated by fatty acids (41). As lipid sensors, they are primarily involved in regulation of lipid metabolism and subsequently in inflammation and atherosclerosis. Ligand activation of PPARα is associated with transcriptional up-regulation of a number of different genes for proteins associated with fatty acid oxidation and lipoprotein metabolism. Thease proteins include acyl coenzyme A oxidase, carnitine palmitoyl transferase, lipoprotein lipase, and apolipoproteins AI and CIII (42). PPARα expression is highest in tissues with high rates of fatty acid catabolism such as liver, kidney, heart, and skeletal muscle. Furthermore, PPARα has been implicated in ϐ oxidation of FAs as well as lipid metabolism. Activation of PPARα has been reported to improve levels of plasma TAG, HDL-C and the overall atherogenic plasma lipid profile, while PPARγ appears to play a role in adipocyte differentiation, lipid storage, fat metabolism, and glucose homeostasis, with

increasing evidence supporting its role as an important modulator of inflammation, atherosclerosis and macrophage differentiation (43). A diverse range of compounds act as ligands for the various PPAR isoforms. This includes both saturated and unsaturated fatty acids, though in general PUFA tend to be more potent. While all three PPAR subtypes have been shown to bind n-3 and n-6 PUFA, affinity appears to be greatest for PPARα, followed by PPARγ and PPARβ (43, 44).

Liver X Receptors

LXRs have been described as sensors of cholesterol in the nucleus since they are activated by increased cholesterol concentrations inside the cell. Oxysterols 22-hydroxycholesterol and 24, 25-epoxycholesterol and long chain fatty acids have been identified as ligands for LXRs (45). There are two LXR family members: LXRα and LXRβ. LXRα is the most highly expressed isoform in the liver while LXRβ is expressed in most cell types, but not in hepatocytes (46). Like PPARs, LXRs bind to response elements as heterodimers with RXR. These transcription factors regulate the expression of genes involved in sterol and fatty acid metabolism, particularly hepatic bile acid synthesis, including adenosine triphosphate-binding cassette protein A1 (ABCA1), apolipoprotein E, lipoprotein lipase, cholesterol ester transfer protein, and phospholipid transfer protein (47). Studies have shown that chronic administration of LXR agonists considerably decrease lesion formation in both low-density lipoprotein receptor (LDLR) and apolipoprotein E knockout– mediated atherosclerosis mouse models (48). Macrophages are known to play key roles in lipid metabolism and atherosclerosis. And

they develop from the bone marrow (49). LXR agonists increase RCT from macrophages by increasing expression of macrophage apolipoprotein E and cholesterol efflux transporters ABCA1 and ABCG1 (ATP-binding cassette, subfamily A, member 1 and ATP-binding cassette, subfamily G, member 1, respectively). ABCA1 is involved in reverse cholesterol transport; therefore, this can be an important part of the mechanism for LXR-dependent protection from atherosclerosis. At site of atherosclerotic lesions within macrophages, excess accumulation of cholesterol converts them into foam cells and accounts for the major portion of lesion deposited cholesterol (50). Thus, by stimulating RCT, LXR reduces foam cell formation and lesion cholesterol content. LXRs also play an important role in the regulation of genes involved lipogenesis as well as carbohydrate metabolism (51, 52). Moreover, evidence suggests that the regulation of lipogenesis by LXR is mediated mainly by its effects on SREBP1c expression (53, 54). PUFA have been reported to inhibit the interaction of oxysterols with LXR and thereby inhibit its activation of target genes (53, 54).

Sterol Regulatory Element-Binding Proteins

A balance between the amount of cholesterol and the amounts of unsaturated and saturated fatty acids in phospholipids is required in order to keep the integrity of cell membranes. Membrane-bound transcription factors, called sterol regulatory element-binding proteins (SREBPs) are responsible to maintain this balance and activate genes that encode enzymes of cholesterol and fatty acid biosynthesis (55). The SREBPs also enhance transcription of the LDLR, which mediates cholesterol uptake from plasma

lipoproteins (53). Unlike other transcription factors, the SREBPs are synthesized as membrane-bound proteins attached to the ER. Transcription of the LDLR occurs when there is low level of cholesterol inside the cells. During this time the SREBPs are transported to the Golgi complex. They are then processed by proteases to release a soluble fragment that enters the nucleus in order to activate transcription of the genes involved in cholesterol biosynthesis such as genes that encode HMG-CoA reductase and LDLR (54).

When LDL-derived cholesterol enters cells, it blocks the transport of SREBPs to the Golgi complex. This leads to blocking the release of the active fragment SREBPs from membranes (53).

SREBPs play a major role in regulating the expression of genes associated with lipid and lipoprotein metabolism (55, 56). Two SREBP genes produce three separate proteins (SREBP1a and 1c from one and SREBP2 from the other) with SREBP1c and SREBP2 being the predominant isoforms in the liver. Furthermore, PUFA have been shown to decrease nuclear SREBP-1 protein levels but it doesn't have any effects on the levels of SREBP2 (57). Therefore, due to the role of SREBP1c in regulating lipogenic gene expression, it may be an important candidate for mediating the effects of PUFA on lipogenesis (58).

In vivo studies have shown that SREBP-1c stimulated transcription of hepatic lipogenic genes in response to insulin and high-carbohydrate feeding, whereas SREBP-2 activated genes involved in cholesterol synthesis (59, 60). The stimulation of SREBP-1c

by LXR consequently activates the lipogenic pathway by activating genes involved in lipid synthesis, including the SCD1 gene (60).

Stearoyl-Coenzyme A Desaturase 1

SCD1 is a delta-9-desaturase, iron-containing enzyme that catalyzes a rate-limiting step in the synthesis of unsaturated fatty acids and is responsible for placing a double bond at carbon 9 of a fatty acid chain. The principal product of SCD1 is oleic acid (18:1(n-9)) that is formed by the desaturation of stearic acid. The ratio of stearic acid to oleic acid has been implicated in the regulation of cell growth and differentiation through its effects on cell membrane fluidity and signal transduction. SCD1 desaturates stearic acid and palmitic acid to produce oleic and palmitoleic acid (16:1(n-7)), respectively (61). Even though many of the effects of SCD1 deficiency are dependent on diet composition and other genetic factors (62), it seems SCD1 deficiency is antiatherogenic. SCD1 deficiency also promotes inflammation and atherosclerosis in mice (63). Studies of a mouse model with a targeted disruption in the SCD1 gene have suggested that SCD1 plays an important role in lipid homeostasis and lipoprotein metabolism. SCD1-deficient mice showed reduced synthesis of lipids, especially TAG, resistance to diet-induced weight gain, and reduced leptin deficiency-induced obesity (64, 65). Moreover, SCD1-deficient mice have low levels of TAG in VLDL (61, 64, 66).

An increased level of SCD1 activity in macrophages is positively correlated with increased palmitoleic acid, which explains the relief from lipid-induced ER stress. Erbay et al. (67) demonstrated endogenous fatty acid synthesis and desaturation of fatty acids

can be highly beneficial for defending ER function when macrophages are exposed to toxic lipids, which may lead to an antiatherogenic effect of SCD1 activity. Previous work has shown that when VLDL hydrolysis was inhibited, SCD1 deficiency significantly reduced VLDL secretion in leptin-deficient *ob*/*ob* mice, suggesting that SCD1 deficiency may also play a role in VLDL-TAG secretion (66). Oleic acid in TAG is a major component of VLDL particles, and any change in the availability of this MUFA may affect VLDL production. Furthermore, oleate produced by SCD1 leads to lipogenic gene expression through fructose-mediated induction suggesting an important role of oleic acid in lipid metabolism (68).

Dietary Intervention and Atherogenic Dyslipidemia

Dietary recommendations to reduce the risk of CVD include lowering energy intakes of total fat, saturated fatty acids (SFA) and *trans*-fatty acids, in order to avoid their effects on raising cholesterol levels (69). The major SFA within beef (myristic acid, 14:0, palmitic acid, 16:0 and stearic acid, 18:0) each have been found to be associated with CHD risk (70), although others suggest that a distinction should be made for stearic acid which has been found to have little cholesterol-raising effects in humans (71, 72).

It has been reported that dietary fatty acids directly regulate the expression of key enzymes involved in fatty acid synthesis such as acetyl coenzyme A carboxylase (ACC), fatty acid synthase (FAS) and SCD1 (73, 74). In animal models it has been shown that PUFA can significantly down-regulate expression of the genes for these enzymes (38, 39). By contrast, it has been suggested that SFA may actually stimulate the expression of

these enzymes (73, 74). PUFAs have been recognized to be capable of depressing the expression of genes for enzymes involved in fatty acid synthesis, including ACC, FAS and SCD (38). These genes appear to be down-regulated by both n-3 and n-6 PUFA though response to n-3 may be more profound (38, 39). *Trans*-unsaturated fatty acids have been considered to be potent in their ability to increase blood concentrations of cholesterol (75).

Epidemiological observations clearly showed that populations with high dietary intakes of SFA had higher plasma cholesterol levels and an increased incidence of CVD (76). The major impact of SFA has been shown to be in increasing LDL-C (72, 77).

Due to the current recommendations, beef and beef fat and their effects on CVD have been examined closely. Though beef fat and coconut oil are both considered to be high in saturated fat, they do not have the same effects on cholesterol levels. When compared to coconut oil, which consist of more medium chain saturated fatty acids (lauric (12:0) and myristic acid (14:0)), beef fat reduced total, LDL, and HDL cholesterol (78). When red meat was tested against fish and poultry, there was no significant difference in plasma cholesterol concentrations between the diets consisting of red meat, fish or poultry (79).

Analysis of a prospective cohort study, the Framingham Heart Study, confirmed that fat content in the diet, after multivariable adjustment for carbohydrate intake and a variety of other potential confounders, did not significantly affect LDL size or TAG levels in either men or women (80). This was true regardless of the quality of fat studied, total fat, SFA, MUFA, or PUFA content. However, a study by Campos et al. (81)

reported an associated reduction of LDL particle size with reduced animal fat intake and increased consumption of carbohydrates. LDL:HDL ratios are reduced when carbohydrate is replaced with fat, even with saturated fat (82). When isocaloric substitutions of carbohydrates for monounsaturated fat or polyunsaturated fat were made in the diets of women, their risk of CVD increased 20 and 60%, respectively (83). Changing the quality of the fat in the diet has been shown to be more beneficial than reducing the total amount of fat in the diet (82, 83). In conclusion, it remains unclear whether having high or low dietary carbohydrate content is more beneficial for cardiovascular health.

In the year 2000, the Nutrition Committee of the American Heart Association moved away from its former insistence on low-fat diets (8-10% of energy) and concluded that diets that provided up to 40% of dietary energy in the form of unsaturated fat were as heart-healthy as low-fat diets. An outcome of this official opinion has been the reevaluation of the nutritional properties of a number of higher fat foods such as dairy, nuts, and dietary oils such as olive oil rich in the monounsaturated fatty acid, oleic acid (84).

Beef Consumption and its Effect on Serum Cholesterol

The relationship of beef consumption to its effect on lipoprotein variants and inflammatory markers related to CVD risk has not been adequately evaluated, particularly in post-menopausal women. Reports linking dietary fat to serum lipoprotein

levels often have been interpreted to mean that the general public, especially those at risk for CVD, should consume diets containing little or no red meat.

Scientists previously concluded that dietary SFA elevate serum cholesterol concentrations, whereas PUFA reduce serum cholesterol concentrations, and MUFA have little or no effect (85, 86). The major MUFA in beef, oleic acid, has been studied in more detail and found to lower LDL-C without affecting the beneficial HDL-C (87, 88). This effect is most convincing when natural foods were used to supplement diets with oleic acid (89, 90). Furthermore, in previous studies conducted in this laboratory, the level of HDL-C significantly increased after consumption of high oleic acid ground beef.

One of the SFA in beef, stearic acid, either has no effect or may actually lower serum cholesterol (91, 92). Typically MUFA constitute 35 to 45% of the total fatty acids in beef produced in the U.S. (88, 93). The wide variation of oleic acid content in market beef has been considered with inconsistent study results, with a decrease (94) in or no effect (95, 96) on serum cholesterol in human subjects.

Benefits of Red Meat Consumption

Consuming moderate amounts of lean red meat, as part of a balanced diet, valuably contributes to intakes of essential nutrients iron, zinc and vitamin B12 and possibly to intakes of n-3 PUFA and conjugated linoleic acid (CLA). Furthermore red meat is long established as an important dietary source of protein (97).

Fatty Acid Composition

Approximately 50% of the intramuscular fat of beef and lamb is made up of unsaturated fatty acids; MUFA, primarily oleic acid and PUFA, predominantly the essential n-6 and n-3 PUFA linoleic acid (LA, C18:2) and alpha-linolenic acid (ALA, C18:3), respectively (98).

The ratio of PUFA to SFA in beef is approximately 0.11 being much lower than the desired dietary ratio of 0.4, due to biohydrogenation of unsaturated fatty acids in the rumen (98). A meta-analysis has shown that increasing the dietary ratio of PUFA:SFA can lead to a reduction in plasma total cholesterol and as a result, there is much research focusing on ways to improve this ratio within meat (99). The fatty acid composition of meat varies by animal breed, age, sex, diet and within the cut of meat (100).

Monounsaturated Fatty Acid

Oleic acid is the main MUFA found in beef (93), but it typically is associated with olive oil and the Mediterranean diet. There are polyphenolic compounds in olive oil that also have an influence on lipid metabolism, in that they reduce HMG-CoA reductase activity (101). However the effect of olive oil on cholesterol metabolism was confirmed by Ruíz-Gutiérrez et al. (102) despite of the beneficial effects of the polyphenols found in olive oil (103). They observed an increase in HDL-C in hypertensive women who consumed a diet enriched with high-oleic sunflower oil or olive oil. Furthermore, a significant decrease in plasma HDL_2 and an increase in plasma HDL_3 cholesterol concentrations were seen with the oleic and interventions. In addition to an increase in

HDL-C, oleic acid reduces the post-secretory oxidation of LDL particles, making them less atherogenic (103).

n-3 Polyunsaturated Fatty Acids

Lean tissue of red meat contains α-linilenic acid and the long chain n-3 PUFA, eicosapentaenoic acid (EPA, 20:5(n-3)), docosapentaenoic acid (DPA, 22:5(n-3)) and docosahexaenoic acid (DHA, C22:6). α-linilenic acid, derived mainly from plant sources, has been associated with a reduced risk of CVD by epidemiological studies (104). Its elongation products, the LCn-3 PUFA, are widely recognized for their many effects on heart health, vasodilation, anti-thrombotic effects and improving platelet aggregation (105). Long chain n-3 PUFA has other beneficial effects such as improving the function of central nervous system, retinal function and the inflammatory response (106). Studies have shown that meat consumers have greater plasma concentrations of long chain n-3 PUFA than vegetarians (107).

Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) is a term used to describe a group isomers of octadecadienoic acid, of which ruminant meat and milk are the major dietary sources (108). Concentrations of CLA have been found to range from 0.37 to 1.08 g/100 g within beef muscle (109).

CLA is formed through the ruminal biohydrogenation of dietary LA and also through an endogenous synthesis pathway from *trans*-vaccenic acid (TVA). The isomer,

18:2cis-9, *trans*-11 which is also known as rumenic acid, is the major and most important CLA isomer found in red meat (110). Its concentration within meat tissue is almost similar to n-3 PUFA (111). CLA has been found to have anti-carcinogenic and anti-atherogenic properties in several animal studies (112, 113). Long chain n-3 PUFA also has been found to favorably alter immune function in humans (114).

Iron

Iron deficiency is one of the most prevalent nutritional deficiencies both in developing and developed countries. It has been estimated that at least 50% of women and children and 25% of men are iron deficient in poor countries. Most of the body's iron is found as a component of a number of proteins, including enzymes, hemoglobin circulating in erythrocytes, and myoglobin in muscle (115, 116). Iron is essential for many cellular processes in the body and, as a component of haemoglobin, is necessary for maintaining adequate transport of oxygen in the blood. Therefore, even mild suboptimal status before the onset of anaemia can impact negatively on health (117). Heme iron found in meat is more bioavailable than non-heme iron found in plant sources; thereby meat consumers maintain better iron status than vegetarians and vegans (118). Furthermore red meat in particular is recognized as a significant source of heme iron compared to poultry and fish (119).

Zinc

Zinc on a molecular level, plays a major role in a variety of biochemical enzymatic processes relevant to maternal, fetal, infant and child health and survival. Women of reproductive age, the fetus, and young children are particularly at risk for deficiencies because of their high requirements for zinc. The main reason for zinc deficiency, particularly in the poor nations of the world, is the low intake of animal source foods (120). In relation to contribution of zinc, beef and lamb contain 4.1 mg and 3.3 mg/100 g tissue (121) and, as a result, have been classified as rich sources (120).

Vitamin B12

The risk of vitamin B12 deficiency is high in vegetarians, particularly in forms such as macrobiotic diets, which contain no foods of animal origin (122). Vitamin B12 is essential for normal blood formation and neurological development and function (123, 124). All naturally occurring vitamin B12 in the diet is derived from bacterial synthesis. Intestinal bacteria synthesize the B12, which is absorbed by animals, mainly ruminants. The main advantage of animal source foods, particularly meat, is the high content and bioavailability of micronutrients; meaning, there is a high level of absorption and utilization by the body because of the presence of heme protein found only in meat, fish and poultry (123). Although some plant foods are relatively high in iron, zinc, or calcium, e.g., spinach and legumes, the micronutrients are poorly absorbed. The essential vitamins play an indirect but essential role in the synthesis of purines and pyrimidines,

transfer of methyl groups, synthesis of proteins from amino acids, and carbohydrate and fat metabolism (123).

Red meat is certainly the major dietary source of B12 in the diet, providing over two thirds of the daily requirement in one 100 g serving (118). B12 is required by active enzymes within the methylation cycle and low intakes of B12 as well as folate and vitamin B6 have been associated with elevated homocysteine, which is a risk factor for CVD and stroke (105, 125). This was confirmed in a cross-sectional study that consumers with high intakes of total meat compared to vegetarians have lower homocysteine levels (105).

Present Study

The objectives of this study were:

1) Evaluate baseline metabolic and inflammatory responses to dietary interventions in men and post-menopausal women.
2) Determine impact of ground beef diets containing different monounsaturated fatty acid:saturated fatty acid (MUFA:SFA) ratios on lipoprotein and inflammatory markers.
3) Examine the effects of ground beef diets containing different MUFA:SFA ratios on gene expression in human peripheral blood mononuclear cells (PBMCs).

My primary hypothesis was improving fatty acid profiles in ground beef by modifying MUFA:SFA ratio in beef through diet would differentially affect CVD risk

meaning premium ground beef compared to conventional ground beef would have beneficial impacts on CVD risk in men and post-menopausal women. Also, dietary induced mechanisms involved in the pathways leading to atherosclerosis were investigated by studying gene expression during ground beef patty consumption.

CHAPTER II

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

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States. It is well known that CVD risk can be influenced by diet, but optimal dietary content of fatty acids continues to be debated. In 2000, the Nutrition Committee of the American Heart Association moved away from its former insistence on low fat diets and concluded that diets that provided up to 40% of dietary energy in the form of unsaturated fat were as heart-healthy as low fat diets (84). An outcome of this official opinion has been the re-evaluation of the nutritional properties of a number of higher fat foods such as dairy, nuts, and dietary oils such as olive oil rich in the monounsaturated fatty acid, oleic acid (126).

The relationship of beef consumption to its effect on lipoprotein variants and inflammatory markers related to CVD risk has not been adequately evaluated, particularly in post-menopausal women. Reports linking dietary fat to serum lipoprotein levels often have been interpreted to mean that the general public, especially those at risk for CVD, should consume diets containing little or no red meat.

Scientists previously concluded that dietary saturated fatty acids (SFA) elevate serum cholesterol concentrations, whereas polyunsaturated fatty acids (PUFA) reduce serum cholesterol concentrations, and monounsaturated fatty acids (MUFA) have little

or no effect (85, 86). The major MUFA in beef, oleic acid, has been studied in more detail and found to lower LDL-cholesterol without affecting the beneficial HDL-cholesterol (87, 127). This effect is most convincing when natural foods were used to supplement diets with oleic acid (89, 90). Furthermore, in previous studies conducted in this laboratory, the level of HDL-cholesterol significantly increased after consumption of high oleic acid ground beef.

One of the SFA in beef such as stearic acid either has no effect or may actually lower serum cholesterol (91, 92). Typically monounsaturated fatty acids constitute 35 to 45% of the total fatty acids in beef produced in the U.S. (88, 93). The wide variation of oleic acid content in market beef has been considered with inconsistent study results, with a decrease (94) in or no effect (95, 96) on serum cholesterol in human subjects. A dose-response relationship was established between the MUFA:SFA ratio in beef that is consumed and plasma lipoprotein cholesterol and inflammatory markers of CVD.

This study focused on the relationship among differences in the ground beef MUFA:SFA ratio and serum total lipoproteins, lipoprotein subfractions and inflammatory markers associated with increased CVD risk. LDL and HDL particle diameter will be used to identify the presence of particularly atherogenic LDL or anti-atherogenic HDL (128-130).

Methods

Overall Design

This study involved 7 women and 5 men (45-75 years of age) who have been assigned randomly to consume ground beef of a specific MUFA:SFA ratio. The ground beef sources included inexpensive chub pack ground beef with a MUFA:SFA ratio of 0.8 and Wagyu ground beef (Heartbrand) with a MUFA:SFA ratio of 1.1. At study entrance, each subject underwent a complete history and physical exam by a physician and baseline measurements of a complete lipid profile including LDL and HDL subfractions, hs-CRP, insulin, and glucose after 12 hours of fasting. Subjects were asked to complete a 4-d diet record. Participants were then randomized to one of two groups: 1) ground beef with MUFA:SFA ratio of 0.8 or 2) 1.1. Ground beef was delivered to individual homes for storage and consumption. Participants were asked to complete two 4-d diet records during the first 5 wk. At the end of 5 wk blood samples was collected. Following the first dietary modification period, the participants restarted a free range diet for 4 wk. During this time they completed a 4-d diet record. At the end of this "washout period", the participants started on the second diet modification for 5 wk. The 5-wk period included the third diet record completion.

Dietary Design

Subjects

The study protocol was reviewed and approved by Texas A&M University Institutional Review Board for the use of human subjects in research and all participants

gave written consent. Nine postmenopausal women and six men were recruited from the local Bryan/College Station, Texas community. Seven women and five men completed the study. The subjects included men over age 45 and women whose last menstrual period was over 1 y prior to enrollment of the study by natural or surgical means with or without ovaries. Subjects did not have history of CVD, stroke or diabetes, had normal liver function tests, normal fasting glucose, and serum total cholesterol of less than 6.72 mmol/L, and not taking lipid lowering drugs. Baseline characteristics of the participants are presented in Table 1. Participants were contacted at least once a week by e-mail or telephone to provide updates and encourage compliance.

Table 1 Baseline characteristics for subjects[1]

Item	Mean	SE
Age, *y*	62.6	2.3
Body weight, *lb*	76.2	6.8
Body mass index, kg/m^2	26.8	0.9
Android fat, *% total fat*	44.5	1.8
Gynoid fat, *% total fat*	41.09	2.6
Total body fat, %	36.1	2.1

[1]Data are means ± SE for 5 men and 7 women.

Preparation of Ground Beef

A survey of the local supermarkets by this laboratory at Texas A&M University has demonstrated that ground beef of the required specifications is readily available. Typical chub pack or ground round has MUFA:SFA ratios ≤ 0.90, whereas branded beef has MUFA:SFA ratios of close to 1.1. Akaushi beef, which was supplied by

HeartBrand, has a MUFA:SFA just over 1.3. This knowledge of different variations in market beef was used to produce the desired fatty acid compositional targets.

The ground beef was transported from local retail outlets on ice to Texas A&M University Rosenthal Meat Science and Technology Center. The ground beef was processed and packaged according to industry standards under HACCP control in the federally inspected Rosenthal Meat Science and Technology Center. Ground beef was formed into 114-g patties, vacuum packaged, and frozen at the Texas A&M University Rosenthal Meat Science and Technology Center. Beef patties were picked up vacuumed packaged and frozen from Texas A&M University to each participant.

Processing of Blood for Analysis

Blood sampling was obtained by certified phlebotomists that were assigned to the research project. Blood was collected from an arm vein prior to initiation of the dietary treatments, at the end of each diet phase. Plasma was harvested from the blood collected with 15% EDTA. Serum for standard lipid assays was collected into serum-separation vacutainer tubes. All serum and plasma samples were stored at -80°C.

Fatty Acid Composition of Plasma and Test Ground Beef

Fatty acids were measured in the baseline plasma, plasma taken after 5 wk of each test beef treatment. Additionally, fatty acid composition, along with total fat and moisture of the dietary ground beef patties were measured. Total lipid was extracted and methylated as described (131, 132). Fatty acid methyl esters were analyzed with a

Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Variam Inc., Walnut Creek, CA). Separation of fatty acid methyl esters was accomplished on a fused silica capillary column CP-Sil88 [100m x 0.25mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands) with helium as the carrier gas (1.2 mL/min). Oven temperatures began at 150°C and were increased to 160°C at a rate of 1°C/min. The oven temperature rose further to 167°C at a rate of 0.2°C/min. The temperature increased a rate of 1.5°C/min to a final temperature of 225°C where it was held for 26 mins. Injector and detector temperatures were at 270°C. Individual fatty acid methyl esters were identified using genuine standards (Nu-check Prep, Inc., Elysian, MN and Sigma-Alderich Co.) and expressed as a g/100 g total fatty acid methyl esters analyzed or as g/100 g hamburger patty. The low-MUFA ground beef patties were made from chub pack ground beef, purchased from a local retail outlet with a MUFA:SFA ratio of 0.9. The high-MUFA patties were made from Akaushi ground beef (Heartbrand Beef, Yoakum, TX) with a MUFA:SFA ratio of 1.3 (Table 2).

Lipoprotein Analysis

Serum aliquots kept at -80°C were sent to SpectraCell Laboratories, Inc. (Houston, TX) for complete lipoprotein density and particle number analyses. A complete "Lipoprotein Particle Profile™" test was provided using the lipoprotein subgroup particle number analysis method. Lipoprotein particles were stained with a fluorescent dye and separated utilizing a patented continuous gradient over a range of d = 1.00 – 1.30 g/cm^3 generated by analytical ultracentrifugation. Once separated, the

fluorescence of the lipoprotein particles was measured in a high performance liquid chromatography type flow system. For processing the fluorescence response was normalized to a cholesterol scale with a proprietary algorithm. Values corresponding to each lipoprotein subgroup at their specific densities were determined using a multiple Gaussian fit/integration routine (133).

Diet Records

Prior to each diet phase, and once during each phase, participants completed a 4-d record (to include one weekend day). The diet records were analyzed for nutrient composition to establish baseline observations, and encourage compliance with total patty consumption requirement. The records were analyzed using Nutribase version 7 (CyberSoft, Phoenix, AZ).

Statistical Analysis

Data were analyzed as a paired *t*-test, comparing baseline (habitual) values to values after 5 wk on the test diet. Thus, each participant served as her/his own control. Because each participant randomly rotated through all three diets, reported values are means ± standard error of the mean (SE) for $n = 12$ observations.

Results

Dietary records indicated that when on a test diet, participants consumed a lesser amount of total MUFA and oleic acid. This was not in patterns consistent with their test

ground beef interventions. Also, there were no significant differences in cholesterol and carbohydrate intake; however protein intake increased significantly with conventional ground beef consumption according to diet analysis (Table 3).

TABLE 2 Fatty acid composition Conventional (Low MUFA) and Premium (High MUFA) ground beef patty[1]

Fatty acid	Low MUFA	High MUFA
g/100-g ground beef patty		
Total lipid	22	17
14:0 (myristic)	0.86	0.59
14:1 (myristoleic)	0.21	0.23
16:0 (palmitic)	6.50	4.89
16:1 (n-7) (palmitoleic)	0.91	0.96
18:0 (stearic)	4.07	2.31
18:1 (*trans*-11) (*trans*-vaccenic)	0.40	0.21
18:1 (n-7) (*cis*-vaccenic)	0.38	0.41
18:1 (n-9) (oleic)	9.72	8.33
18:2 (n-6) (linoleic)	0.54	0.41
MUFA:SFA ratio	0.8	1.1

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

TABLE 3 Total daily energy intake per day from major nutrients for habitual diets (Baseline) and for test diets of women rotated through test ground beefs low in monounsaturated fatty acids (Conventional) or high in monounsaturated fatty acids (Premium)[1]

Nutrient	Baseline	Conventional	Premium
Total energy, kcal	1,876 ± 142	1,926 ± 223	1,814 ± 358
Protein, g	59.00 ± 8.06	89.76 ± 8.06**	71.11 ± 8.1
Carbohydrate, g	231 ± 34.1	212 ± 38.6	236 ± 58.3
Fat, g	64.3 ± 7.58	78.7 ± 9.69	67.5 ± 17.1
Saturated fat, g	20.5 ± 2.67	27.3 ± 3.88	23.8 ± 6.28
Monounsaturated fat, g	21.09 ± 2.95	27.1 ± 3.1	22 ± 5.64
Polyunsaturated fat, g	10.4 ± 1.37	11.7 ± 1.68	11.2 ± 3.4
Trans-unsaturated fat, g	0.85 ± 0.67	0.25 ± 0.12	0.34 ± 0.28
Cholesterol, mg	283 ± 65.1	293 ± 40.6	206 ± 58.9
Oleic acid, g	18.04 ± 2.47	23.0 ± 2.8	17.7 ± 4.47
Linoleic acid, g	7.49 ± 1.07	8.75 ± 1.54	9.18 ± 3
Linolenic acid, g	0.83 ± 0.15	0.89 ± 0.12	0.58 ± 0.13
Eicosapentaenoic acid, g	0.07 ± 0.03	0.04 ± 0.02	0.004 ± 0.002
Docosahexaenoic acid, g	0.14 ± 0.07	0.09 ± 0.04	0.007 ± 0.003
Fiber, g	13.7 ± 1.6	13.6 ± 2.29	15.0 ± 3.23
Sugar, g	90.9 ± 24.8	75.4 ± 23.2	87.9 ± 22.8
Sodium, mg	2,987 ± 402	2,595 ± 491	2,286 ± 397
Potassium, mg	2,374 ± 185	2596 ± 322	2,265 ± 513
Vitamin A, IU	4,399 ± 855	4236 ± 1008	4,258 ± 1974
Vitamin C, mg	188.14 ± 91.13	100.75 ± 29.14	82.01 ±31.26
Calcium, mg	573 ± 66.6	720 ± 121	588 ± 105
Iron, mg	16.3 ± 2.98	14.82 ± 2.85	16.55 ± 4.2
Thiamin, mg	1.47 ± 0.24	1.39 ± 0.24	1.59 ± 0.36
Riboflavin, mg	1.76 ± 0.26	1.94 ± 0.28	1.86 ± 0.46
Niacin, mg	29.0 ± 8.62	21.04 ± 2.96	19.53 ± 5.32
Folate, µg	380.7 ± 79.1	348.7 ± 68.8	343.8 ± 100.0

[1]Data were derived from 4-d diet records, including one weekend day, collected during each test period. Data for the habitual diet were obtained at baseline before patty consumption began. Data are means ± SE for 12 men and women. ** $P \leq 0.05$

Total cholesterol concentration decreased after consumption of both type of beef patties but the changes were not significant. LDL-C concentrations was decreased after conventional ground beef treatment and increased after Premium ground beef treatment. However, the changes were not significant. HDL-C concentration was significantly decreased after premium ground beef consumption and increased after conventional beef patty consumption (Table 4 and Figure 1). Serum VLDL-C and TAG concentrations

were increased with the consumption of the conventional ground beef patties and decreased with the consumption of premium ground beef patties while RLP and IDL particle concentrations increased after conventional ground beef consumption; however none of these changes were significant (Table 5 and Figure 2).

TABLE 4 Major lipoprotein cholesterol concentrations (mmol/L) for men and women rotated through test ground beefs low in monounsaturated fatty acids (Conventional) or high in monounsaturated fatty acids (Premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
Total cholesterol, mmol/L	5.55 ± 0.28	5.37± 0.24	5.34 ±0.27	5.08 ±0.20
LDL, mmol/L	5.55 ± 0.28	5.37 ± 0.24	3.14 ± 0.23	5.08 ± 0.2**
HDL, mmol/L	1.58 ± 0.11	0.76 ± 0.16	1.80 ± 0.11	1.51 ± 0.06**

[1] Data are means ± SE for 12 men and women. ** $P \leq 0.05$

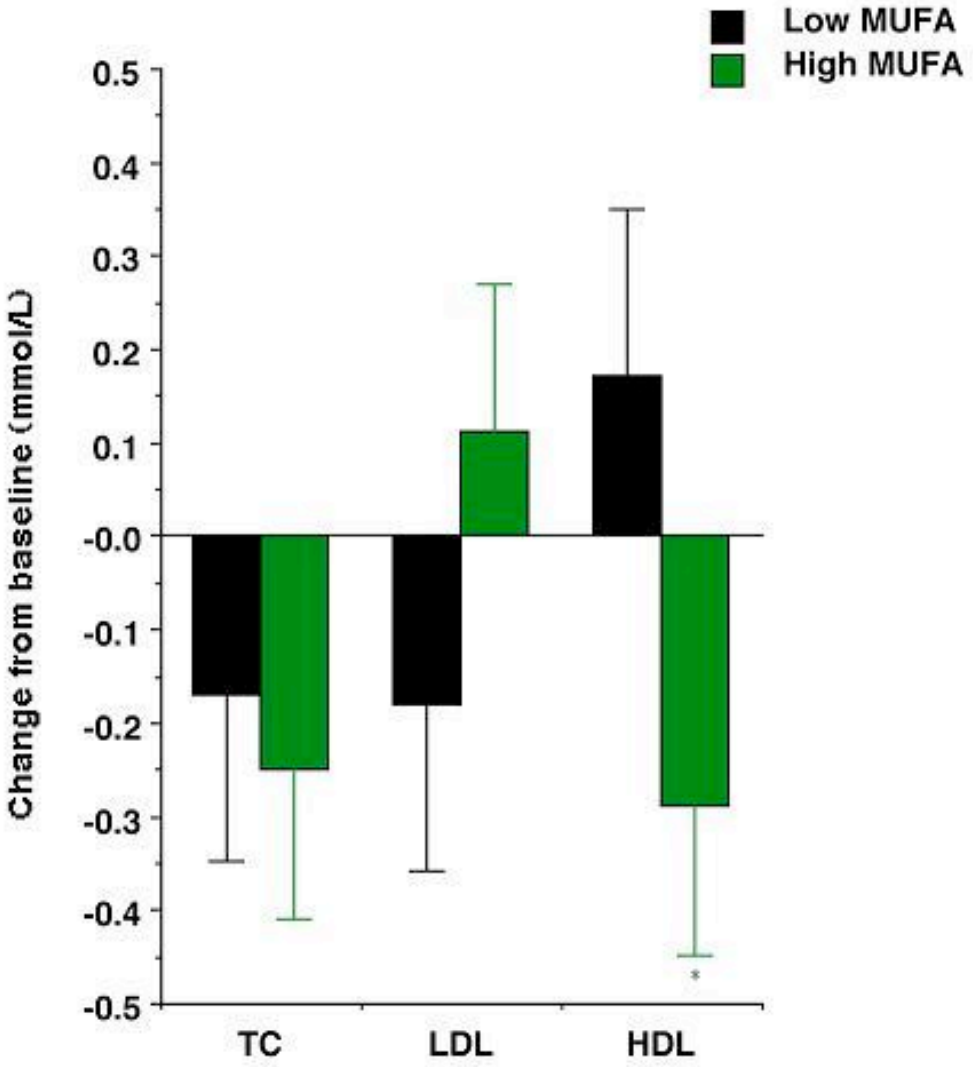

FIGURE 1 Changes from baseline in total cholesterol, LDL, and HDL cholesterol concentration (mmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, *n* = 12. * $P \leq 0.01$ (the significant change is between mean differences of low and high MUFA consumption).

TABLE 5 TAG, VLDL, RLP, and IDL cholesterol concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
VLDL, mmol/L	0.35 ± 0.03	0.31 ±0.03	0.47 ±0.05	0.35 ±0.04
RLP, mmol/L	0.81 ±0.06	0.94 ±0.07	0.90 ±0.1	0.91 ±0.1
IDL, mmol/L	0.72 ±0.05	0.85 ±0.06	0.79 ±0.09	0.81 ±0.09
TAG, mmol/L	1.02 ±0.2	0.78 ±0.06	1.06 ±0.11	0.87 ±0.09

[1] Data are means ± SE for 12 men and women.
There were no significant differences among phases ($P > 0.1$)

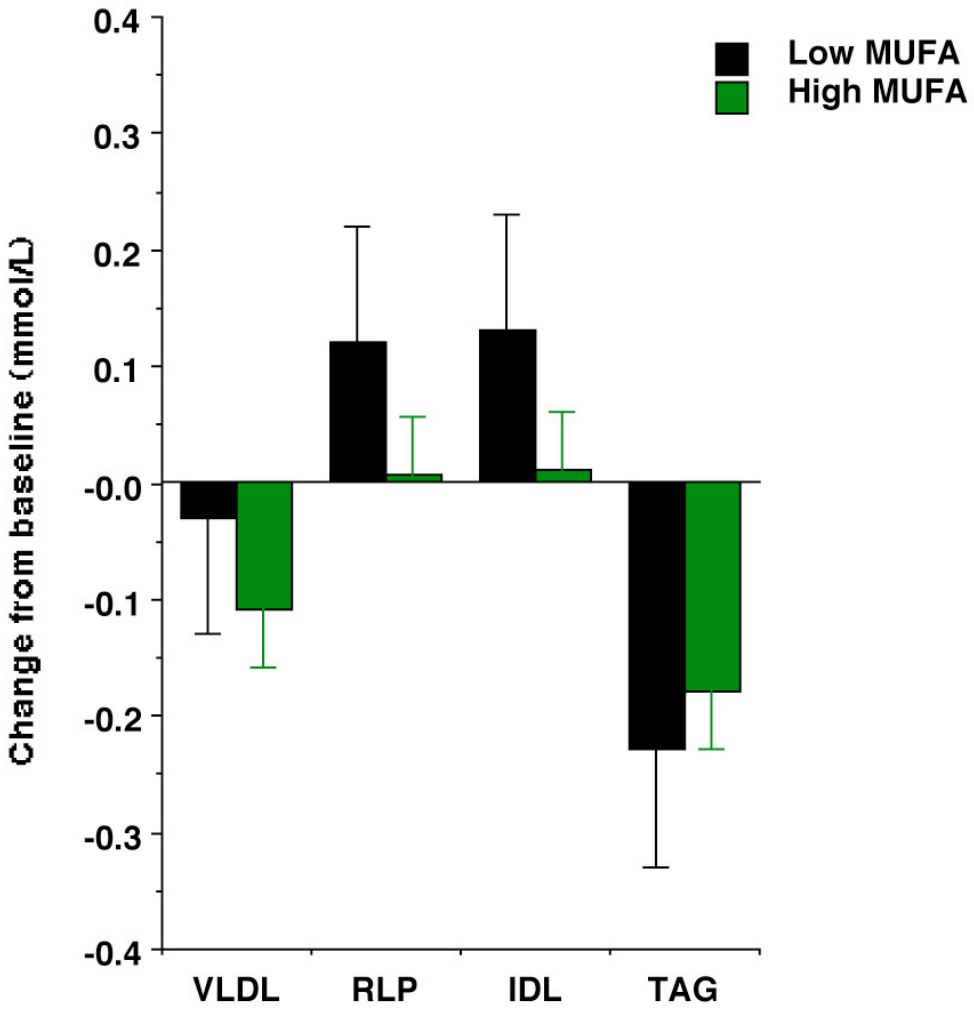

FIGURE 2 Changes from baseline in triglycerides (TAG), intermediate lipoprotein (IDL), very low density lipoprotein (VLDL) and remnant lipoprotein (RLP) cholesterol concentration (mmol/L) of women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, *n* = 12.

LDL III, LDL IV, and LP(a) particle concentration did not show a significant change with consumption of both beef patty types (Table 7 and Figure 3). LDL particle size increased significantly with conventional ground beef consumption. No significant change was observed in LDL particle size after premium ground beef consumption (Table 6 and Figure 4).

TABLE 6 LDL III, LDL IV, and Lp(a) lipoprotein cholesterol concentrations and LDL particle size for men and women rotated through test ground beefs low in monounsaturated fatty acids (Low MUFA) or high in monounsaturated fatty acids (High MUFA)[1]

Lipoprotein	Low MUFA Baseline	Low MUFA Final	High MUFA Baseline	High MUFA Final
Dense LDL III, mmol/L	0.59 ± 0.06	0.53 ±0.03	0.62 ±0.09	0.53 ±0.03
Dense LDL IV, mmol/L	0.20 ±0.01	0.20 ±0.02	0.21 ±0.02	0.21 ±0.02
Lp(a), mmol/L	0.63 ±0.19	0.65 ±0.19	0.63 ±0.18	0.50 ±0.14
LDL (nm)	20.11 ±0.02	20.20 ±0.03**	20.10 ±0.04	20.16 ±0.03

[1] Data are means ± SE for 12 men and women.
** $P \leq 0.05$

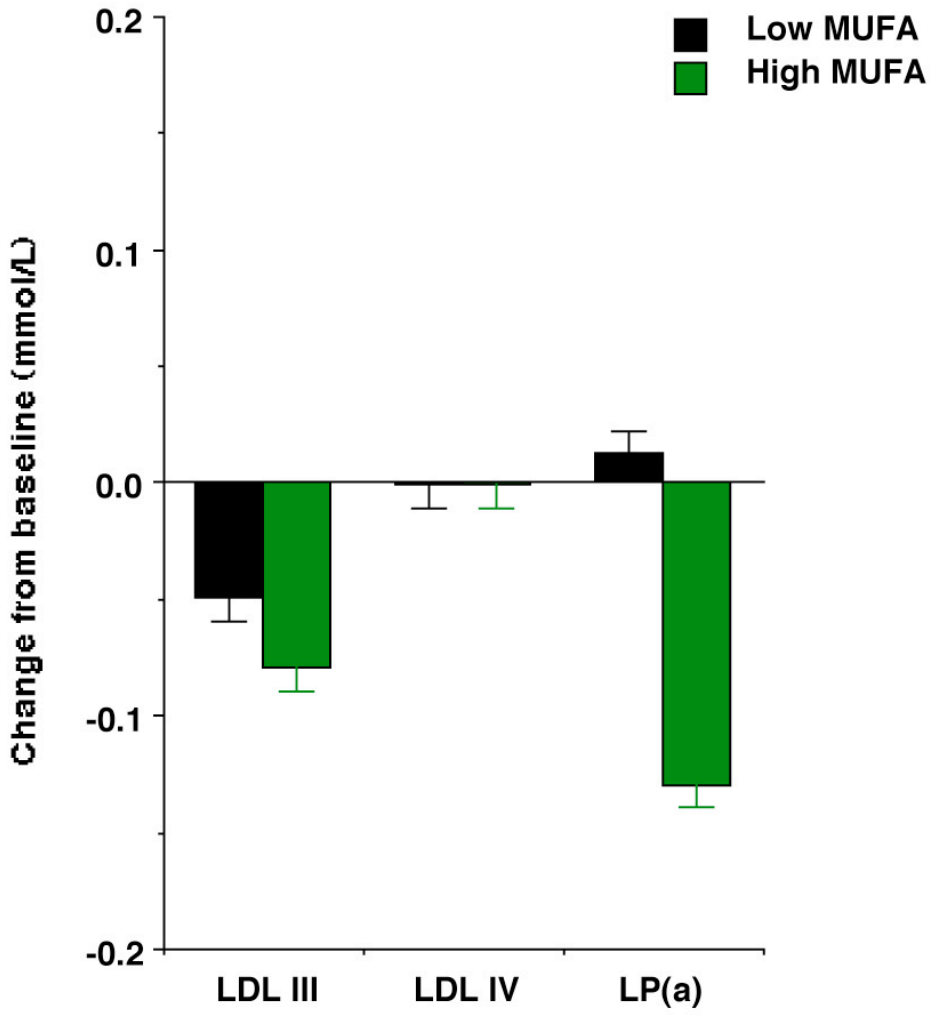

FIGURE 3 Changes from baseline in LDL III, LDL IV, and Lp(a) cholesterol concentration (mmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, *n* = 12.

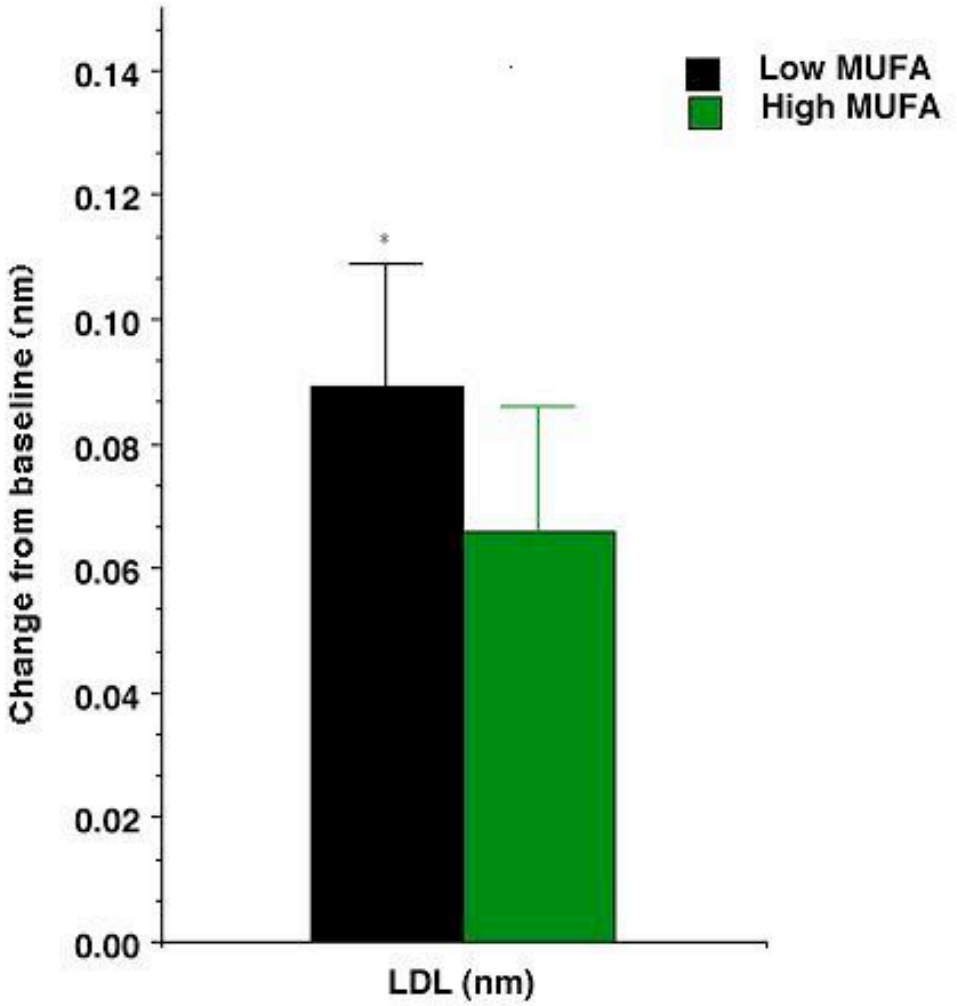

Figure 4 Changes from baseline in LDL particle size (nm) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, n = 12. * $P \leq 0.01$ (the significant change is between mean differences of low and high MUFA consumption).

HDL subfraction cholesterol concentration did not change significantly after consumption of either beef patty. HDL 2a and HDL2b decreased with premium ground beef consumption while HDL 3c increased after both premium and conventional ground beef consumption. The changes however were not significant (Table 7 and Figure 5).

TABLE 7 HDL subfraction cholesterol concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
HDL_{2a}, mmol/L	0.22 ± 0.05	0.22 ±0.02	0.25 ±0.04	0.22 ±0.03
Bouyant HDL_{2b}, mmol/L	0.70 ±0.11	0.68 ±0.07	0.70 ±0.09	0.68 ±0.08
HDL_{3c}, mmol/L	0.72 ±0.03	0.75 ±0.03	0.70 ±0.02	0.73 ±0.02

[1] Data are means ± SE for 12 men and women. There were no significant differences among phases ($P > 0.1$)

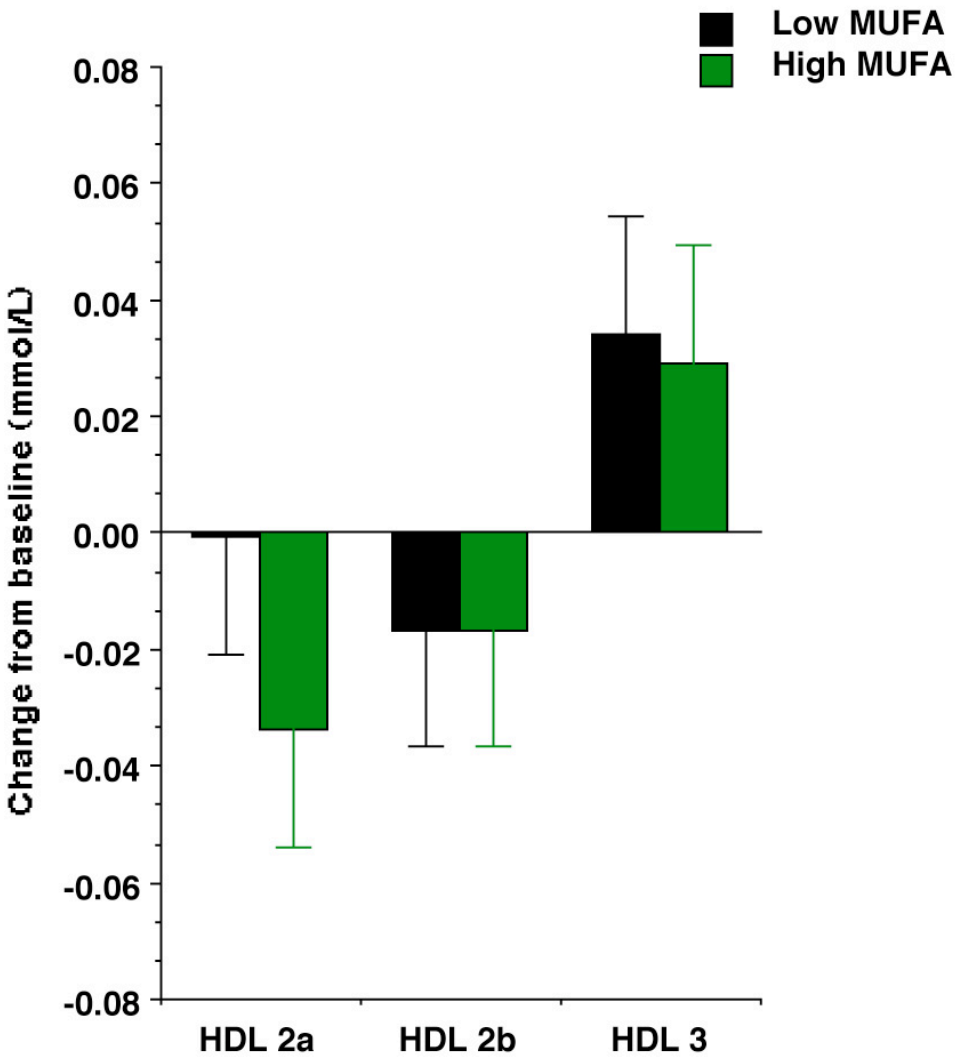

FIGURE 5 Changes from baseline in HDL subfractions cholesterol concentration (mmol/L) for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, *n* = 12.

Conventional ground beef patty consumption had no significant effect on VLDL particle concentration. However, VLDL decreased after consumption of either premium or conventional ground beef (Table 8 and Figure 6).

TABLE 8 VLDL and RLP particle concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
VLDL, nmol/L	52.66 ± 5.93	44.83 ± 5.17	70 ± 8.64	55.25 ± 7.70
RLP, nmol/L	134.58 ± 10.92	148.66 ± 13.65	148.41± 17.12	148.41 ± 16.26

[1] Data are means ± SE for 12 men and women.
There were no significant differences among phases ($P > 0.1$)

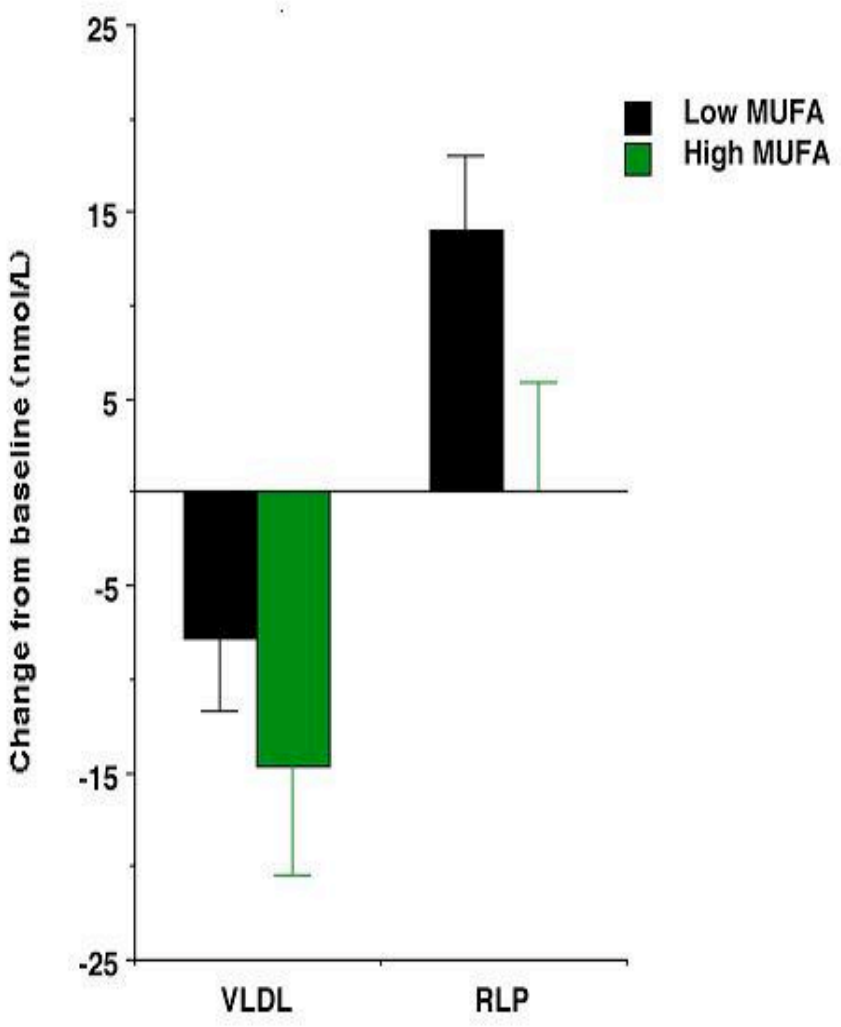

FIGURE 6 from baseline in VLDL and RLP particle concentration (nmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, n = 12

Though LDL cholesterol particle number was increased with the conventional ground beef intervention and decreased with premium ground beef consumption. However, none of these changes were significant. LDL III and LDL IV particle number concentration showed no significant changes after each diet (Table 9 and Figure 7).

TABLE 9 LDL and LDL subfraction particle concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
LDL Total, nmol/L	851.33 ± 55.08	903.5 ± 50.74	879.5 ± 68.60	866.5 ± 58.68
Dense LDL III, nmol/L	216.66 ± 25.31	196 ± 13.23	227.75 ± 34.64	196.66 ± 13.92
Dense LDL IV, nmol/L	99.416 ± 6.93	99.91 ± 10.97	103.91 ± 12.03	103 ± 10.47

[1] Data are means ± SE for 12 men and women.
There were no significant differences among phases ($P > 0.1$)

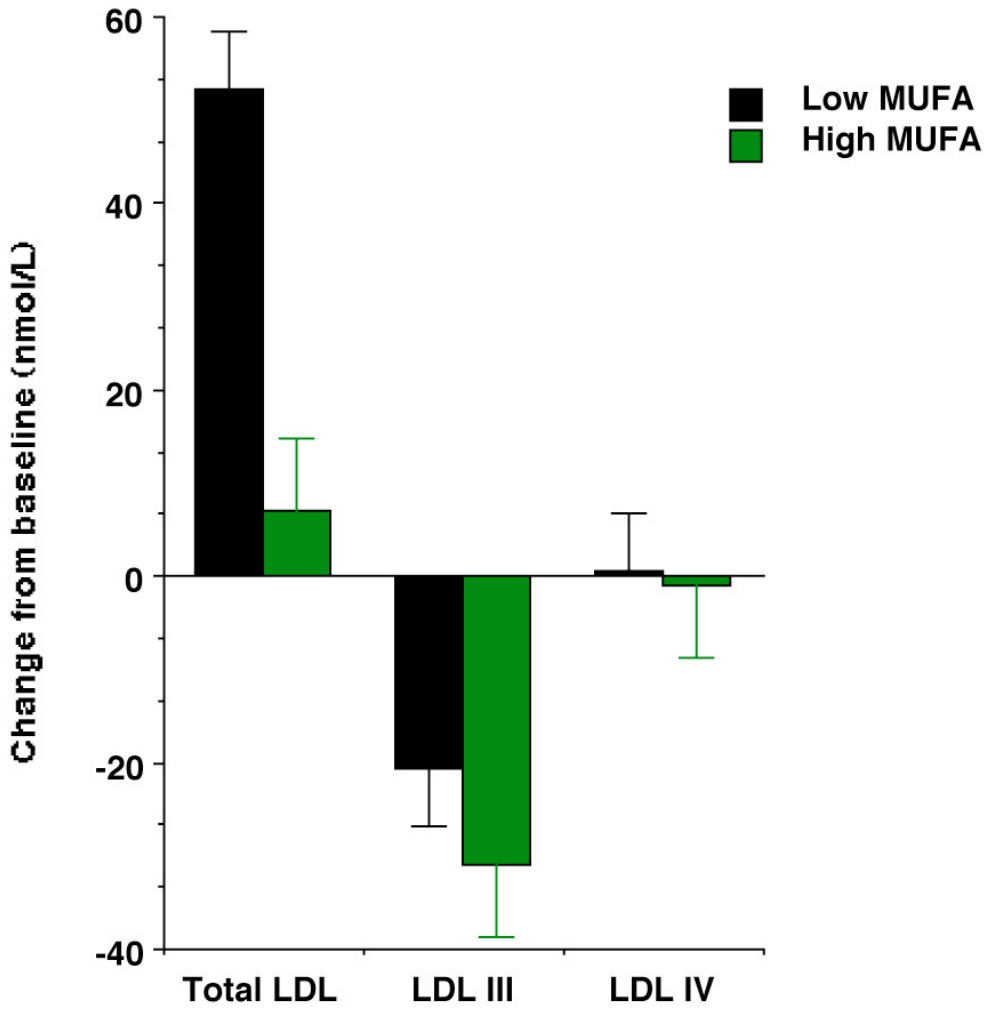

FIGURE 7 Changes from baseline in LDL subfraction particle concentration (nmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, *n* = 12.

Total HDL and buoyant HDL_{3b} particle number concentration showed no significant changes with the consumption of either conventional or premium ground beef patties (Table 10 and Figure 8).

TABLE 10 HDL particle concentrations for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
HDL Total, nmol/L	11256 ± 795.36	11526 ± 581.83	11322.42 ± 467.00	11322.67 ± 439.95
Bouyant HDL_{3b}, nmol/L	2441.91 ± 399.46	2379.33 ± 277.84	2459.08 ± 329.41	4061.41 ± 1722.05

[1] Data are means ± SE for 12 men and women. There were no significant differences among phases ($P > 0.1$)

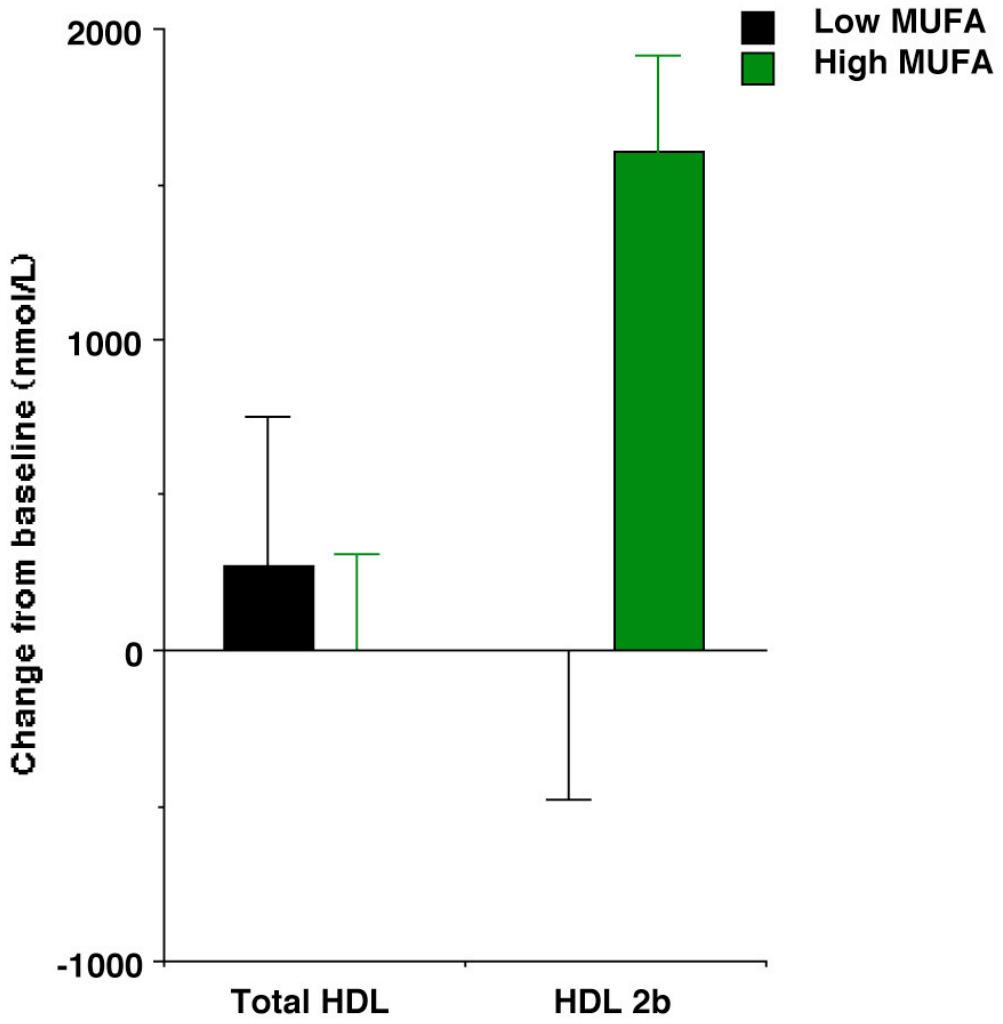

FIGURE 8 Changes from baseline in HDL particle concentration (nmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

No significant differences were seen in inflammatory markers, C-reactive protein (hs-CRP) or homocystine. Insulin also remained unchanged (Table 11). However, homocysteine concentration differed significantly between different diets (Figure 9).

TABLE 11 C-reactive protein, homocysteine, and insulin levels pre- and post-test patty consumption[1]

	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
hs-CRP, mg/L	0.32 ± 0.08	0.36 ±0.09	0.40 ± 0.10	0.30 ±0.04
Insulin, uIU/ml	8.65 ± 1.61	8.76 ±1.85	9.87 ± 2.22	11.92 ± 2.24
Homocysteine, µmol/L	9.52 ± 0.94	10.60 ± 0.75	10.71 ±1.07	9.21 ± 0.67

[1] Data are means ± SE for 12 men and women.
There were no significant differences among phases ($P > 0.1$)

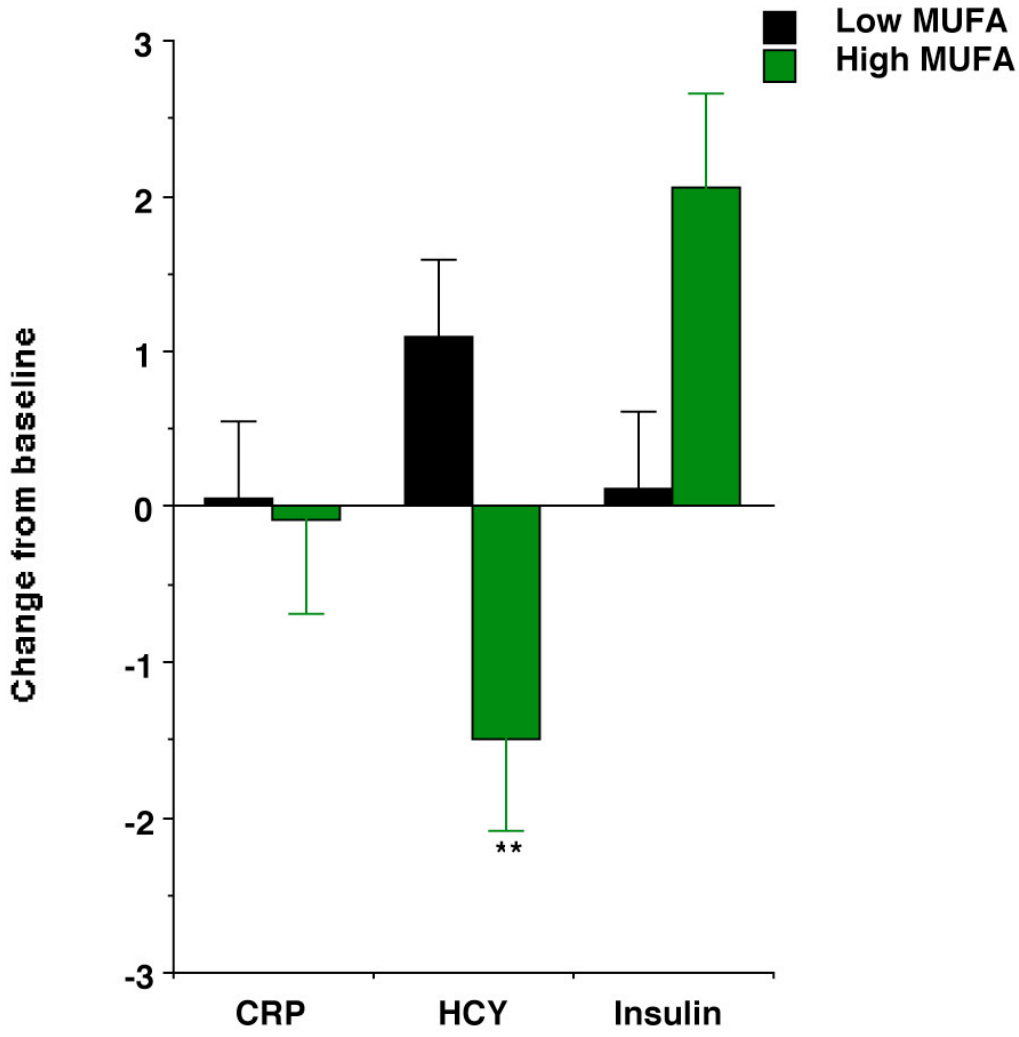

Figure 9 Changes from baseline in HDL particle concentration (nmol/L) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, n = 12. ** $P \leq 0.01$ (the significant change is between mean differences of low and high MUFA consumption).

Along with lipoprotein concentration, particle density and size was also measured, but no significant changes were seen (Table 12).

TABLE 12 Particle density and size pre- and post-test patty consumption[1]

	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
HDL mean density	1.09 ± 0.002	1.08 ±0.005	1.09 ±0.002	1.09 ±0.002
LDL mean density	1.03 ±0.0001	1.02 ±0.0002**	1.03 ±0.0003	1.03 ±0.0003

[1] Data are means ± SE for 12 men and women. ** $P \leq 0.05$

While plasma concentration of palmitic acid significantly increased with premium ground beef consumption, linoleic acid decreased significantly after premium ground beef treatment. (Figure 10 and Table 13); furthermore, stearic and oleic acid concentration were increased after both types of beef patty treatment; however, the change was not statistically significant.

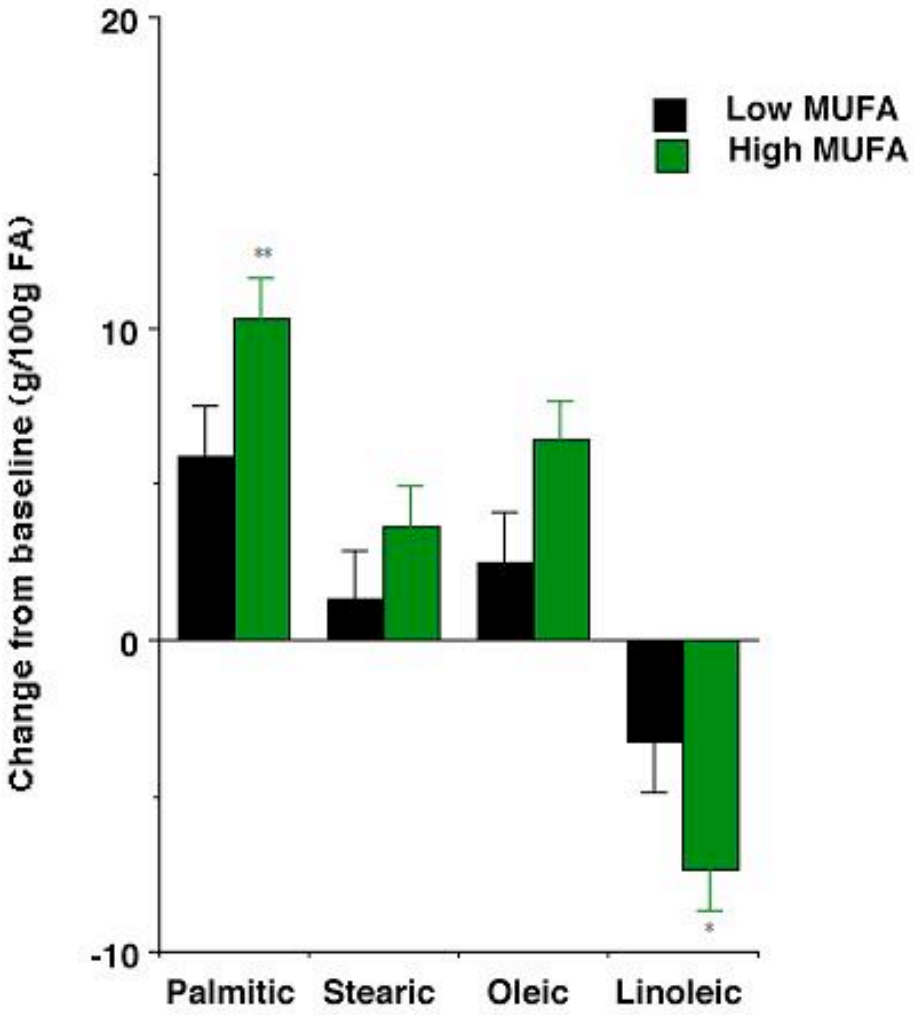

FIGURE 10 Changes from baseline in major plasma fatty acid of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, *n* = 12. * P ≤ 0.10; ** P ≤ 0.05 (the significant change is between mean differences of low and high MUFA consumption).

TABLE 13 Plasma fatty acid concentrations (g/100 g fatty acids) of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Fatty Acid	Pre Conventional	Post Conventional	Pre Premium	Post Premium
14:0	0.35 ± 0.05	0.94 ± 0.1	0.2 ± 0.05	0.25 ± 0.06 *
16:0	24.40 ± 1.35	25.62 ± 2.94	16.93 ± 1.42	17.50 ± 1.55 **
16:1	1.72 ± 0.18	2.13 ± 0.30	1.26 ± 0.24	1.19± 0.20
18:0	15.22 ± 1.93	10.08 ± 1.48	10.48 ± 0.45	10.09 ± 0.82
18:1c9	28.62 ± 1.60	22.53 ± 2.43	22.10 ± 1.43	20.81 ± 0.78
18:1c11	2.06 ± 0.16	4.03 ± 2.22 ***	1.67 ± 0.08	1.62 ± 0.07
18:2	24.12 ± 2.20	36.20 ± 1.67	33.54 ± 2.02	38.24 ± 1.83
18:3	0.52 ± 0.06	0.80 ± 0.10	0.68 ± 0.08	0.66 ± 0.07
20:1	0.14 ± 0.02	0.14 ± 0.01*	0.15 ± 0.01	0.22 ± 0.01

TABLE 13 Continued

Fatty Acid	Pre Conventional	Post Conventional	Pre Premium	Post Premium
20:4	4.05 ± 0.96	10.87 ± 1.53	11.80 ± 0.72	11.97 ± 1.00
20:5	0.48 ± 0.06	0.70 ± 0.1	0.99 ± 0.13	0.78 ± 0.12
22:0	0.32 ±0.09	1.49 ± 1.15	0.47 ± 0.04	0.32 ± 0.04
22:6	1.30 ±0.28	1.97 ± 0.37	2.36 ± 0.19	2.33 ± 0.30

[1] Data are means ± SE for 12 men and women.
* $P \leq 0.10$; ** $P \leq 0.05$; ***$P \leq 0.01$

Tables 14 and 15 present correlations between plasma fatty acids and lipoprotein cholesterol concentrations. HDL cholesterol concentration was significantly decreased with premium ground beef consumption and was positively correlated with erucic acid (22:1) and negatively correlated with α-linolinic acid (18:3), linoleic (18:2) and eicosatetraenoic acid fatty acid (20:4). LDL cholesterol concentration was negatively correlated with erucic acid (22:1). Though plasma CRP level was not significant within each treatment, its concentration was positively correlated with oleic acid, lignocenic acid (24:0) and EPA, eicosapentaenoic acid (20:5). Though **V**LDL concentration did not change significantly but it was increased due to conventional ground beef diet and depressed with premium ground beef consumption. VLDL concentration was positively correlated with the *trans* isomer of oleic acid, elaidic acid (18:1*cis*11), and it was negatively correlated with stearic acid and oleic acid plasma concentration. HDL3 was negatively correlated with lignocenic acid (24:0). Furthermore, LDL mean density significantly increased with conventional ground beef consumption which was negatively correlated with linoleic acid (18:2).

TABLE 14 Simple correlations between plasma fatty acids and cholesterol fractions, triglycerides, hs-CRP, insulin and homocysteine[1]

	Total Cholesterol	LDL	HDL	TAG	hs-CRP	Insulin	Homocysteine	VLDL	RLP	IDL
14:0	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
16:0	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
18:0	NC	NC	NC	NC	NC	NC	NC	-0.34	NC	NC
18:1	NC	NC	NC	NC	0.34	NC	NC	-0.38	NC	NC
18:1*cis*11	NC	NC	NC	NC	NC	NC	NC	0.98	NC	NC
18:2	NC	NC	-0.37	NC	NC	NC	NC	NC	NC	NC
18:3	NC	NC	-0.38	NC	NC	NC	NC	NC	NC	NC
20:4	NC	NC	-0.24	NC	NC	NC	NC	NC	NC	NC
22:1	NC	-0.58	0.30	NC	NC	NC	NC	NC	NC	NC
20:5	NC	NC	NC	NC	0.32	NC	NC	NC	NC	NC
24:0	NC	NC	NC	0.32	0.34	NC	NC	NC	NC	NC

[1] The correlations are estimated by Pairwise method. All r values stated are significant, $P \leq 0.05$; NC = no correlation

TABLE 15 Simple correlations between plasma fatty acids and cholesterol subfractions, HDL mean density, LDL particle size and density[1]

	LDL III	LDL IV	HDL 2b	HDL 2a	HDL 3	HDL mean density	LDL mean density	LDL mean size	Lp(a)
16:0	NC	NC	NC	NC	NC	NC	NC	NC	NC
16:1	NC	NC	NC	NC	NC	NC	NC	NC	NC
18:0	NC	NC	NC	NC	NC	NC	NC	NC	NC
18:1c9	NC	NC	0.29	NC	NC	NC	NC	NC	NC
18:1c11	NC	0.45	NC	NC	NC	NC	NC	NC	NC
18:2	NC	NC	-0.34	NC	NC	NC	-0.35	NC	NC
18:3	NC	NC	NC	NC	NC	NC	NC	NC	NC
20:4	NC	NC	NC	NC	-0.32	NC	NC	NC	NC
20:5	NC	NC	0.33	NC	NC	NC	NC	NC	NC

[1] The correlations are estimated by Pairwise method.
All r values stated are significant, $P \leq 0.05$; NC = no correlation

In order to determine whether there had been a phase effect among plasma lipoprotein and fatty acid concentration, the interaction between diet and phase was also studied. Data were analyzed by a 2-way ANOVA method using JMP statistical software.

LDL, HDL and total cholesterol concentration along with the rest of lipoprotein particles did not show a significant phase and diet interaction (Table 16 and Figures 11-16). There was a significant change in the level of HCY due to diet; however, there is an interaction between phase and diet, which means changes in HCY due to diet, is not independent of phase (Table 17).

Plasma CRP level did not change significantly after either premium or conventional ground beef intervention; also, there was no significant phase and diet interaction in the level of CRP (Table 17).

Insulin concentration of men and women rotated through either test ground beefs, conventional or premium, did not change significantly after each phase and the interaction between diet and phase did not show a significant change as well (Table 17).

Table 16 Major lipoprotein plasma concentration of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1] in each phase and the interaction between diet and phase.

		Conventional		Premium		Effect		
	Phase	Phase 1	Phase 2	Phase 1	Phase 2	Diet (D)	Phase (P)	D x P
Total Cholesterol	Baseline (B)	5.62 ±0.29	4.85 ±0.25	5.24 ±0.44	5.65 ±0.26	NS	NS	NS
	Final (F)	5.30 ±0.35	5.19 ±0.38	5.30 ±0.29	5.88 ±0.45			
	F-B	-0.01 ±0.49	-0.29 ±0.49	-0.19 ±0.62	-0.34 ±0.08			
LDL	Baseline (B)	3.43 ±0.38	2.98 ±0.16	3.12 ±0.37	3.48 ±0.33	NS	NS	NS
	Final (F)	3.42 ±0.28	3.34 ±0.33	3.21 ±0.25	3.72 ±0.40			
	F-B	-0.23 ±0.36	-0.15 ±0.32	0.12 ±0.58	0.10 ±0.10			
HDL	Baseline (B)	1.76 ±0.36	1.56 ±0.14	1.70 ±0.15	1.62 ±0.21	NS	NS	NS
	Final (F)	1.54 ±0.15	1.61 ±0.12	1.75 ±0.15	1.69 ±0.19			
	F-B	0.36 ±0.45	0.03 ±0.19	-0.24 ±0.18	-0.36 ±0.15			

[1] Data are means ± SE for 12 men and women.
NS = not significant

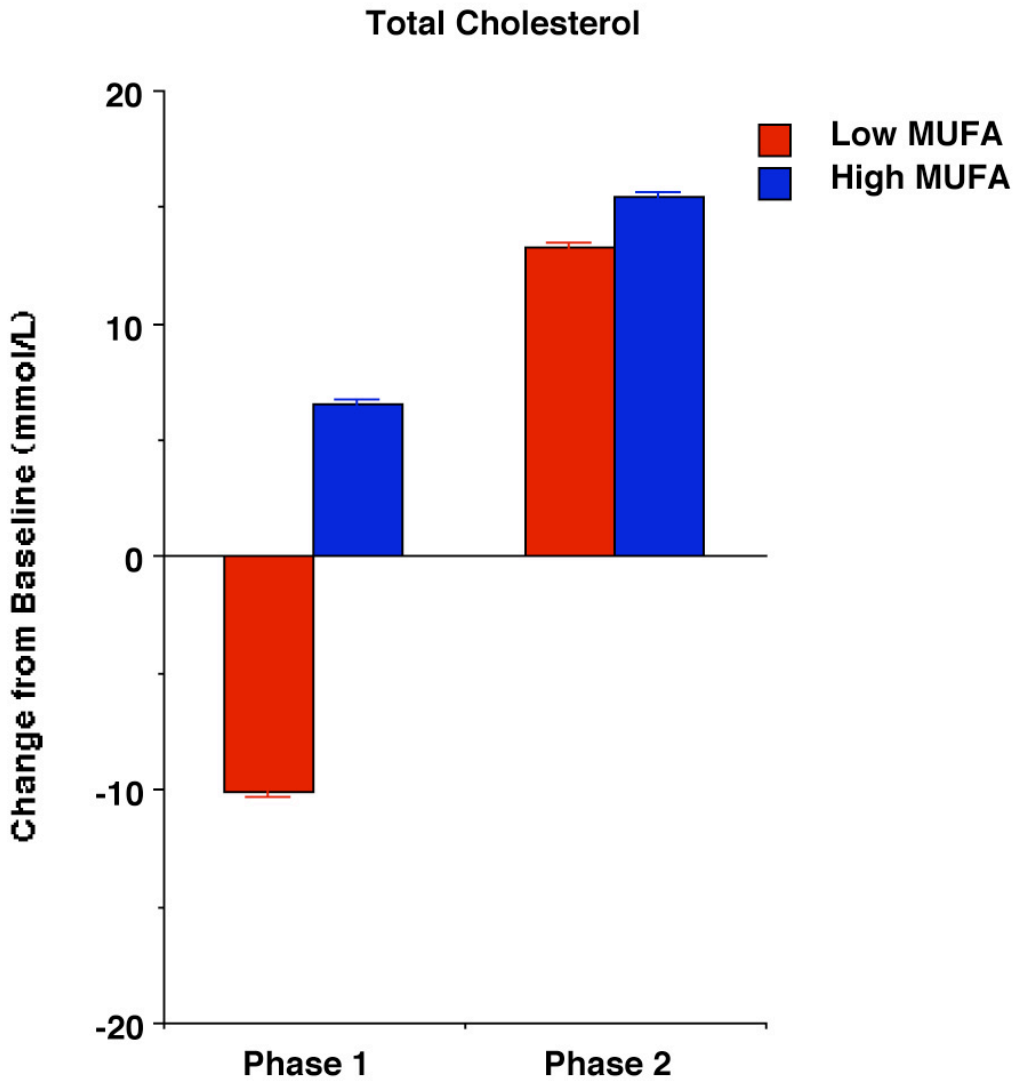

Figure 11 Changes from baseline in total cholesterol concentration (mmol/L) of men and women after beef treatment in each phase. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases (*P* > 0.1)

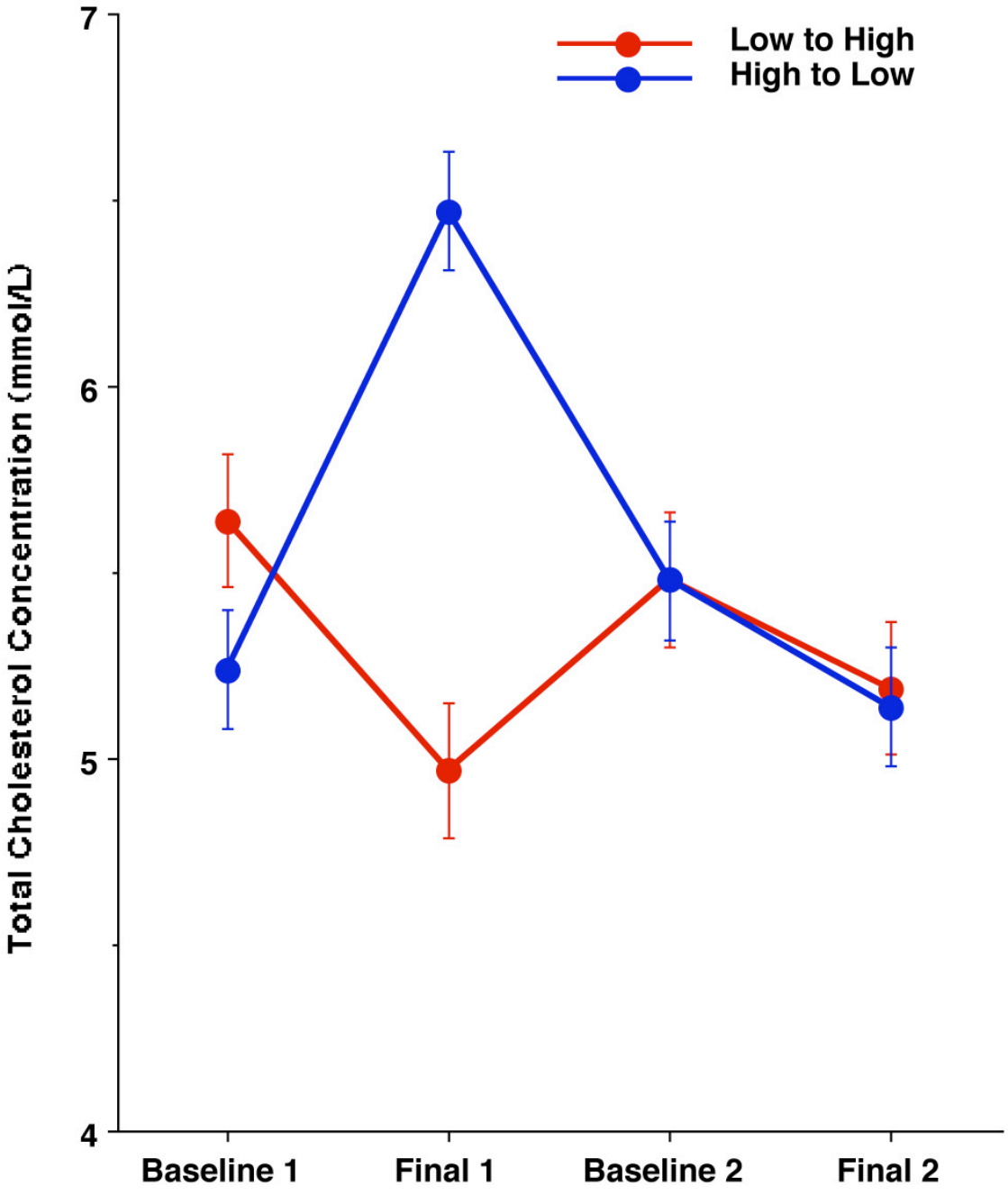

Figure 12 Changes from baseline in total cholesterol concentration (mmol/L) of men and women in each phase including wash out period. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases ($P > 0.1$)

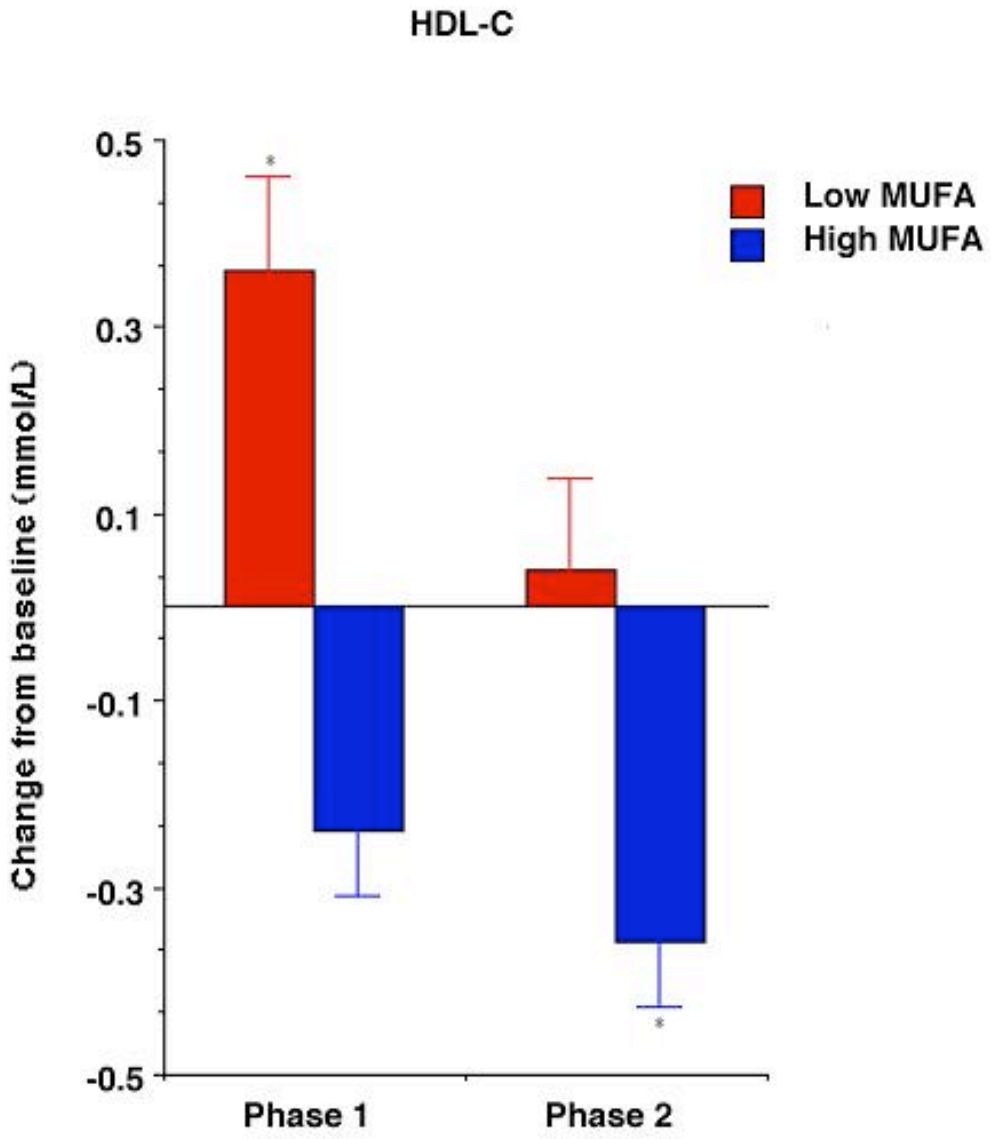

Figure 13 Changes from baseline in HDL concentration (mmol/L) of men and women after beef treatment in each phase. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases (*P* > 0.1)

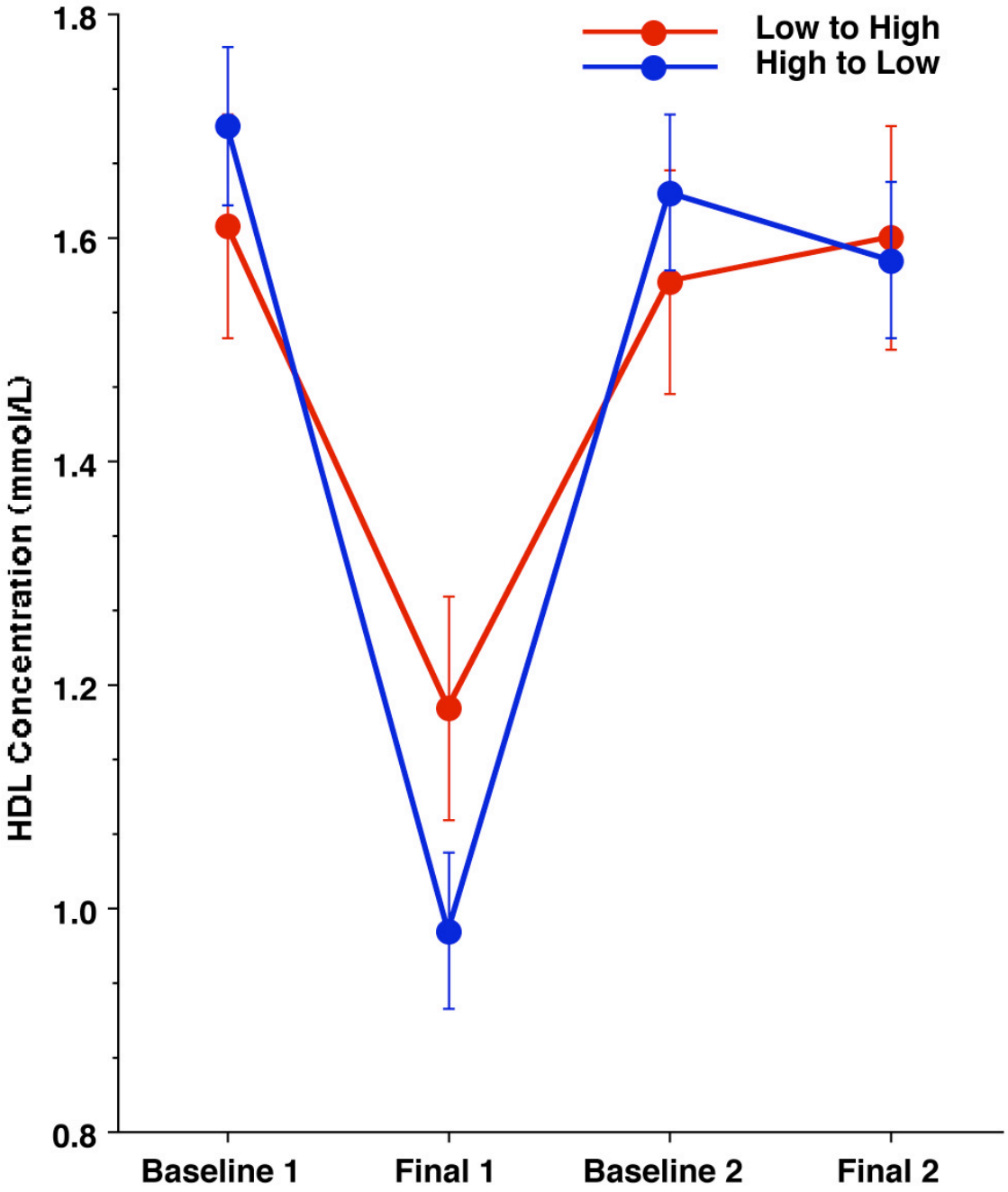

Figure 14 Changes from baseline in HDL concentration (mmol/L) of men and women in each phase including wash out period. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases (*P* > 0.1)

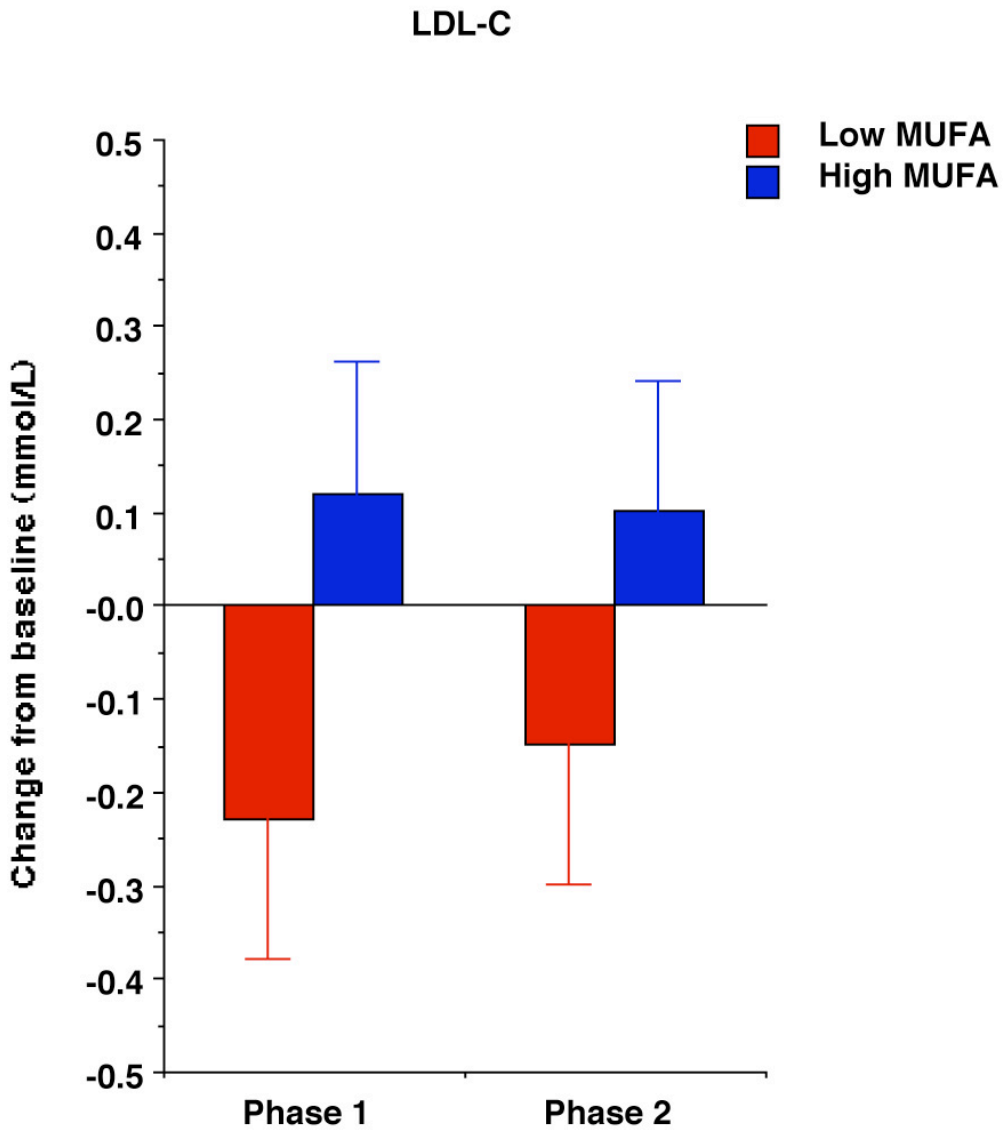

Figure 15 Changes from baseline in LDL concentration (mmol/L) of men and women after beef treatment in each phase. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases ($P > 0.1$)

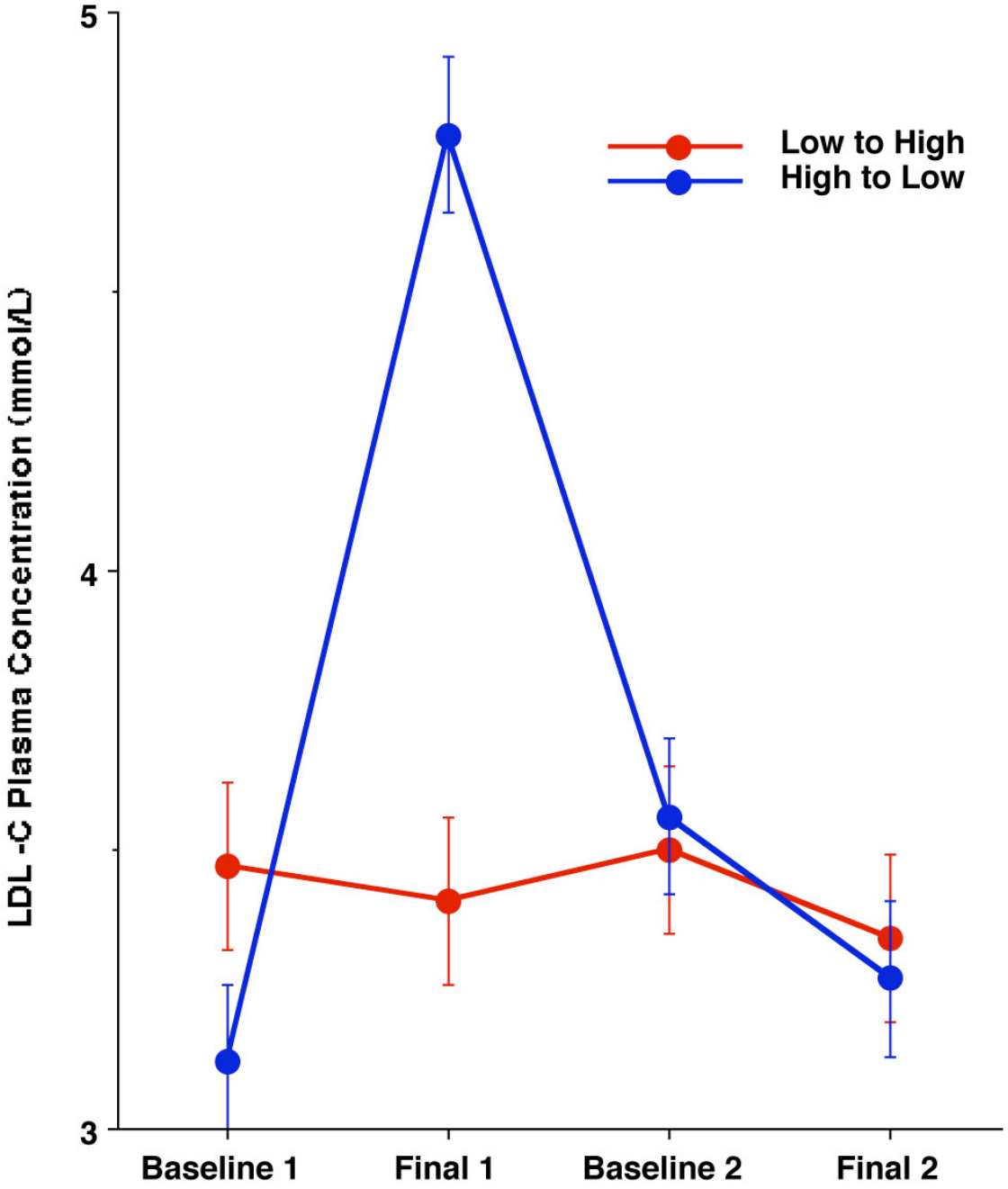

Figure 16 Changes from baseline in LDL concentration (mmol/L) of men and women in each phase including wash out period. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases ($P > 0.1$)

Table 17 CRP, HCY and insulin concentration of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1] in each phase and the interaction between diet and phase

	Phase	Conventional		Premium		Effect		
		Phase 1	Phase 2	Phase 1	Phase 2	Diet (D)	Phase (P)	D x P
CRP	Baseline (B)	0.21 ±0.05	0.41 ±0.12	0.43 ±0.15	0.35 ±0.16	NS	NS	NS
	Final (F)	0.17 ±0.02	0.51 ±0.14	2.823 ±0.40	0.24 ±0.47			
	F - B	-0.04 ±0.05	0.10 ±0.10	-0.08 ±0.09	-0.11 ±0.17			
HCY	Baseline (B)	7.06 ±0.28	11.29 ±1.22	11.30 ±1.37	9.90 ±1.80	**	*	**
	Final (F)	10.76 ±0.90	10.50 ±1.15	9.60 ±1.07	8.68 ±0.66			
	F - B	3.70 ±1.10	-0.78 ±0.40	-1.70 ±1.19	-1.22 ±1.48			
Insulin	Baseline (B)	7.62 ±1.81	9.39 ±2.53	5.72 ±0.55	15.68 ±4.19	NS	***	NS
	Final (F)	12.82 ±3.8	5.87 ±0.54	13.16 ±3.54	10.20 ±2.33			
	F - B	5.20 ±2.23	-3.51 ±2.77	7.42 ±3.69	-5.48 ±3.43			

[1] Data are means ± SE for 12 men and women.
* $P \leq 0.10$; ** $P \leq 0.05$; ***$P \leq 0.01$; NS = not significant

Major plasma fatty acids were also studied in order to test for diet and phase interaction. As it is shown on Table 19 there was no significant interaction between diet and phase in plamitic, stearic, oleic and linoleic acid (Table 18 and Figures 17-20).

Table 18 Major plasma fatty acid concentration of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1] in each phase and the interaction between diet and phase.

Fatty acid	Phase	Conventional		Premium		Effect		
		Phase 1	Phase 2	Phase 1	Phase 2	Diet (D)	Phase (P)	D x P
Palmitic	Baseline (B)	20.87 ±2.02	18.32 ±2.71	14.12 ±1.11	15.19 ±1.73	NS	NS	NS
	Final (F)	24.44 ±2.69	25.93 ±3.87	24.37 ±1.51	22.63 ±3.02			
	F - B	3.56 ±3.58	7.60 ±3.80	4.19 ±2.34	10.47 ±3.93			
Stearic	Baseline (B)	10.46 ±0.81	21.76 ±1.16	10.50 ±0.58	20.32 ±1.35	NS	NS	NS
	Final (F)	15.37 ±4.50	23.24 ±3.36	15.11 ±1.32	19.91 ±1.63			
	F - B	4.91 ±5.20	-1.92 ±2.65	7.42 ±1.29	2.32 ±1.97			
Oleic	Baseline (B)	24.13 ±2.86	1.56 ±0.14	20.64 ±1.30	1.62 ±0.21	NS	NS	NS
	Final (F)	28.07 ±3.81	1.61 ±0.12	29.02 ±1.06	1.69 ±0.19			
	F - B	3.93 ±5.84	1.48 ±4.24	10.56 ±1.74	3.66 ±3.82			
Linoleic	Baseline (B)	29.24 ±3.34	40.18 ±1.18	36.00 ±2.18	35.63 ±4.61	NS	NS	NS
	Final (F)	27.06 ±4.53	35.66 ±2.53	22.01 ±1.93	37.94 ±0.74			
	F - B	-0.04 ±0.05	3.66 ±9.04	-12.45 ±2.61	1.84 ±12.88			

[1] Data are means ± SE for 12 men and women.
NS = not significant

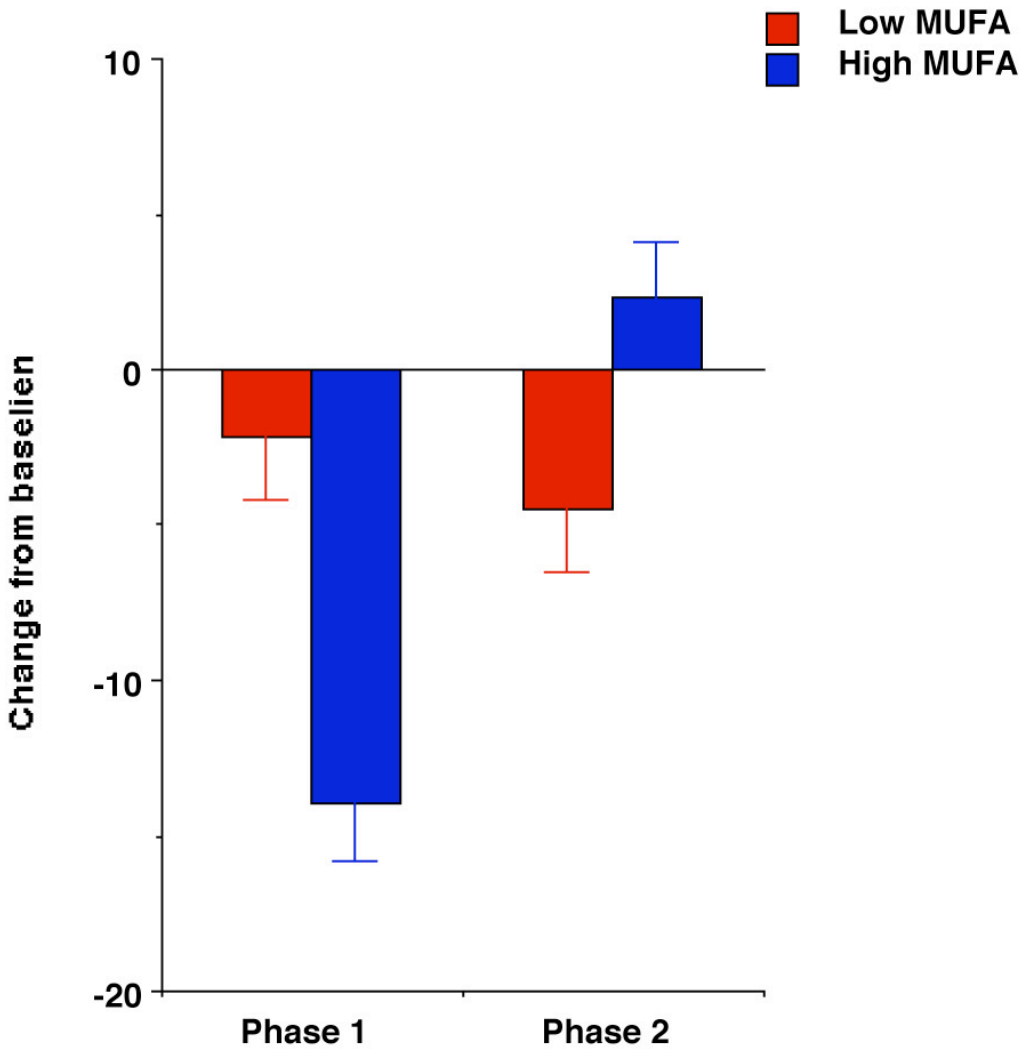

Figure 17 Changes from baseline in Linoleic acid concentration (g/100g fatty acid) of men and women after beef treatment in each phase. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases ($P > 0.1$)

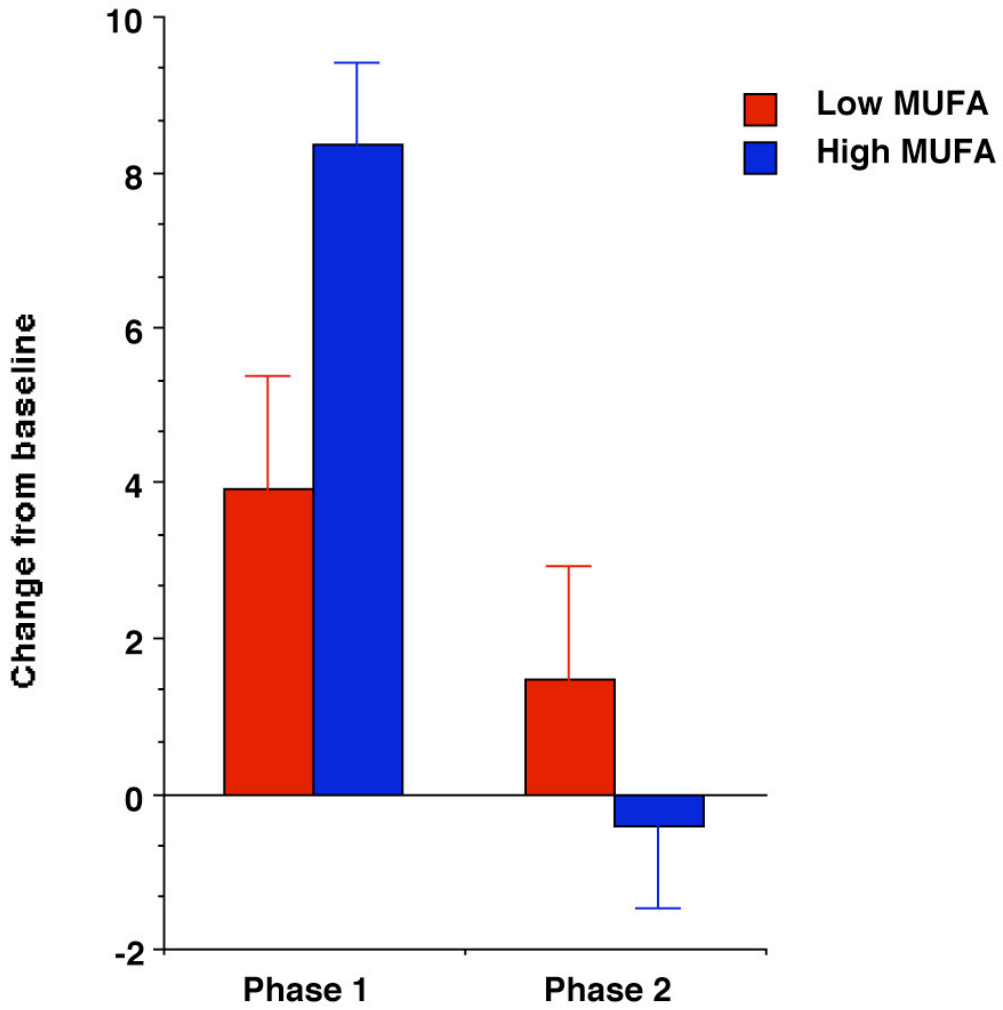

Figure 18 Changes from baseline in Oleic acid concentration (g/100g fatty acid) of men and women after beef treatment in each phase. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases ($P > 0.1$)

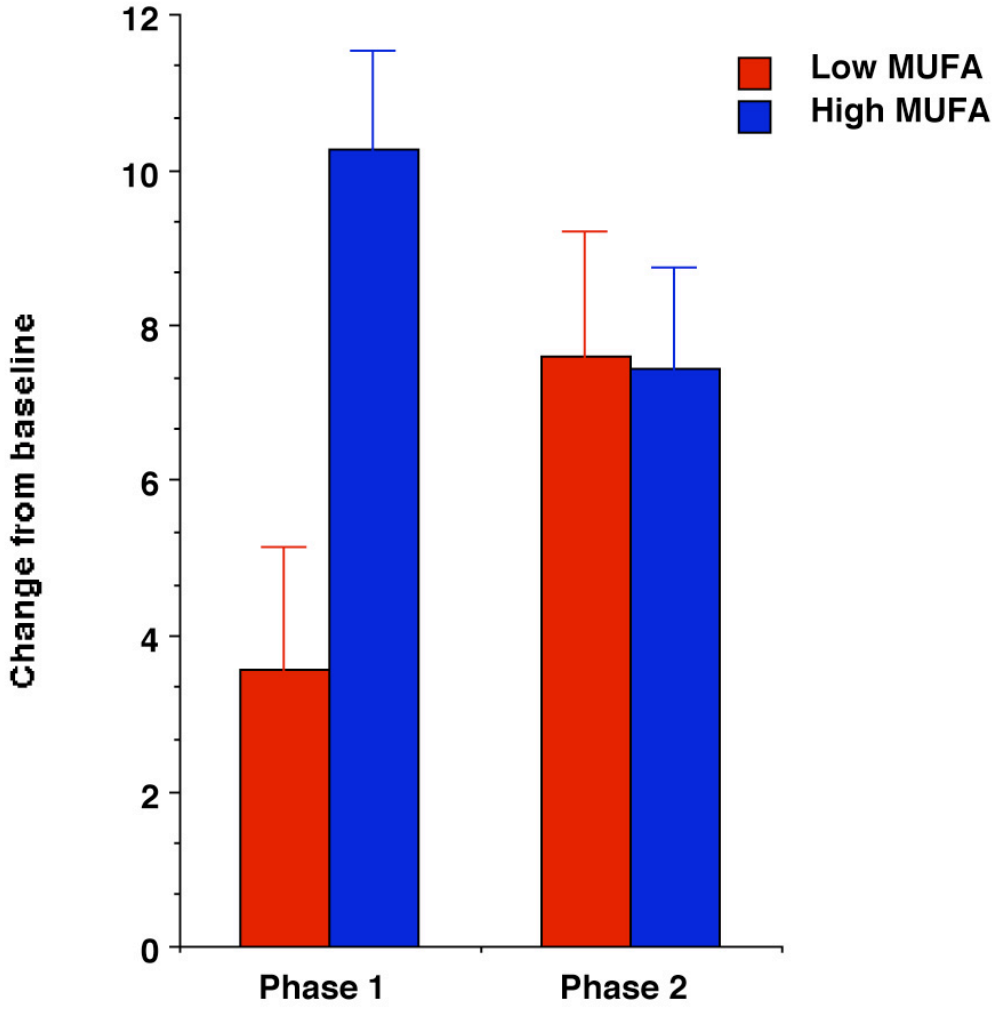

Figure 19 Changes from baseline in Palmitic acid concentration (g/100g fatty acid) of men and women after beef treatment in each phase. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases ($P > 0.1$)

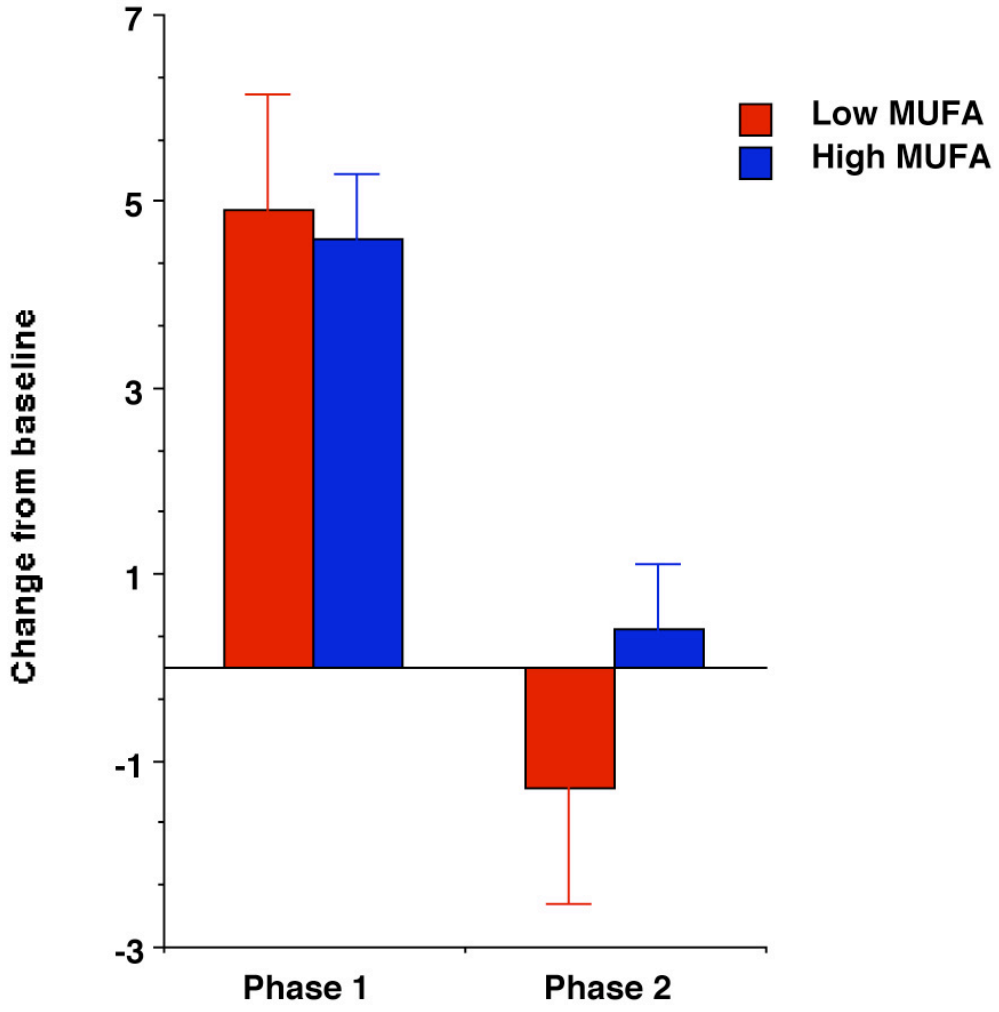

Figure 20 Changes from baseline in Stearic acid concentration (g/100g fatty acid) of men and women after beef treatment in each phase. Values are expressed as mean ± SEM; n=12. There were no significant differences among phases ($P > 0.1$)

Discussion

Diet has profound effects on the development of atherosclerosis. The importance of the fatty acid composition of dietary fats in coronary artery disease rates was first recognized by the classic Seven Countries Study (134) and the Japan-Honolulu-San Francisco Study (135). Both of these cross-population studies reported that total fat, SFA, and cholesterol intakes were strongly and positively associated with coronary artery disease risk. Furthermore, they showed that MUFA intake is strongly and negatively associated with coronary artery disease mortality when adjustments were made for SFA and cholesterol. Also, it was shown that the ratio of MUFA to SFA was a better predictor than when each was considered separately (136).

Studies of the Mediterranean diet or of olive oil might incorrectly be considered studies of monounsaturated fatty acids. In fact, the Mediterranean diet involved a set of complex, nutritional components including fruits and vegetables. Furthermore, olive oil also includes an unsaponifiable fraction, that should be taken into consideration. In spite of this, the Seven Countries Study indicated there might be an inverse relationship between monounsaturated fatty acids and coronary mortality. Moreover, the American prospective studies of healthcare professionals and nurses have shown an estimated reduction of 19% in the risk of coronary disease when the MUFA intake was increased by 5% (as a percentage of the total energy intake) (83).

In this study, I focused on the impact of the dietary MUFA, specifically oleic acid (18:1, n-9) content on total lipoproteins, lipoprotein subfractions and inflammatory markers associated with increased CVD risk. This was implemented through a

comparison between the intake of conventional ground beef, and the intake of premium ground beef which was naturally modified to have an elevated MUFA:SFA ratio.

The conventional ground beef consisted of lower MUFA:SFA ratio in comparison to the premium ground beef in each patty; however, the total fat was higher in conventional ground beef. This caused total SFA and MUFA intake to be greater in the conventional ground beef diet as compared to premium ground beef diet.

In this study, I observed a significant decrease in HDL-C and an increase in LDL-C with premium ground beef consumption. Also, the opposite effect (although not significant) was seen with conventional ground beef diet. This was in contrast to previous studies that have shown increases in HDL-C concentration after premium ground beef consumption (137). However, the main difference between this study and the previous studies was the concentration of oleic acid in premium ground beef was lower than in previous studies. Because the conventional ground beef contained more oleic acid than the premium ground beef, these results indicate that the beneficial effect of oleic acid intake may be apparent even when combined with higher total fat intake and higher SFA.

It has been shown that above a certain threshold, SFA intake causes a clear increase in plasma HDL-C level (138). Although the physiopathological significance of this increase is not well established, our results were consistent in terms of HDL-C increase with conventional ground beef diet. In addition to the increase of HDL-C, LDL-C decreased with conventional ground beef diet. This may be attributed to the concentration of oleic acid in conventional ground beef diet. Furthermore, in a different

study (82) it was shown that the LDL-C/HDL-C ratio is more favorably influenced by replacing SFA with unsaturated fatty acids than by reducing SFA alone (82).

An earlier study showed that a low-fat diet significantly lowered HDL-C, whereas high- MUFA diet did not (87). It was indicated that a solid-food diet rich in MUFA was equivalent to a low-fat, high-carbohydrate diet for cholesterol lowering, without reducing HDL-C (87). A similar response was reported in which a low-fat, high-carbohydrate diet caused a reduction of HDL-C levels, whereas a diet high in MUFA did not (139). However, neither of those studies examined the effects of higher MUFA intake in combination with higher total fat intake.

Both ground beef treatments caused a decrease in total plasma cholesterol, TAG, and VLDL-C. High TAG is associated with a number of adverse metabolic risk factors, including the preponderance of small, dense LDL (140). Furthermore, based on meta-analysis of prospective studies from the epidemiological literature, TAG is a risk factor for CVD, independent of HDL-C (141). In this study lower plasma TAG after both conventional and premium ground beef consumption suggests a negative association with ground beef consumption and CVD. Moreover, in this study the effect of ground beef consumption on serum cholesterol was beneficial given that total cholesterol concentration was depressed after both ground beef interventions. It has been found that each 1% rise in serum cholesterol is predicted to increase the risk of CVD by almost 2% (159). When VLDL TAG are hydrolyzed in plasma, the cholesterol, phospholipids, and proteins are transferred to higher density lipoproteins such as HDL and LDL, leaving a small, dense remnant lipoprotein (RLP). RLP are further metabolized to smaller, more

dense lipoproteins called IDL. RLP and IDL particles were measured after each ground beef intervention, and their plasma concentrations was decreased after both treatments. This also supports my contention that beef consumption in general lowers risk factors for CVD, given that an increase in the concentration of RLP particles is considered a risk factor for CVD (87, 88).

LDL-III decreased after both ground beef interventions, although this change was not statistically significant. It is believed that small, dense LDL particles are a contributing factor to atherosclerosis based on evidence that smaller LDL are taken up less readily by LDL receptors (15) are more suitable to penetrate arterial tissue (16), and are oxidized at a greater rate than larger LDL particles (17). There was a significant increase in LDL particle size after consuming conventional ground beef, suggesting that higher concentration of oleic acid in beef improves lipoprotein profile in men and women. This was confirmed by a significant increase in LDL mean density after conventional ground beef intervention. There was no change in LDL-IV, the densest form of LDL with both ground beef consumption. These results suggest that consumption of premium or conventional ground beef either decrease or has no effect on the concentration of small and dense LDL particles. Lp(a) increased but not significantly after either ground beef intervention. Lp(a) is similar to LDL particles, but also contains apolipoprotein(a). Lp(a) particles accumulate in atherosclerotic lesions; therefore, it is considered to be a strong risk factor for CVD. However, Lp(a) levels are dependent on genetically regulated synthesis and not particle clearance. Plasma concentrations of HDL subfractions did not change significantly with either ground beef

intervention. HDL-2a cholesterol and HDL-2b cholesterol concentration decreased after either ground beef consumption; however, HDL-3 plasma concentration increased as did HDL-3 particle concentration with premium ground beef consumption. Antioxidant activity of HDL particles increases in the order $HDL_{2b}<HDL_{2a}<HDL_{3a}<HDL_{3b}<HDL_{3c}$ with the antioxidant activity of HDL_{3c} most effective at the late stages of oxidation (142). These findings indicate that small, dense HDL particles protect LDL against oxidative stress, thereby, suggesting that premium ground beef consumption had protective effects against oxidative stress.

MUFA are antiatherogenic by decreasing the susceptibility of LDL to oxidation, improving endothelial function and reducing inflammation marker levels and platelet aggregation (143). In this study, premium ground beef consumption resulted in a decrease in the plasma CRP concentration and an increase in plasma homocysteine concentration as compared to conventional ground beef consumption. This change was not statistically significant, but it is consistent with the notion that ground beef with a higher MUFA:SFA ratio can be anti-atherogenic and have anti-inflammatory effects through involvement of inflammatory proteins or biomarkers that are associated with CVD. Therefore, this study and previous studies in this laboratory lead me to conclude that the quality of beef fat influences the risk of CVD and inflammation.

The different results between each ground beef intervention and their effects on plasma HDL-C and LDL-C concentrations could be related to several factors, including participant characteristics, genetic factors and other dietary components. Furthermore, several other studies that have focused on the effects of high-MUFA consumption on

CVD and atherosclerosis risk factors have shown an improvement in the level of lipoproteins; however, most of those studies focused on the Mediterranean life style, including olive oil consumption (101) or the replacement of carbohydrate or SFA by MUFA in diet (87). Different results in lipoprotein concentrations in this and a previous study suggest that the beneficiary effects in terms of lowering risk factors for CVD associated with increasing MUFA:SFA ratio in ground beef can be related to other factors in addition to improvement of plasma HDL-C. For instance, lowering plasma total cholesterol, TAG, VLDL-C, RLP-C and increasing small, dense HDL3 might be effective in lowering risk factors for CVD. Furthermore, the amount of MUFA intake may be more important in terms of improving lipoprotein profile in comparison to dietary modification of MUFA:SFA ratio.

.

CHAPTER III

THE EFFECTS OF GROUND BEEF DIETS CONTAINING DIFFERENT MUFA:SFA RATIOS ON GENE EXPRESSION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

Introduction

The effects of PUFA on human immune cells have been examined in several intervention trials (144-147) PUFA intake has been shown to decrease the expression of genes of liver X receptor (LXR) signaling and increase the expression of genes related to cellular stress responses. In a recent study increased MUFA intake had a significant effect on ATP-binding cassette A1 (ABCA1) gene expression and similar effects on sterol regulatory element binding transcription factor 1 (SREBF1) and LXR gene expression compared with PUFA intake (128). In this study, peripheral blood mononuclear cell gene expression in response to dietary lipids was investigated.

Peripheral blood mononuclear cells (PBMNC) play a major role in the development of atherosclerosis and were chosen for this study to examine the effects of high-oleic acid ground beef consumption on gene expression in an intervention study. The genes that were selected in this study were Stearoyl-coA desaturase-1 (SCD-1), LDL receptor (LDLR), SREBF1, ABCA1, ATP-binding cassette G1 (ABCG1), and mediator complex subunit 1 (MED1). These genes are involved in lipid metabolism (SCD-1, LDLR and MED1) and cholesterol metabolism (SREBF1, ABCG1 and ABCA1). They are also part of LXR signaling pathways and play a major role in

reverse cholesterol transport in macrophages and have been shown to be affected by different dietary fatty acids (148).

The selection of genes was based on their relation to atherosclerosis as well as the responses observed in previous studies (147). A long-term effect of consuming virgin olive oil on peripheral blood mononuclear cell gene expression has been investigated previously (148). In a similar study the consumption of high-fat shakes varying in dietary fatty acid composition was shown to alter gene expression profiles in PBMCs (147). None of those studies, however, investigated the differences between a premium high MUFA:SFA ground beef and a conventional low MUFA:SFA ground beef consumption on PBMC gene expression.

Stearoyl-CoA Desaturase

Pathway analysis has identified several candidate genes that are associated with fatty acid composition. For example, SCD-1 is an iron-containing enzyme that catalyzes a rate-limiting step in the synthesis of unsaturated fatty acids (149). SCD-1 is responsible for the conversion of saturated fatty acids to their Δ9 desaturated counterparts and can be influenced by diet. The major product of SCD is oleic acid, which is formed by the desaturation of stearic acid. The ratio of stearic acid to oleic acid has been implicated in the regulation of cell growth and differentiation through its effects on cell membrane fluidity and signal transduction (88, 150). A nonsynonymous mutation in SCD1 of Japanese-Black cattle was identified by Taniguchi and others. The mutation was associated with the saturation level of fatty acids and fat melting

temperature (151). Cattle show a dramatic change in the concentration of MUFA and the amount of marbling after being grain-fed. SCD1 is the main pathway involved (152). Even though many of the effects of SCD1 deficiency are dependent on diet composition and other genetic factors (59), it seems SCD1 deficiency is antiatherogenic. SCD1 deficiency also promotes inflammation and atherosclerosis in mice (60).

Adenosine Triphosphate Binding Cassette A1

ABCA1 is involved in the efflux of cholesterol from cells to HDL particles (153). Mutations in ABCA1 have also been associated with a less severe form of familial hypoalphalipoproteinemia (154, 155). ABCA1 and ABCG1 are two members of the ABC family of transporter proteins. These genes are induced in lipid-loaded macrophages (156). A mutation in the ABCA1 gene results in loss of function and leads to Tangier disease (157). Fibroblasts from Tangier patients have impaired ability to donate cholesterol to apolipoprotein AI (apoAI). This suggests that ABCA1 plays a major role in cellular cholesterol efflux. Recent studies have provided evidence that the nuclear receptors LXRa and LXRb mediate the lipid induction of both ABCG1 and ABCA1 (158).

The molecular mechanisms responsible for the intracellular localization, substrate specificity, and functional activity of ABCG1 are still poorly understood. ABCG1 expression is up-regulated by RXR-specific ligands as well as lipoprotein-derived lipids, and some oxysterols (159). This mechanism takes place via the LXR/RXR pathway (159). The active involvement of ABCG1 in macrophage reverse

cholesterol transport suggests its potential role in the process of foam cell formation. ABCG1 may be part of a lipid transport system that counterbalances excessive influx of cholesterol into macrophages. Because ABCG1 is expressed ubiquitously in human tissues, its role in cellular lipid homeostasis may not be limited to macrophages (156).

Mediator Complex Subunit 1 (MED1)

MED1 plays a key role in PPAR-induced adipogenesis. MED1 interacts with nuclear receptors such as PPARc and other transcriptional activators. Peroxisome proliferator-activated receptor-c, a nuclear receptor, when overexpressed in liver stimulates the induction of adipocyte-specific and lipogenesis-related genes and causes hepatic steatosis. MED1 is a key component of Mediator complex and is required for RNA polymerase II–dependent gene transcription (160). Mediator involves in transmitting signals from transcriptional activators that are also nuclear receptors. It has been reported that the liver-specific knock-out of MED1 impairs the ligand-dependent activation of PPAR target genes (161). Furthermore, MEFs derived from MED1 KO embryos show reduced PPAR-mediated adipogenesis (161). MED1 is known to mediate strong ligand-dependent interactions between the Mediator complex and many nuclear receptors, including members of the PPAR family. It was demonstrated that PPAR-mediated adipogenesis depends on MED1 (161).

Low Density Lipoprotein Receptor (LDLR)

LDLR is a cell-surface receptor that recognizes the apoprotein B100 that is embedded in the phospholipid outer layer of LDL particles. This receptor also recognizes the apoE protein found in chylomicron remnants and VLDL remnants (IDL). LDLR is associated with clathrin-coated pits, and when it binds LDL it is internalized into acidic endosomes where it can get separated from its ligand. The ligand then gets degraded in lysosomes, and the receptor returns to the cell surface (162). Mutations in the LDLR gene might lead to elevated plasma cholesterol levels, resulting in artherosclerosis and coronary heart disease (163).

The cholesterol that is carried by LDL and generated within the lysosome is known to be responsible for suppressing HMG CoA reductase activity. This suppression of transcription of the HMG CoA reductase gene occurs through pathways involving sterol regulatory element-binding protein (SREBP) (55). Another role of the LDL-derived cholesterol is activation of a cholesterol-esterifying enzyme, acyl CoA: cholesterol acyltransferase (ACAT), in order for the excess cholesterol to be stored as cholesteryl ester droplets in the cytoplasm (164). LDL also inhibits the SREBP pathway, which leads to suppression of transcription of the LDL receptor gene (55). Therefore, when cell cholesterol level increases, the production of LDL receptors is reduced (55). This role of the LDL-derived cholesterol in addition to suppression of the HMG CoA reductase transcription can be part of the regulatory responses that lead to a decrease cholesterol input from plasma as well as from endogenous synthesis (165).

Objectives of this study were to determine whether Premium ground beef intake has an impact on the expression of genes associated with lipid and cholesterol metabolism in order to better understand the underlying mechanisms involved with CVD.

Methods

PBMC Isolation

PBMCs were isolated using Vacutainer Cell Preparation Tubes according to the manufacturer's instruction (Plumolab Medical Supplies).

RNA Extraction from White Blood Cells

Whole blood was centrifuged at 1690×*g* for 30 min. White cells within the buffy coat fraction of the blood sample was collected after the centrifugation. White cells was washed with phosphate buffered saline (PBS), centrifuged at 970×*g* for 15 min, resuspended in Ultra-spec, and then stored at -80ºC until RNA isolation. Total RNA was then extracted from the Ultra-spec. Total RNA concentration and purity (ratios A260/A280 and A260/A230) were estimated by spectrophotometry (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, DE).

Reverse-transcription and Real-time qPCR

cDNA was generated from total RNA samples using the High Capacity cDNA RT Kit (Applied Biosystems, Foster City, CA), according to the manufacturer's

instructions. All generated cDNA was stored at -80ºC prior to quantification by PCR. Real-time PCR was completed in the Animal and Food Science Department, Texas Tech University, Lubbock, TX. Eukaryotic 18S rRNA was used as an endogenous gene expression control.

Statistical Analysis

Data were analyzed by paired *t*-test comparing baseline (habitual) values to values after 5 wk on the test diet. Also a 2-factorial ANOVA was used by JMP (John's Macintosh Project) statistical software to examine the interaction between diet and phase with regards to each gene expression. Each participant served as her/his own control. Because each participant randomly rotated through all three diets, reported values are means ± standard error of the mean (SE) for $n = 12$ observations.

Results

After consuming the Premium ground beef test diet there was an increase in the expression of the genes ABCA1, ABCG1, LDLR and SCD-1; however, this change was not significant. With the exception of SCD1, all these genes were down-regulated after consumption of conventional ground beef. Both SREBF1 and MED1 were down-regulated after each beef patty treatment but the effect was significantly greater after consuming the conventional ground beef. (Table 19, and Figures 21-22).

TABLE 19 Relative gene expression for men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Gene	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
ABCA 1	1.56 ± 0.39	0.89 ± 0.39	1.61 ±0.47	1.89 ±0.40
ABCG 1	2.19 ±0.56	1.60 ± 0.92	2.026 ± 0.57	2.99 ±0.66
SCD 1	1.15 ± 0.34	1.21 ± 0.60	0.41 ±0.10	1.34 ±0.41**[a]
SREBF 1	2.04 ± 0.62	0.20 ± 0.08***[b]	1.93 ± 0.56	1.02 ± 0.54
MED 1	1.22 ± 0.39	0.54 ± 0.23	1.54 ± 0.54	1.41 ± 0.36
LDLR	1.26 ± 0.36	0.39 ± 0.16*[b]	1.29 ± 0.43	1.52 ± 0.38

[1] Data are means ± SE for 12 men and women; * $P \leq 0.1$; ** $P \leq 0.05$; *** $P \leq 0.01$
[a] p-value for the comparison between premium baseline and premium final
[b] p-value for the comparison between conventional baseline and conventional final

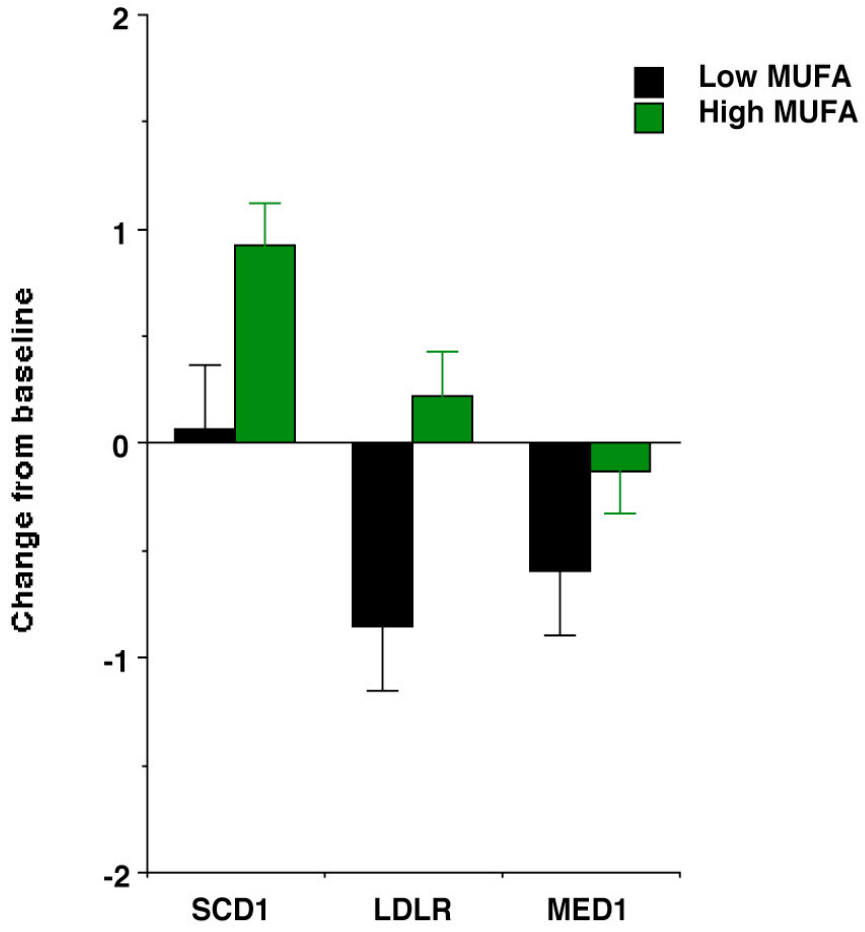

FIGURE 21 Changes from baseline in SCD1, LDLR and MED1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

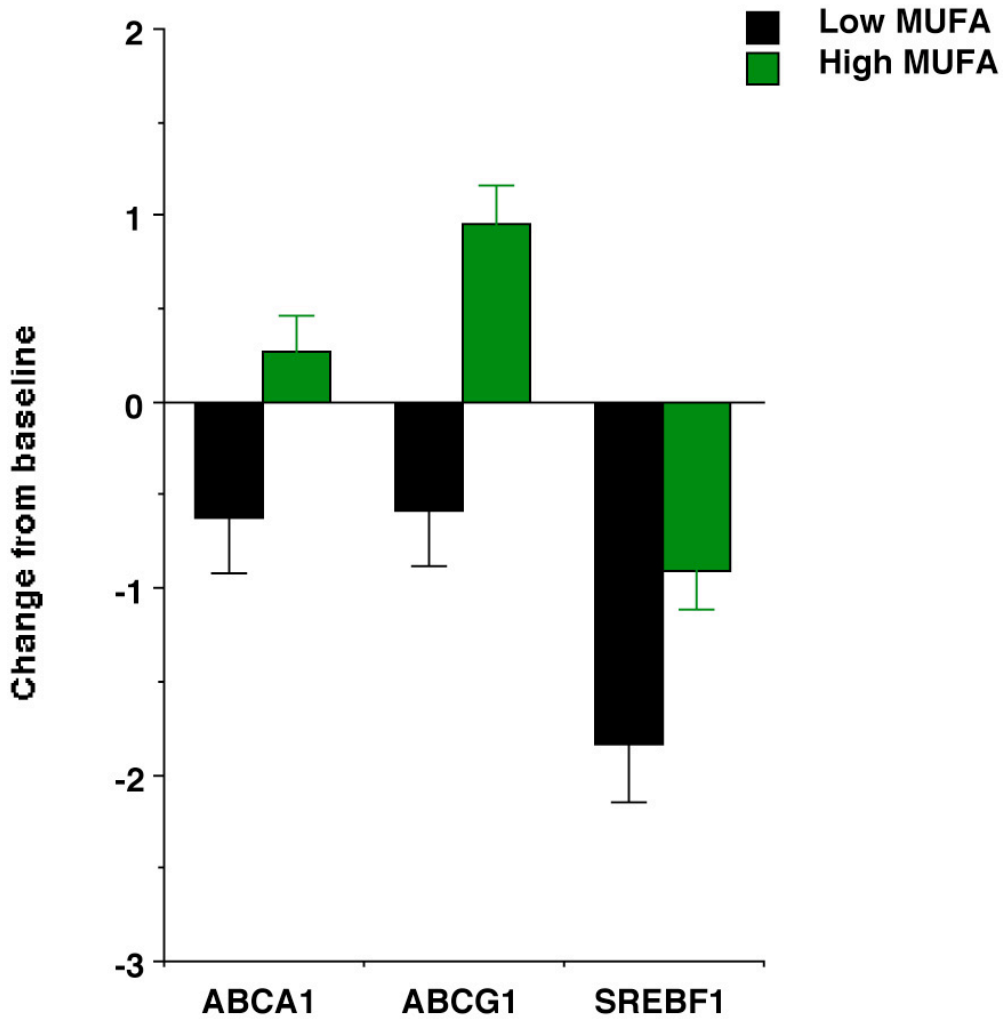

FIGURE 22 Changes from baseline in ABCA1, ABCG1 and SREBF1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium). Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

In order to test for the interaction effect of diet and phase within each group of participant, a 2 way ANOVA was performed, the data were analyzed, and p-values were obtained. As it is shown in Table 20, SCD1 expression was significantly up-regulated with premium ground beef consumption and has a statistically significant interaction with phase suggesting that the result from diet is not independent of phase. Furthermore, other genes that were studied showed no significant phase interaction with diet effect (Table 20 and Figures 23-28).

TABLE 20 Relative gene expression for men and women consumed test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1] in each phase and the interaction of phase and diet.

Genes	Phase	Conventional		Premium		Effect		
		Phase 1	Phase 2	Phase 1	Phase 2	Diet (D)	Phase (P)	D x P
ABCA1	Baseline (B)	0.377 ±0.48	2.33 ±0.40	0.658 ±0.40	2.961 ±0.48	NS	***	NS
	Final (F)	1.70 ±0.48	0.313 ±0.40	2.823 ±0.40	0.583 ±0.48			
	F - B	2.03 ±0.98	-2.01 ±0.56	1.93 ±0.44	-2.37 ±0.8			
ABCG1	Baseline	0.493 ±0.80	3.404 ±0.67	0.743 ±0.67	3.823 ±0.80	NS	***	NS
	Final	3.482 ±0.80	0.263 ±0.67	4.627 ±0.67	0.706 ±0.80			
	F - B	3.50 ±1.87	-3.14 ±0.67	3.68 ±0.49	-3.11 ±0.58			
SCD1	Baseline	0.166 ±0.51	1.853 ±0.43	0.423 ±0.43	0.396 ±0.51	***	***	NS
	Final	2.829 ±0.51	0.068 ±0.43	1.798 ±0.43	0.702 ±0.51			
	F - B	3.03 ±0.99	-1.78 ±0.40	1.01 ±0.45	0.30 ±0.56			

TABLE 20 Continued

Genes	Phase	Conventional		Premium		Effect		
		Phase 1	Phase 2	Phase 1	Phase 2	Diet (D)	Phase (P)	D x P
SREBF1	Baseline	2.140 ±0.76	1.984 ±0.64	1.204 ±0.64	2.968 ±0.76	NS	NS	NS
	Final	0.471 ±0.76	0.016 ±0.64	1.616 ±0.64	0.187 ±0.76			
	F - B	-0.69 ±1.78	-1.96 ±0.59	0.44 ±1.19	-2.78 ±1.10			
MED1	Baseline	0.366 ±0.54	1.842 ±0.45	0.649 ±0.45	2.796 ±0.54	NS	***	NS
	Final (F)	1.109 ±0.54	0.135 ±0.45	1.786 ±0.45	0.883 ±0.54			
	F - B	1.35 ±0.79	-1.70 ±0.58	0.99 ±0.41	-1.91 ±0.84			
LDLR1	Baseline	0.394 ±0.45	1.879 ±0.38	0.575 ±0.38	2.295 ±0.45	NS	***	NS
	Final (F)	0.821 ±0.45	0.097 ±0.38	2.179 ±0.38	0.598 ±0.45			
	F - B	1.05 ±0.78	-1.78 ±0.49	1.53 ±0.52	-1.69 ±0.71			

[1] Data are means ± SE for 12 men and women; *** $P \leq 0.01$

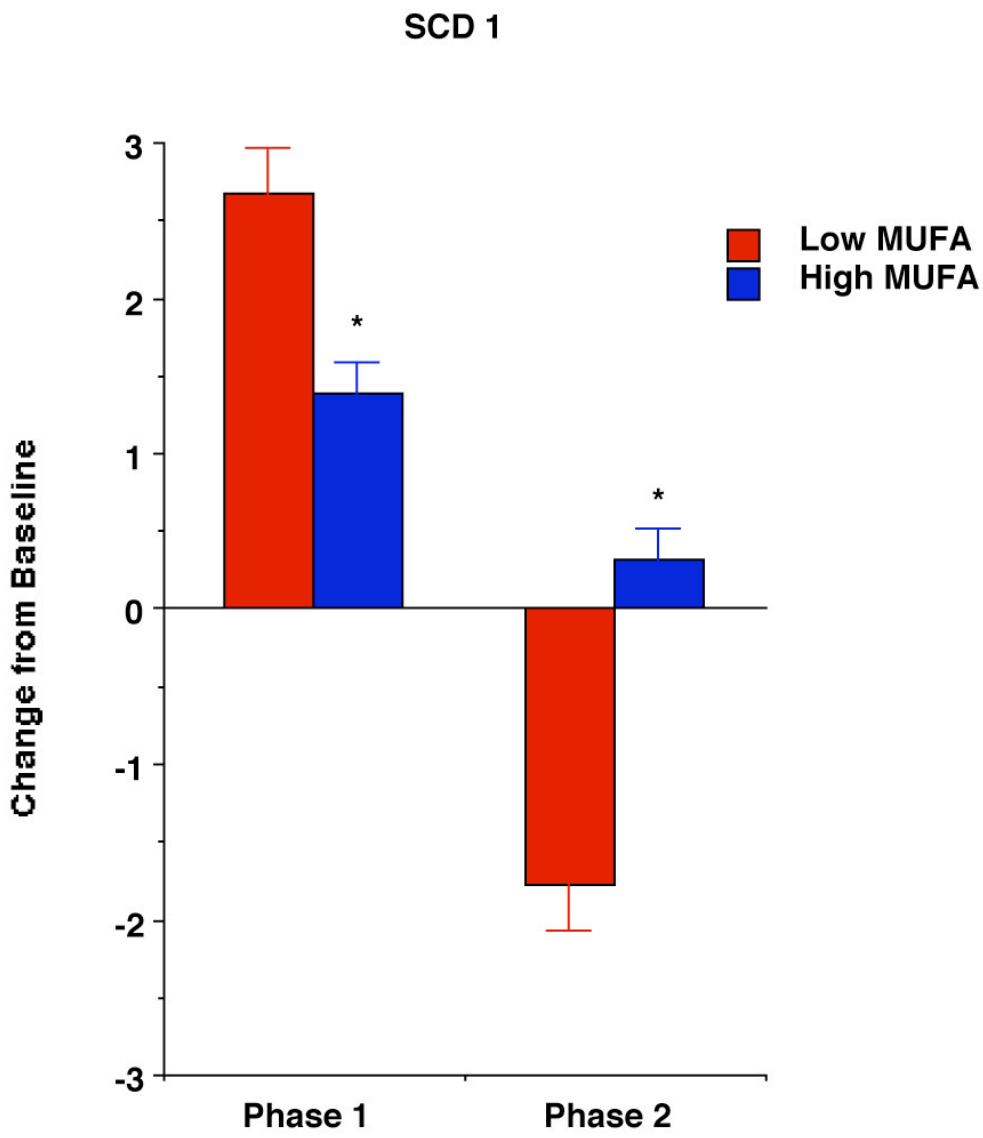

FIGURE 23 Changes from baseline in SCD1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase. * $P \leq 0.01$

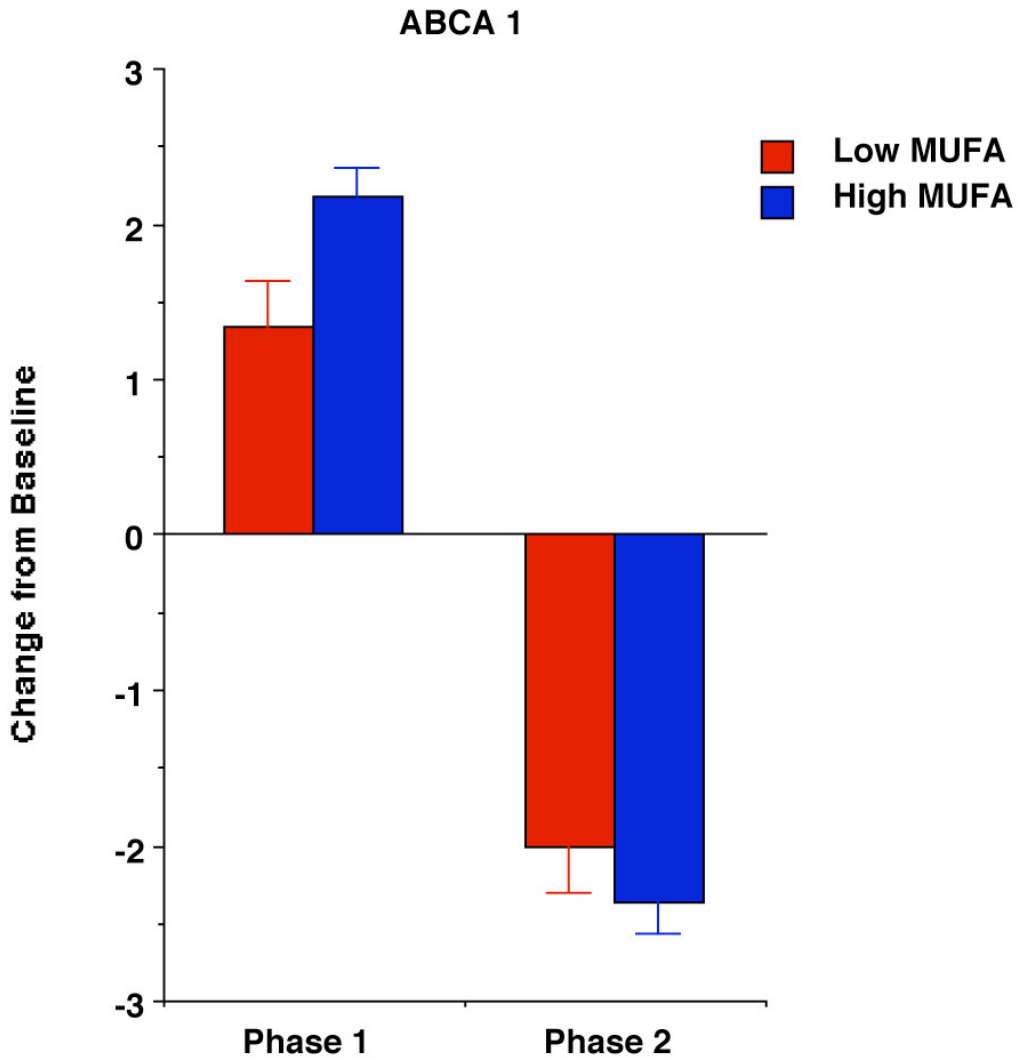

FIGURE 24 Changes from baseline in ABCA1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

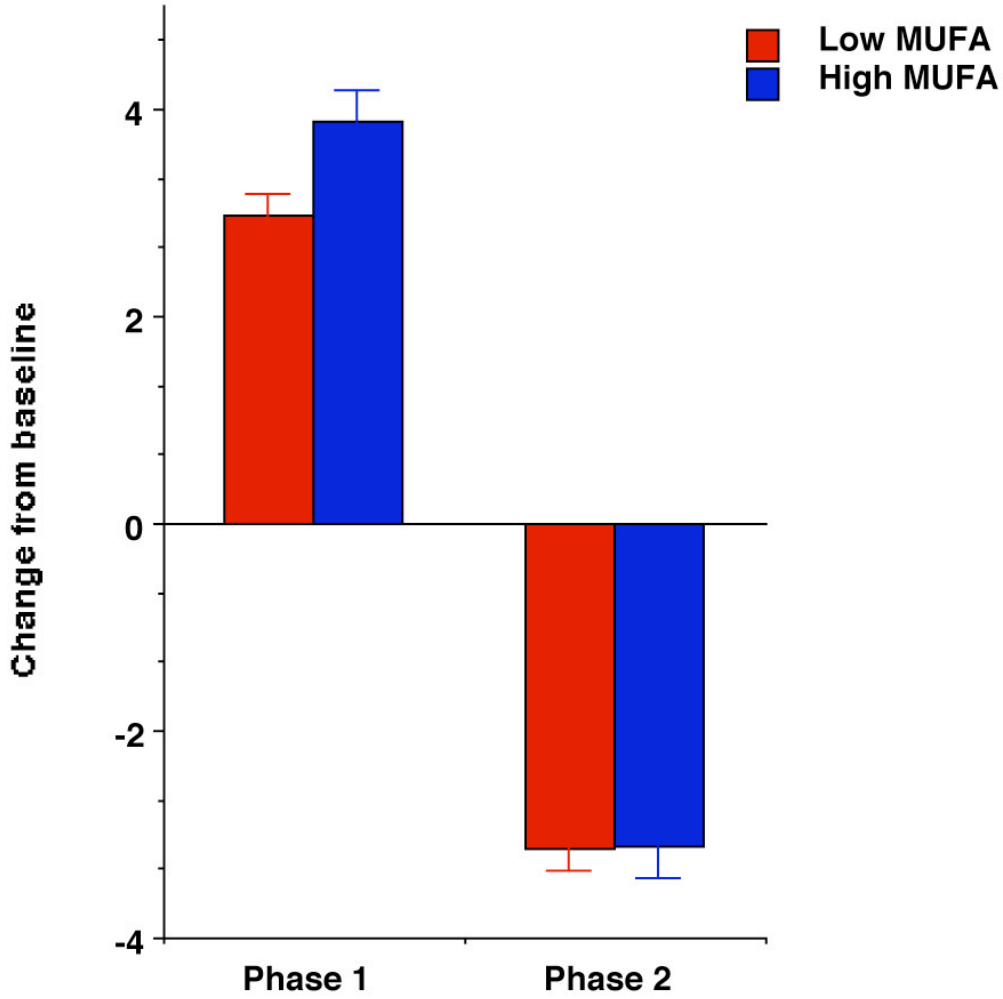

FIGURE 25 Changes from baseline in ABCG1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

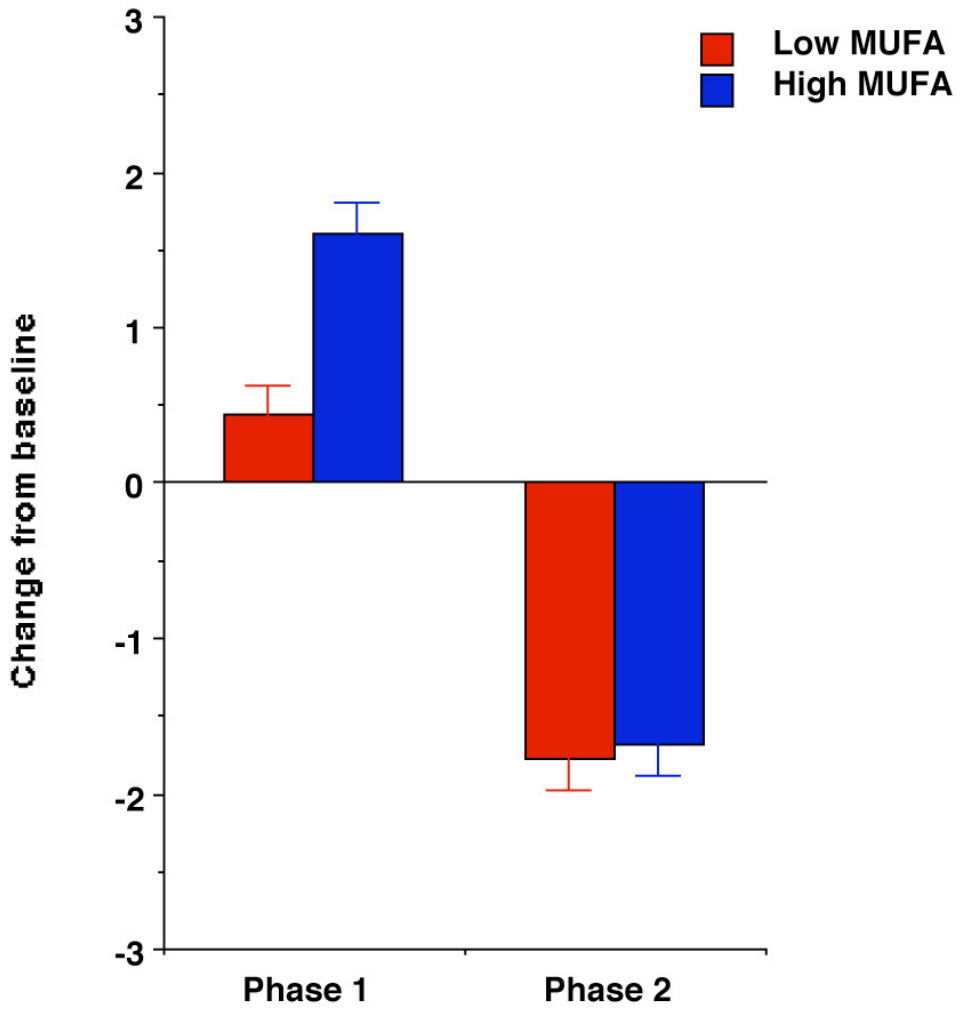

FIGURE 26 Changes from baseline in LDLR relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

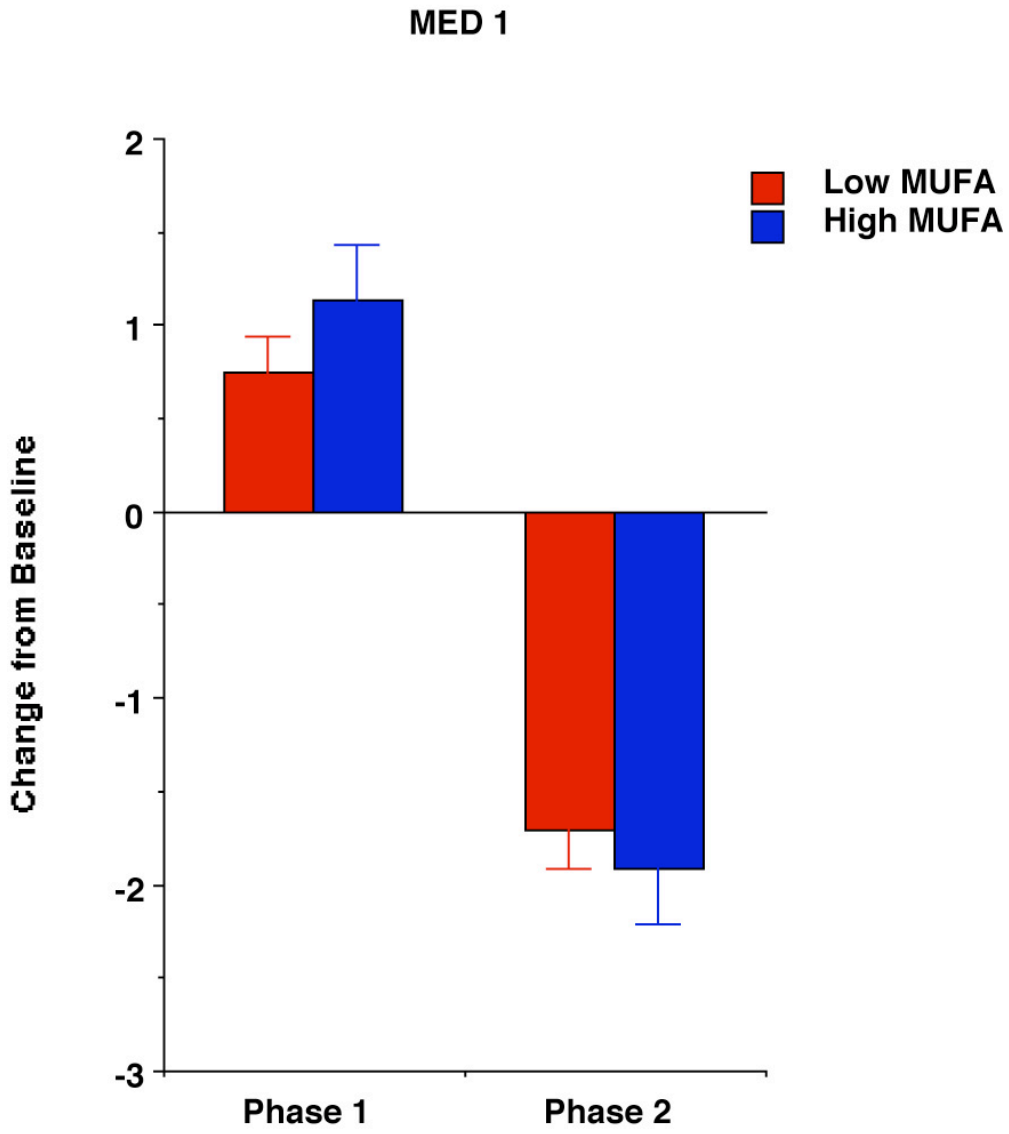

Figure 27 Changes from baseline in MED1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P*> 0.1)

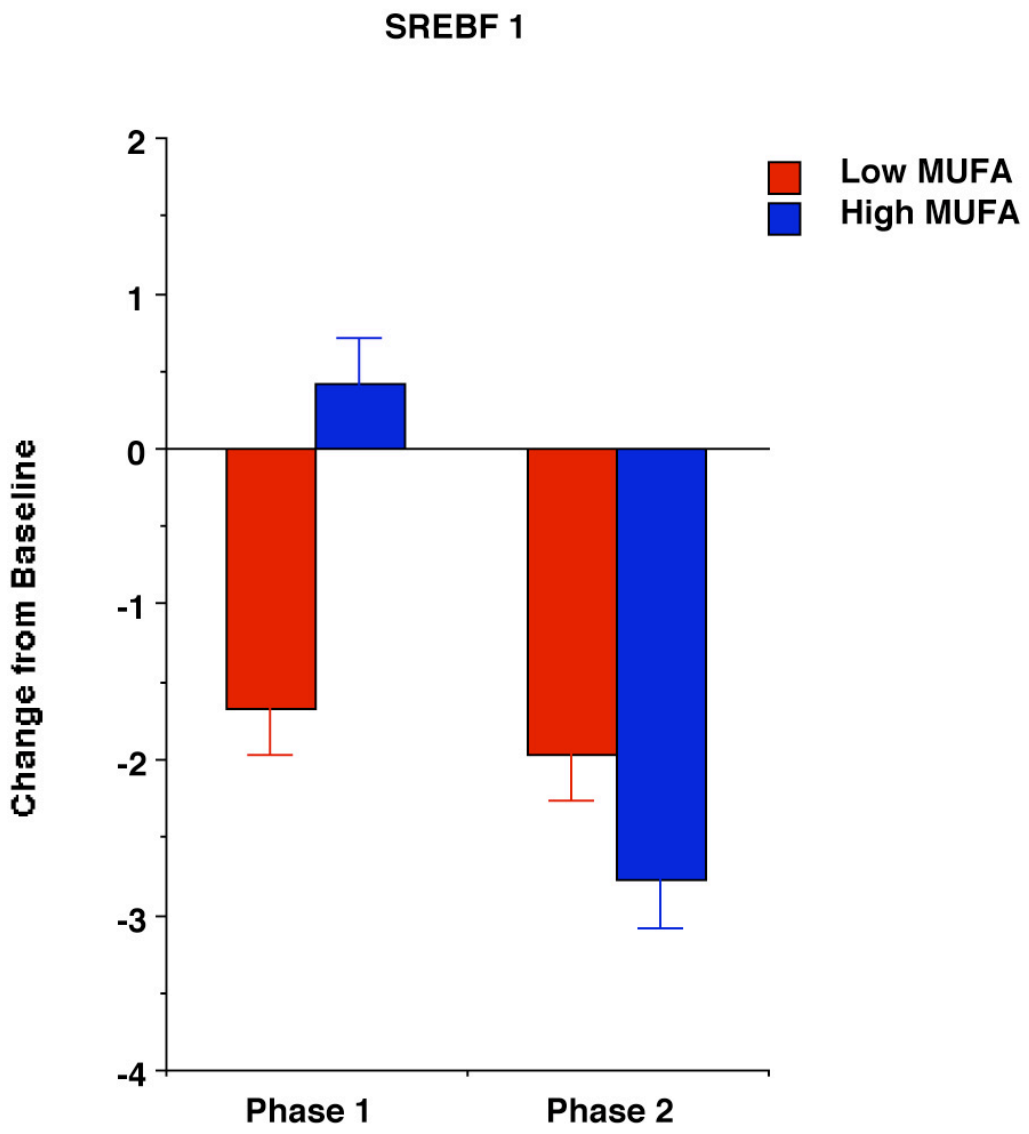

FIGURE 28 Changes from baseline in SREBF1 relative expression for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in each phase. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

Simple correlation among gene expression, plasma lipoproteins and fatty acids was obtained using multivariate Pairwise method by JMP statistical software. There were no significant correlations among gene expression and LDL, HDL and total cholesterol (Table 21). Also, other particles and fatty acids that are not listed in Table 19 had no correlation with gene expression. There was a negative correlation between ABCA1 expression and myristic acid (14:0), which was also negatively correlated with the expression of LDLR and SREBF1. Furthermore, Lp(a) had positive correlation with both ABCG1 and SREBF1. There was also a negative correlation between ABCA1 expression and the concentration of plasma HDL2a. Insulin had positive correlation with ABCA1 and ABCG1 gene expression (Table 21).

Discussion

The effects of different fatty acids on gene expression in immune cells in humans has been studied through microarray analysis as well as in an ex-vivo designed study (128). After postprandial consumption of milk shakes containing either 70% of energy from fat in the form of PUFA, saturated fatty acids, or MUFA, genes that changed differentially included LXR, ABCA1, SREBF1, cJUN, GSTP1, and the PPARα target gene PDK4 (128). PUFA, MUFA, and SFA consumption resulted in differential gene expression. PUFA intake decreased the expression of genes in liver X receptor signaling, whereas SFA intake increased the expression of these genes (128). PUFA intake also increased the expression of genes related to cellular stress responses. However, MUFA intake had an intermediate effect on several genes. For instance, the MUFA shake

intervention caused down-regulation on ABCA1, SREBF1 and LXR expression 6 h postprandially (128).

TABLE 21 Simple correlations between gene expression, plasma major fatty acids, cholesterol subfractions and plasma insulin

	VLDL	HDL2a	LP(a)	Insulin	14:0	16:0	18:0	18:1
ABCA1	NC	-0.26	NC	0.27	-0.29	NC	NC	NC
ABCG1	NC	NC	0.24	0.24	NC	NC	NC	NC
LDLR	NC	NC	NC	NC	-0.29	NC	NC	NC
SCD1	-0.24	NC	NC	NC	NC	NC	NC	NC
SREBF1	NC	NC	0.34	NC	-0.37	NC	NC	NC
MED1	NC	NC	NC	NC	NC	NC	NC	NC

[1] The correlations are estimated by Pairwise method.
All r values stated are significant, $P \leq 0.05$; NC = no correlation

The comparable but lower effects observed after MUFA intake than after PUFA intake for some genes had been described previously after a long-term intervention of 6 months (166). However, whole-genome microarray analyses were not performed after the MUFA shake intervention in those studies; therefore, conclusions regarding the total effects of MUFA on the whole transcriptome could not be drawn.

In this study I investigated many of same genes (128) to investigate the effects of ground beef interventions differing in MUFA:SFA ratio and total MUFA content on

PBMC gene expression in a cross-over study. Although it has been shown previously that MUFA consumption can alter expression of other genes in PBMC postprandially (148, 167), those studies focused on the effects of virgin olive oil on gene expression. Olive oil recently has been acknowledged as being more than a monounsaturated fat, due to its phenolic content, which provides benefits for plasma lipid concentrations and depresses oxidative damage (168). Therefore, none of those studies have investigated the interaction between MUFA intake and gene expression through dietary beef intervention.

MUFA typically constitute 35 to 45% of the total fatty acids in beef produced in the U.S. (88, 93). The major MUFA in beef, oleic acid, has been studied in more detail and found to lower LDL-C without affecting the beneficial HDL-C (87, 127). However, the relationship between beef MUFA intake and the expression of genes related to CVD has not been evaluated.

The selection of genes in this study was based on their relation to atherosclerosis as well as the responses seen in previous studies. The genes that were selected in this study were SCD1, LDLR, SREBF1, ABCA1, ABCG1 and MED1. These genes are involved in lipid metabolism (SCD1, LDLR and MED1) and cholesterol metabolism (SREBF1, ABCG1 and ABCA1). They are also part of LXR signaling pathways, play a major role in reverse cholesterol transport in macrophages, and have been shown to be affected by different dietary fatty acids. After consuming the premium ground beef test diet, there was a small but not significant increase in the expression of the genes ABCA1, ABCG1, LDLR.

SCD1 gene expression, however, increased significantly after consumption of the premium ground beef. SCD1 is responsible for the conversion of saturated fatty acids to their Δ9 desaturated counterparts (88, 150). The primary product of SCD is oleic acid, which is formed by the Δ9 desaturation of stearic acid. It was reported previously that hepatic Δ9 desaturation of saturated fats such as stearic acid by SCD1 was a major step in mediating their ability to induce hepatic lipogenesis (74). Furthermore, Enoch et al. (169) demonstrated that palmitoyl-CoA and stearoyl-CoA have similar substrate properties for SCD1 and that oleoyl-CoA inhibits SCD1 in rat hepatocytes. The two types of ground beef tested in this study provided different amounts of SCD1 substrates (palmitic and stearic acid). The conventional ground beef patty provided up to 10.6 g SCD1 substrates and 9.7 g of potentially SCD1 inhibitory oleic acid, whereas the premium ground beef provided 2.9% less (7.2 g) SCD1 substrate and 1.2% less (8.3 g) inhibitory oleic acid.

These data suggest that PBMC SCD1 expression was sensitive to the composition of ground beef. When MUFA intake was high enough it had an inhibitory effect and could be negatively associated with SCD1 expression regardless of the higher amount of SFA. Thus, lower MUFA intake in the premium ground beef diet was associated with increase in SCD1 expression, whereas with conventional ground beef diet SCD1 expression was not affected. This occurred in the presence of higher SCD1 substrates, suggesting that the inhibitory effect of oleic acid on SCD1 was independent of the amount of palmitic and stearic acid in diet.

With the exception of SCD1, all the other genes were down-regulated after consumption of conventional ground beef. SREBF1 and MED1 were down-regulated after either beef intervention. For SREBF1, the effect was significantly greater after consuming the conventional ground beef. Furthermore, LDLR expression decreased significantly with the consumption of conventional ground beef and increased with premium beef patty consumption.

Both LDLR and SREBF1 are involved in cholesterol metabolism (128), suggesting a differential effect of conventional and premium ground beef on cholesterol metabolism through LDLR and SREBF1. LDLR is associated with clathrin-coated pits, and when binding to LDL, it is internalized into acidic endosomes where it can be separated from its ligand. The cholesterol that is carried by LDL and generated within the lysosome is known to be responsible for suppressing HMG-CoA reductase activity. Such suppression of transcription of the HMG-CoA reductase gene occurs through pathways involving sterol regulatory element-binding protein (SREBP). LDL also inhibits the SREBP pathway, which leads to suppression of transcription of the LDLR gene. Sterol regulatory element-binding protein-1 (SREBP1) is transcription factor involved in fatty acid synthesis and cholesterol metabolism (170). SREBP1 is encoded by the SREBF1 gene, which is associated with obesity, insulin resistance, type 2 diabetes and HIV-related hyperlipoproteinemia (170-173). These results indicated that SREBF1 expression was significantly down-regulated after the conventional ground beef intervention, and the concentration ground beef contained more MUFA per patty than the premium ground beef. These data are consistent with the previous study in which caused a de-

crease in SREBF1 after MUFA intake (128). Furthermore, ABCA1 and ABCG1 were both down-regulated with the consumption of the conventional ground beef. Though the change was not significant, the down-regulation of these genes was in agreement with the results from the previous study (128) in which postprandial gene expression after MUFA or PUFA intake. In that study, PUFA shake intake *in vivo* down-regulated the expression of ABCA1 and ABCG1, and similarly, PBMC *ex vivo* incubation with DHA decreased ABCA1 expression. These data were all consistent with several *in vitro* studies that have shown that PUFA intake can decrease the expression of ABCA1 and ABCG1 in macrophages (174, 175). Therefore, I conclude that MUFA intake might have similar effects on the genes related to cholesterol efflux that PUFA intake does. Furthermore, SFA shake consumption resulted in an increased expression of genes involved in LXR signaling, such as ABCG1, ABCA1 and SREBF1 (128). Contrary to those data, consumption of conventional ground beef, which was also higher in SFA as well as MUFA compared to premium ground beef, caused a decrease in the expression of these genes. This suggests that increasing MUFA intake might diminish the negative effects of SFA that have been associated with CVD.

CHAPTER IV

THE EFFECTS OF GROUND BEEF DIETS CONTAINING DIFFERENT MUFA:SFA RATIOS ON GENE EXPRESSION AND CVD MARKERS IN WOMEN WITH A COMPARISON TO MEN

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality and account for a third of all deaths of women worldwide and causes half of all deaths of women over 50 years of age in developing countries (176). Though many important studies over the past two decades have developed preventive interventions and effective therapies for CVD, most of them included only men or limited numbers of women (177, 178).

Extrapolating recommendations for prevention and treatment of CVD from studies conducted predominantly on men to women may not be appropriate, because the symptoms of CVD and response to therapy are different in men and women (177, 178). Only recently, significant gender-related differences in prevalence, presentation and management of CVD have been evaluated (179). Many population studies indicated that total cholesterol measurements are higher in men until the fifth decade of life; however, women have greater values beyond this age (179). Furthermore, gender differences in HDL-C concentration diminish with advancing age. Women on average experience a relatively mild decrease in HDL-C during menopause (180, 181). Manolio et al. (182) reported that HDL-C was negatively associated with coronary artery disease in younger women and men as well as older (65 years) women (182). Despite the fact that the major

CVD risk factors are the same in both sexes, gender-specific differences should be taken into consideration. These differences are related to different outcomes in response to dietary interventions. There is also considerable gender-related variability in the prevalence and outcome related to CVD risk factors.

CVD still is considered to be a male disease. Rates of CVD increase with age in both sexes and are higher in men than in women (183). Although many studies have focused on the relationship between coronary disease and the effects of lowering cholesterol in men, there still are not sufficient recommendations for prevention of coronary disease that would distinguish between men and women.

The distribution of risk markers was shown to be different in men and women. Different dose responses for cholesterol, cigarette smoking, diastolic blood pressure, and social class in both sexes have been detected. In despite of the similarity in relative risks in men and women the absolute risk are very much lower in women and the reasons underlying the lower incidence of CVD in women remain unclear. One contributing factor might be lower frequency of risk markers in women than in men. In a study done by Christopher G. Isles et al. (184) women compared to men smoked less and had similar blood pressure but women still had higher plasma cholesterol concentration. Furthermore, women were more likely to be obese and of lower social class. However, the lower level of smoking among women could not explain the difference in death rates because non-smoking women had only half the coronary mortality of non-smoking men (184).

Moreover, there is evidence of sex differences in endothelial function which may explain differences in the prevalence of CVD in women relative to men (185, 186). Women have greater microvascular dysfunction relative to men (187) and chest discomfort and chest pain have been linked to greater endothelial dysfunction in women (188). There is also evidence of direct sex hormone effects on endothelial function; for instance, the menstrual cycle has been shown to modulate endothelial function in healthy women. Furthermore, many studies have indicated that there is an increase in endothelium-dependent vasodilation as women pass from the follicular phase to the luteal phase (189, 190) a period related to estrogen surge (191). Altogether, in comparison to healthy, age-matched men, healthy women tend to have better endothelial function. However, during menopause, women show a decline in endothelial function such that their endothelial deterioration rapidly catches up with that of men (192, 193). It has been found that there are specific sex differences in the expression of hormone receptors such as estrogen, progesterone and androgen receptors all of which have been found to be abundance in human vascular endothelium (194). Hormone replacement therapy directly stimulates endothelial receptors. It can also indirectly modify molecules known to affect the endothelium, e.g., lipoproteins, homocysteine (195).

In this study risk markers for CVD in men and women after consumption of different ground beef patty preparation were measured. Also, the relative expression of PBMC genes related to cholesterol and lipid metabolism was studied. Based on these data, a comparison between male and female participants was completed. The objective of this study was to determine a relationship between dietary fatty acids and serum

cholesterol, HDL, LDL and other CVD risk indicators such as the inflammatory marker homocysteine, CRP and insulin in men and women. In addition, the effect of dietary fatty acids on expression of genes related to CVD and their differences between men and women was evaluated to establish gender-related differences.

Methods

Subjects

Among subjects recruited for the study men were over age of 45 and women whose last menstrual period was over 1 y prior to enrollment of the study by natural or surgical means with or without ovaries. Subjects did not have history of CVD, stroke or diabetes, had normal liver function tests, normal fasting glucose, and serum total cholesterol of less than 6.72 mmol/L, and must not be taking lipid lowering drugs. Baseline characteristics of the participants are found in Table 1.

Statistical Analysis

A 2-factorial analysis using JMP statistical software allowed us to see if there was an interaction between gender and diet.

Results

There were no significant changes in the concentration of plasma LDL, HDL and total cholesterol within the group of male participants; however, a significant decrease was seen in women's plasma HDL concentration with Premium ground beef diet (Tables

22-23 and Figures 29-31). Furthermore, VLDL plasma concentration increased significantly within male participants after conventional ground beef treatment but no change was seen among women (Tables 24-25 and Figures 32-33). Moreover, HDL2b concentration was lower after consumption in the male group. HDL2b particle concentration was also significantly changed with Conventional ground beef diet in the male group but no change was seen in the female participants regarding HDL2b plasma concentration. There were no significant changes in LDL particle size among women and men (Tables 26-27 and Figures 34-36).

In addition to lipoprotein changes, CRP level change was also dependent on gender; however, there was a significant interaction between gender, phase and diet. Homocysteine level in women did not differ significantly when compared with men. Furthermore gene expression studies didn't differ between men and women.

Discussion

There are sex-specific differences in the processes underlying risk factor injury and atherosclerotic responses that are responsible for the different presentation of CVD in men and women. Therefore, measuring risk factors for this disease through dietary interventions may help elucidate these differences.

Women may tolerate CVD risks better than men. It has been found that, in women, any given level of risk marker was associated with lower CVD rates than in men (184). This protection might be conferred by female sex hormones. In the same study, even in women over age of 60, CVD rates were substantially lower than in men of

similar age, suggesting that natural estrogen may protect for many years beyond menopause (184).

TABLE 22 Major lipoprotein cholesterol concentrations (mmol/L) for men rotated through test ground beefs low in monounsaturated fatty acids (Conventional) or high in monounsaturated fatty acids (Premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
Total cholesterol, mmol/L	5.77 ± 0.58	5.30 ± 0.45	5.30 ± 0.51	5.10 ± 0.24
LDL, mmol/L	3.93 ± 0.52	3.47 ± 0.42	3.15 ± 0.43	3.26 ± 0.18
HDL, mmol/L	1.36 ± 0.06	1.52 ± 0.14	1.70 ± 0.18	1.52 ± 0.08

[1] Data are means ± SE for 5 men.
There were no significant differences among phases ($P > 0.1$)

TABLE 23 Major lipoprotein cholesterol concentrations (mmol/L) for women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
Total cholesterol, mmol/L	5.39 ± 0.27	5.42 ± 0.30	5.36 ± 0.33	5.07 ± 0.31
LDL, mmol/L	3.17 ± 0.17	3.19 ± 0.19	3.14 ± 0.27	3.25 ± 0.31
HDL, mmol/L	1.75 ± 0.17	1.92 ± 0.25	1.88 ± 0.14	1.50 ± 0.09**

[1] Data are means ± SE for 7 women. ** $P \leq 0.05$

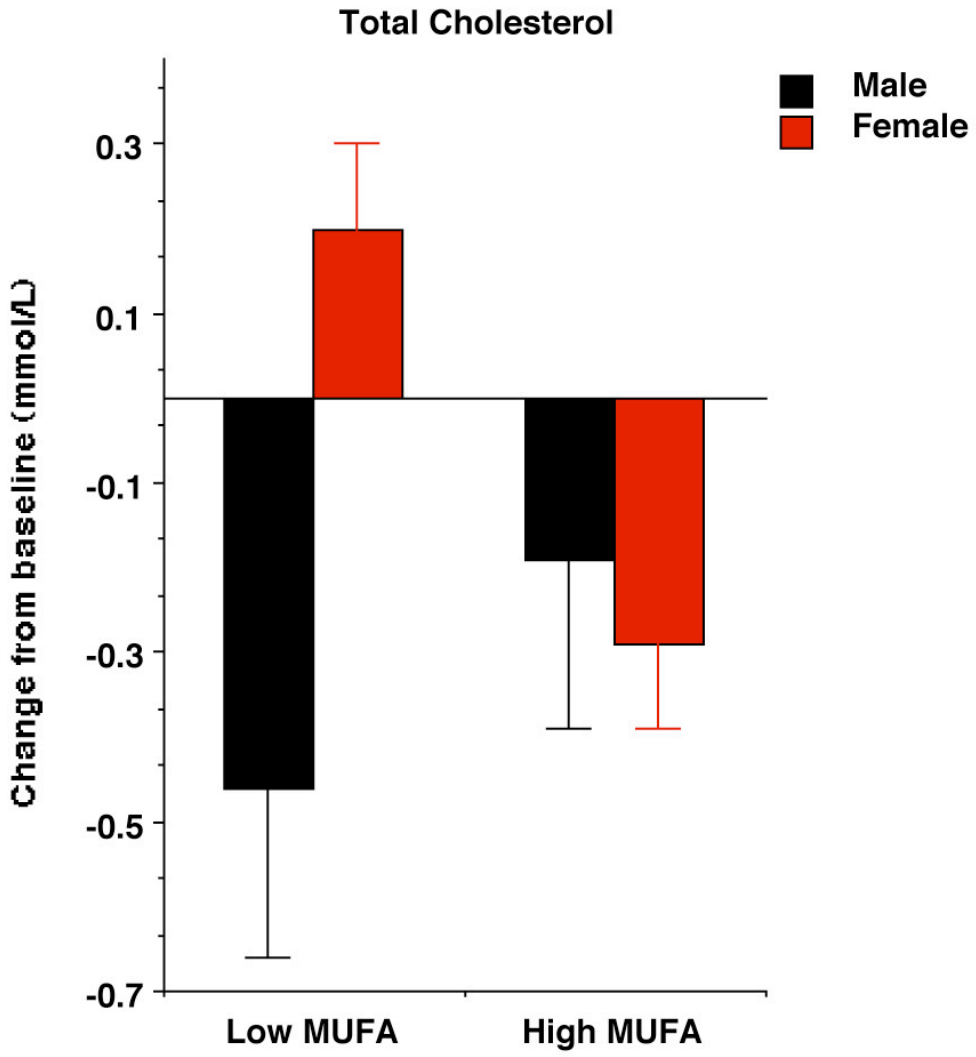

Figure 29 Changes from baseline in plasma total cholesterol concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

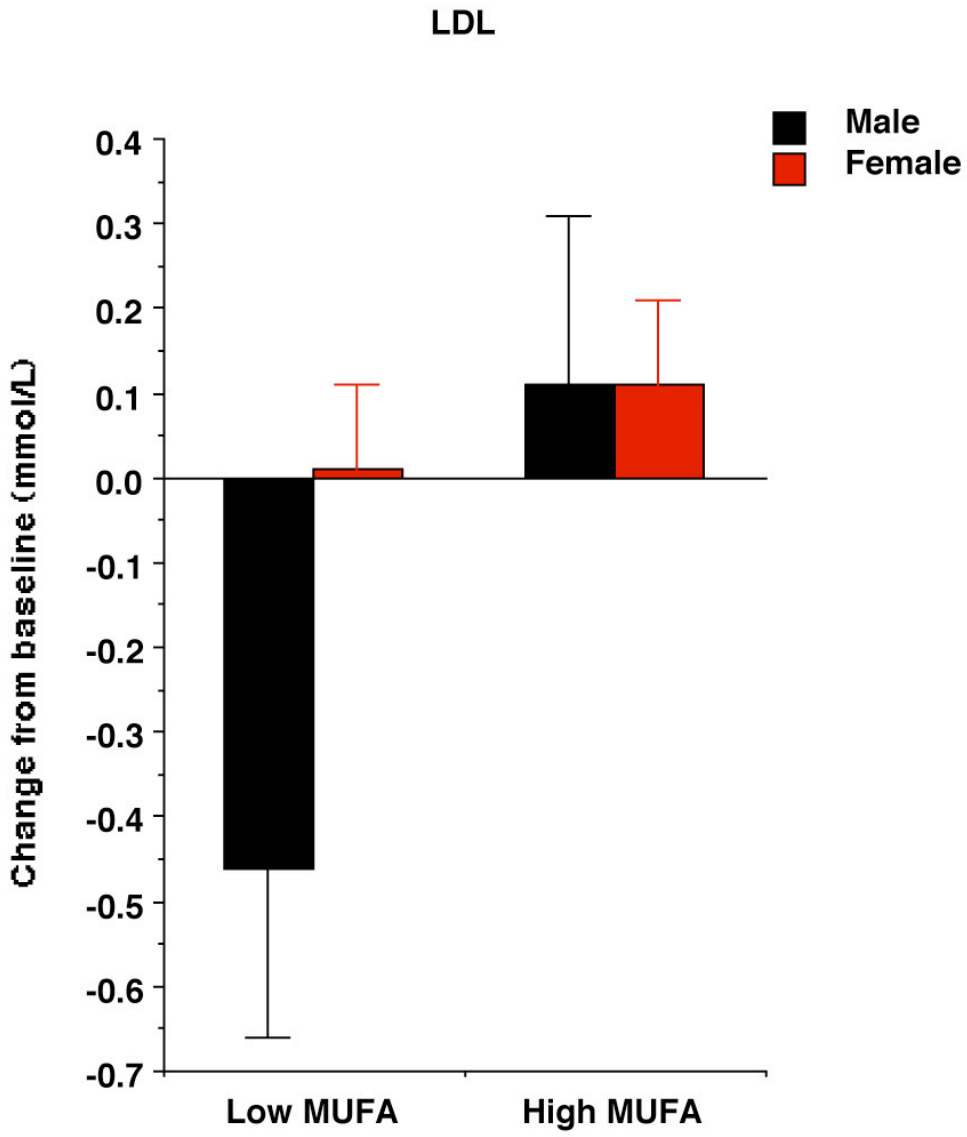

Figure 30 Changes from baseline in plasma total LDL concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

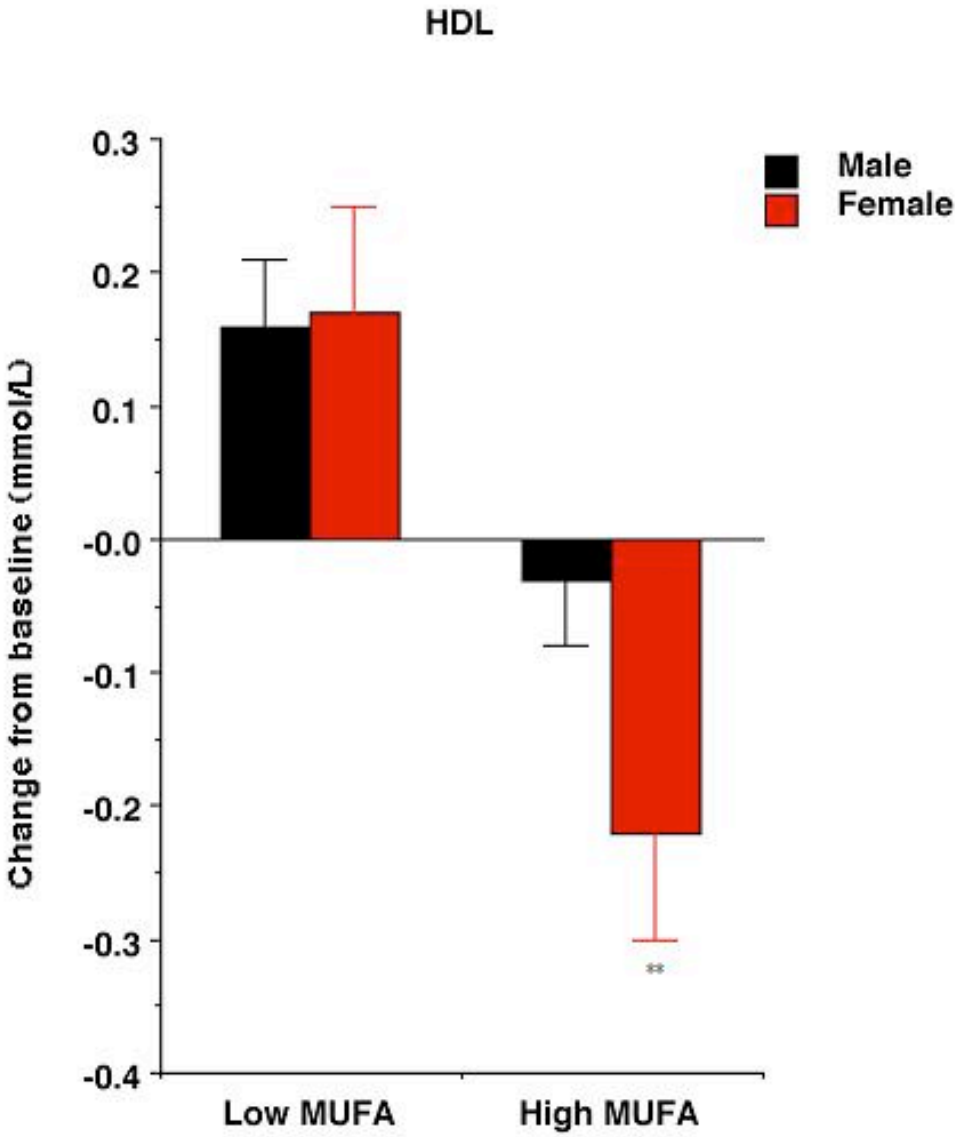

Figure 31 Changes from baseline in plasma total HDL concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women. Values are expressed as mean ± SEM, $n = 12$. ** $P \leq 0.05$

TABLE 24 VLDL and HDL2b concentrations for men rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
VLDL, mmol/L	0.48 ± 0.08	0.55 ± 0.08*	0.55 ± 0.08	0.54 ± 0.06
HDL2b, mmol/L	0.41 ± 0.07	0.36 ± 0.04	0.55 ± 0.10	0.30 ± 0.03

[1] Data are means ± SE for 5 men. * $P \leq 0.1$

TABLE 25 VLDL and HDL2b concentrations for women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
VLDL, mmol/L	0.85 ± 0.16	0.77 ± 0.11	0.81 ± 0.14	0.79 ± 0.13
HDL2b, mmol/L	0.31 ± 0.04	0.28 ± 0.04	0.41 ± 0.06	0.40 ± 0.07

[1] Data are means ± SE for 7 women. There were no significant differences among phases ($P > 0.1$)

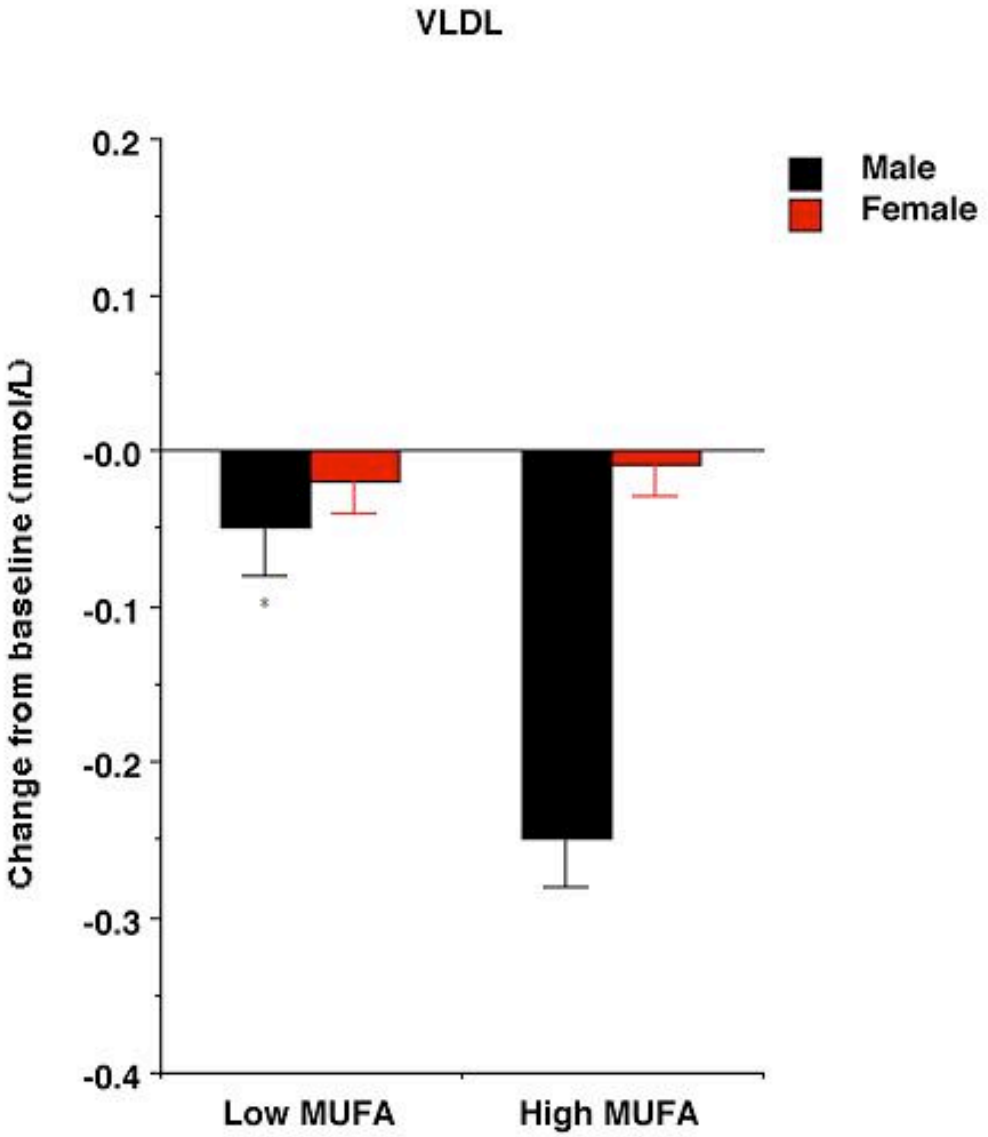

Figure 32 Changes from baseline in plasma total VLDL concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women. Values are expressed as mean ± SEM, $n = 12$. * $P \leq 0.1$

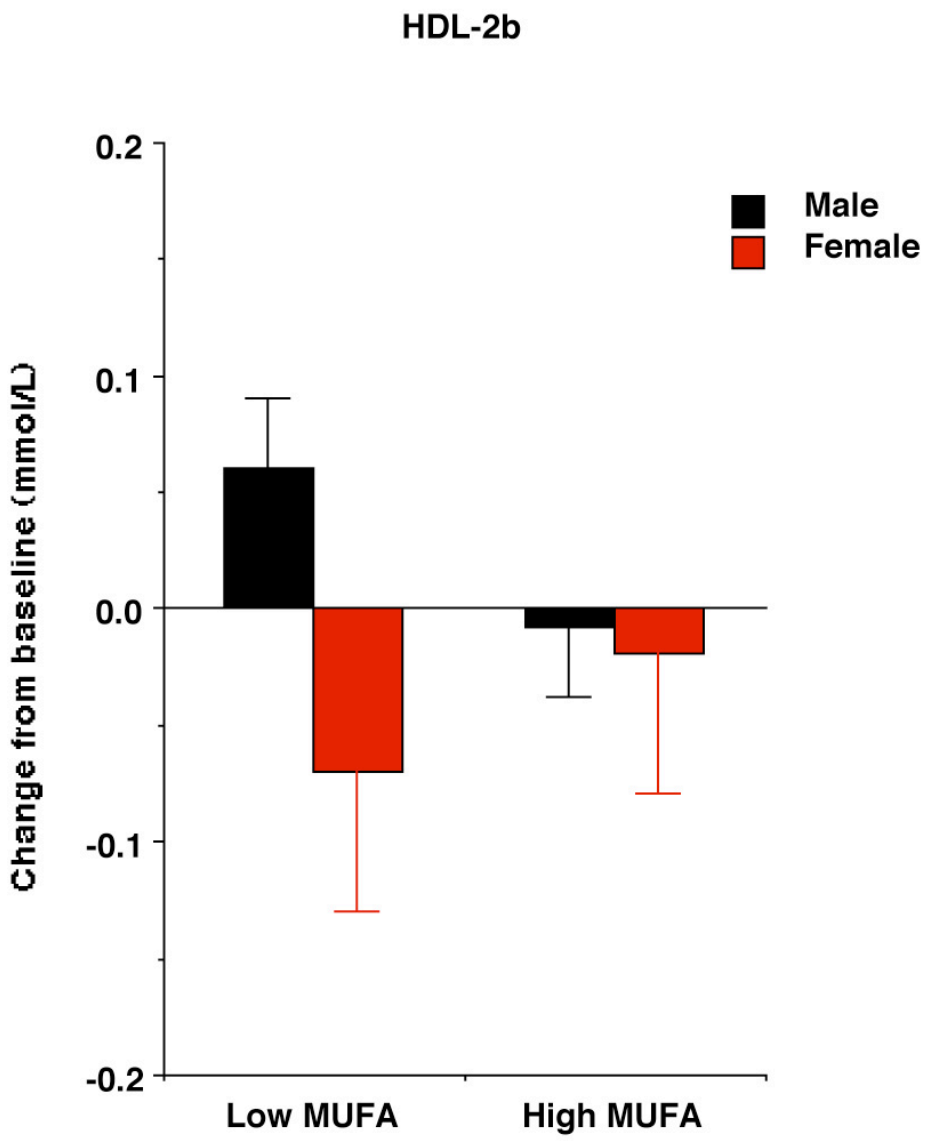

Figure 33 Changes from baseline in plasma total HDL-2b concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

TABLE 26 HDL and HDL2b particle concentrations and LDL particle size for men rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
HDL, nmol/L	9791 ± 763	10836 ± 932	10522 ± 530	10609 ± 431
HDL2b, nmol/L	1694 ± 282	1935 ± 305**	1930 ± 303	1898 ± 220
LDL (nm)	20.12 ±0.04	20.20 ±0.05	20.05 ±0.09	20.14 ±0.06

[1] Data are means ± SE for 5 men. ** $P \leq 0.05$

TABLE 27 HDL and HDL2b particle concentrations and LDL particle size for women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium)[1]

Lipoprotein	Conventional Baseline	Conventional Final	Premium Baseline	Premium Final
HDL, nmol/L	12302 ± 1128	12019 ± 743	11893 ± 648	11832 ± 644
HDL2b, nmol/L	2975 ± 591	2696 ± 397	2836 ± 490	2749 ± 457
LDL (nm)	20.11 ±0.04	20.20 ±0.04	20.13 ±0.01	20.18 ±0.04

[1] Data are means ± SE for 7 women.
There were no significant differences among phases ($P > 0.1$)

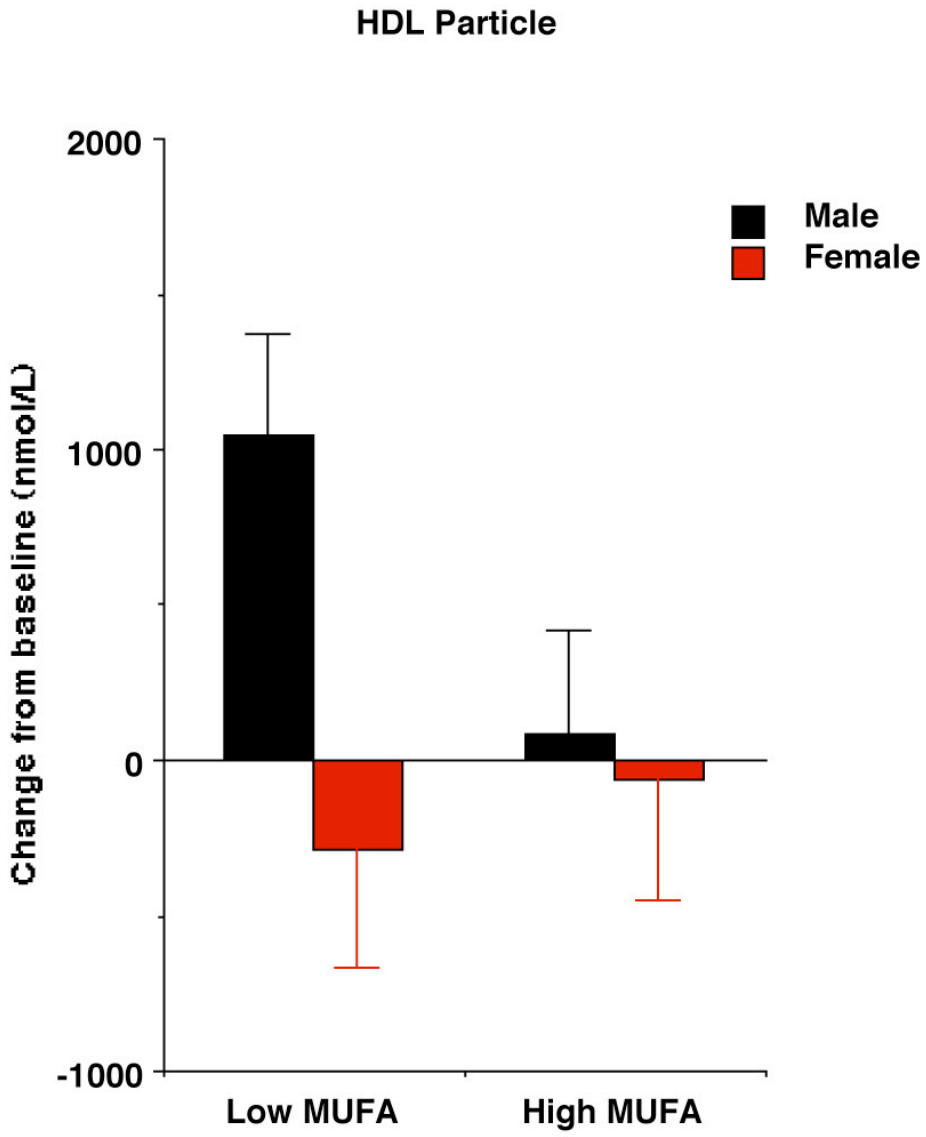

Figure 34 Changes from baseline in plasma total HDL particle concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

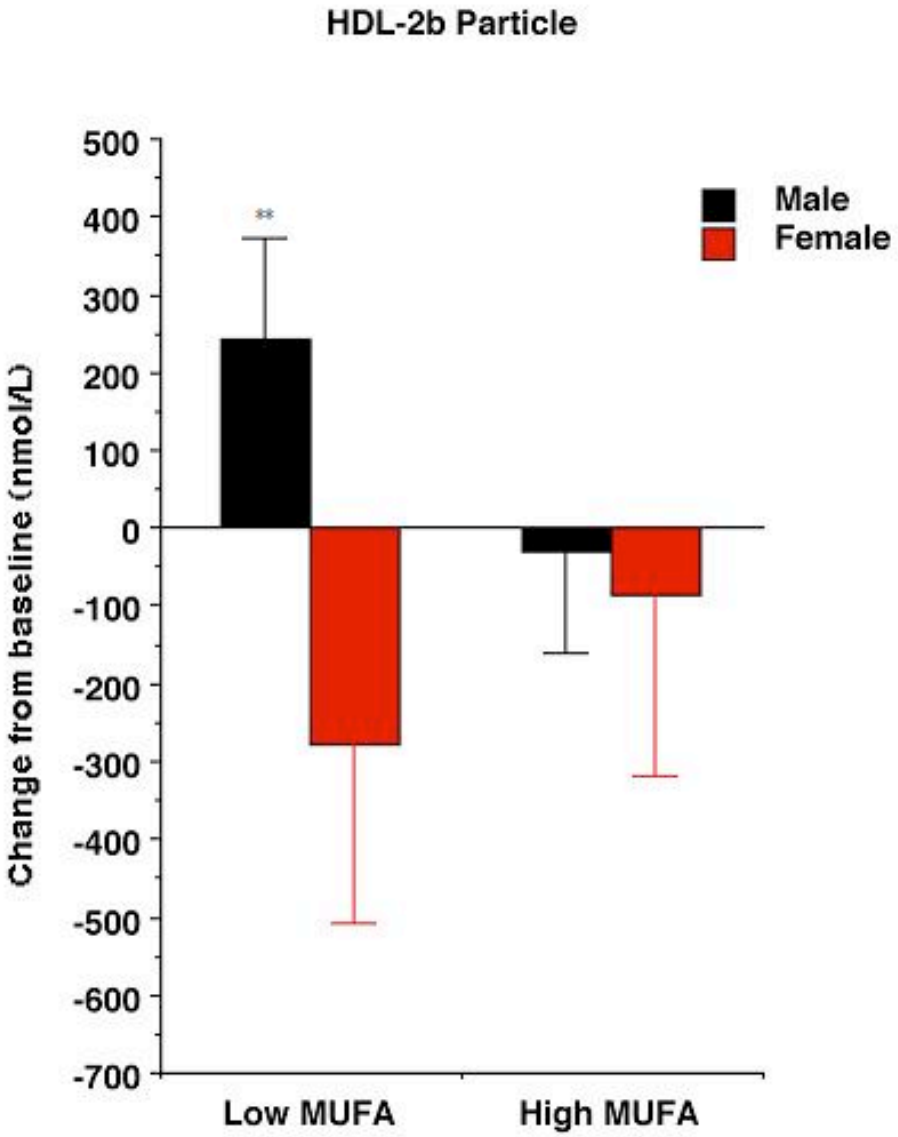

Figure 35 Changes from baseline in plasma total HDL-2b particle concentration for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women. Values are expressed as mean ± SEM, n = 12. ** $P \leq 0.05$

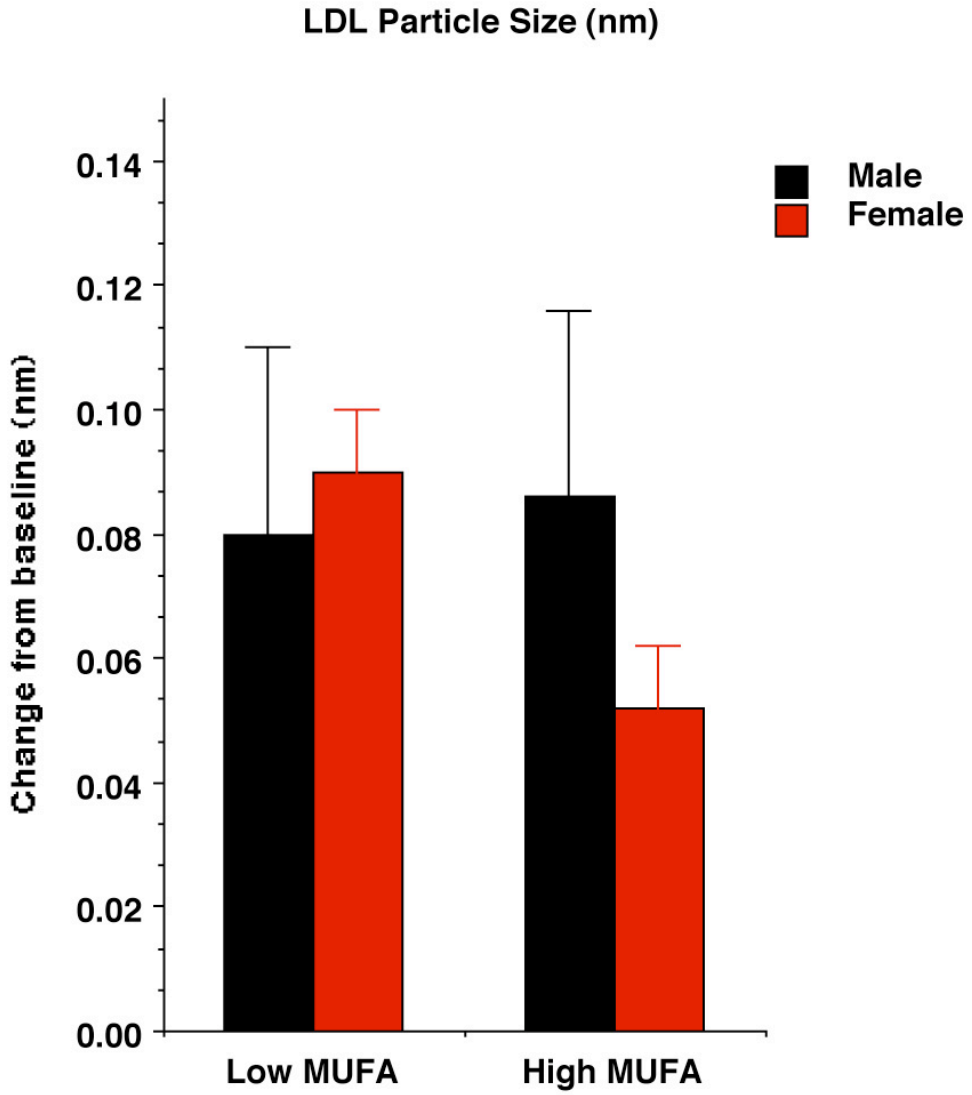

Figure 36 Changes from baseline in plasma total LDL particle size for test diets of men and women rotated through test ground beefs low in monounsaturated fatty acids (conventional) or high in monounsaturated fatty acids (premium) in men and women. Values are expressed as mean ± SEM, *n* = 12. There were no significant differences among phases (*P* > 0.1)

Several epidemiological and clinical data have provided evidence that there are gender-specific variations in CVD risk. Diabetes, hypothyroidism and depression manifest gender-related differences and are associated with increased CVD risk (196). Women for instance, are more likely than men to experience an episode of depression and are more subsequently diagnosed with the metabolic syndrome compared to men (196). Furthermore, studies have suggested a relationship between thyroid disease and coronary artery disease (197). However, the prevalence of hypothyroidism is higher in women and increases with age (197).

Because the relationship between hypercholesterolemia and CVD has not yet been completely clarified, researchers have been investigating other potential contributors. Some of these contributors include sex hormones, vasodilators, vascular endothelial growth factor (VEGF), angiotensin II, vitamin D, plasma lipoprotein-C concentration, adipocyte hormones and inflammatory markers such as CRP, homocysteine, ferritin and insulin (198).

In this study, there was a significant change in HDL-C concentration with premium ground beef consumption. However, the change was significant in women but not in men suggesting that different this ground beef intervention elicited a greater impact in women than in men. This might have been caused by various factors that differ between men and women such as hormonal and/or genetic factors. In pre-menopausal women, endogenous female sex hormones, especially estrogens, are cardioprotective via multiple mechanisms including increased HDL-C, decreasing LDL-C, and release of vasodilators such as nitric oxide (NO) and prostacyclin (PGI2) from vessel walls. This

results in lowering of blood pressure and a decrease in platelet aggregation (199). However, after menopause, blood pressure increased in women to levels even higher than those observed in men (200). In addition, in post-menopausal women, there was an increase in the prevalence of the metabolic syndrome, elevated body weight, dyslipidemia, hyperinsulinemia, and hypertension (201). Therefore, these data are in agreement with previous studies in that women may exhibit higher risk factors for CVD after menopause and in comparison to age-matched men.

HDL2b particle concentration significantly increased after conventional ground beef consumption in men but no significant changes were observed in women with either ground beef interventions. Furthermore, VLDL-C increased significantly in men after consumption of conventional ground beef, whereas no significant change was seen in women. Several studies have shown that certain fatty acid species are preferentially utilized over others for VLDL assembly and secretion. For instance when McA-RH7777 cells were cultured in the presence of exogenous oleic acid, TAG-rich VLDL were secreted many fold higher as compared to no oleic acid supplementation (23-25).

My results were consistent with the previous in vitro studies given that men in our study showed an increase in VLDL-C with consumption of conventional ground beef which had higher MUFA content compared to premium ground beef. VLDL-C and TAG correlate strongly with LDL density and they are inversely related to HDL-C, especially the HDL_2 subfraction (21, 22). However, these changes were as result of an increase in free fatty acid that was secondary to insulin resistance in those studies. In this

study there was no increase in TAG concentration in either men or women with either type of ground beef intervention.

Plasma CRP concentration was not significantly changed after either ground beef intervention in men or women, although there was an interaction between gender and diet on plasma CRP. There was also a significant interaction between gender, phase and diet. This suggests that there was a phase effect and therefore there was no significant differences between men and women according to different types of diet.

CRP is an acute-phase reactant that was originally described in 1930 (202). CRP was described as a CVD risk factor in men, noting that high plasma concentrations were associated with a two-fold increased risk of stroke and a three-fold increased risk of myocardial infarction (203). Further studies confirmed its efficacy as a predictive factor for CVD in women as well. These studies showed an increase in CRP levels above 3.0 mg/L was associated with elevated age-adjusted incidence rates of future cardiovascular incidents (203). CRP did not differ between men and women (203). Therefore, data from our study were consistent with the previous studies given that CRP changes don't differ between genders.

Plasma insulin concentrations were not changed by either ground beef intervention in either men or women. In women, insulin resistance and diabetes are associated with greater CVD, including up to a 6-fold increase in myocardial infarction risk, as compared to men (who have a four-fold increased risk of myocardial infarction in the setting of diabetes) (204, 205).

Homocysteine, regarded as a biomarker strongly associated with CVD (35) did not change significantly in women or men with ground beef intervention. Elevated homocysteine levels are normally caused by either nutritional deficiencies in vitamin cofactors (folate, vitamin B12, and vitamin B6) or by genetic defects in the enzymes involved in homocysteine metabolism (37). These data suggest that neither conventional ground nor premium ground beef intake caused an increase in the pro-inflammatory marker, homocysteine, in either men or women.

No significant gender-related changes were observed in the expression of the genes ABCA1, ABCG1, SCD1, LDLR, MED1 and SREBF1 between men and women. This suggests that the significant changes in SCD1, LDLR and SREBF1 that was described previously was not gender-dependent.

Considerable research remains to be done to document the interaction between nutritional factors and their role in gender-related cardiovascular differences because several clinical observations which demonstrated the importance of sex-specific differences in the clinical presentation of CVD. It is of significance to establish gender differences in CVD as this may avoid considerable delays in diagnosis and treatment of CVD in women. Furthermore, because diet has been shown to have considerable impact on CVD, studies comparing CVD risk factors and dietary influence in men and women could provide a better understanding the underlying risk factors related to CVD.

CHAPTER V

CONCLUSIONS

Dietary recommendations to reduce the risk of CVD are to lower the contribution to daily energy intakes of total fat, SFA and *trans*-fatty acids, in order to avoid their effects on raising cholesterol levels (61). Epidemiological studies have shown that red meat consumption is associated with an increased risk of CVD (206, 207); however, the data to prove this claim remain unclear (208). Consuming moderate amounts of lean red meat, as part of a balanced diet, valuably contributes to intakes of essential nutrients iron, zinc and vitamin B12 and possibly to intakes of n-3 PUFA and conjugated linoleic acid (CLA). Furthermore red meat is long established as an important dietary source of protein (96). However, recommendations for a "heart healthy" diet do not encourage red meat consumption most likely due to its saturated fat content (209). The aim of this study was to improve meat quality by naturally modifying the fatty acids content in beef and to evaluate the effects of different MUFA:SFA ratios and total MUFA content in beef on CVD risk factors. Six candidate genes were selected to be studied according to their role in cholesterol metabolism and being involved in the inflammatory pathways which lead to atherosclerosis. The results from each study are summarized in Table 28.

In the first study, a significant increase was observed in LDL-C concentration and a significant decrease was seen in HDL-C after premium ground beef consumption. LDL particle size increased significantly after consuming the conventional ground beef (Table 28).

TABLE 28 Overall effects of conventional (low-MUFA) and premium (high-MUFA) ground beef on major cholesterol fractions, triglycerides and gene expression

Study	Gender	Substance	Conventional ground beef	Premium ground beef
1	**Men, Women n = 12**	Total C	NC[1]	NC
		LDL-C	NC	↑
		HDL-C	NC	↓
		VLDL-C	NC	NC
		TAG	NC	NC
		LDL (nm)	↑	NC
		LDL density	↓	NC
2	**Men, Women n = 12**	ABCA1	NC	NC
		ABCG1	NC	NC
		SCD1	NC	↑
		SREBF1	↓	NC
		MED1	NC	NC
		LDLR	↓	NC
3	**Men n = 5**	HDL-C	NC	NC
		VLDL-C	↑	NC
		HDL2b	↑	NC
	Women n = 7	HDL-C	NC	↓
		VLDL-C	NC	NC
		HDL2b	NC	NC

[1] NC = no change, ↑ = increased, ↓ = decreased

LDL mean density was depressed after conventional beef patty intervention. Other lipoproteins and cholesterol fractions did not show significant changes after either dietary beef interventions (Table 28). Gene expression study showed a consistent pattern

with lipoprotein concentration and most of the selected genes were down-regulated and up-regulated with conventional and premium ground beef consumption respectively. However, the significant changes were observed in SCD1, SREBF1 and LDLR (Table 28). Finally, there was a significant change in HDL-C concentration with premium ground beef consumption. However, the change was significant in women but not in men. Furthermore, HDL2b particle concentration significantly increased after conventional ground beef consumption in men, but no changes were observed in women with either ground beef interventions. In addition, VLDL-C increased significantly in men after consumption of conventional ground beef, whereas no significant change was seen in women (Table 28).

These results have confirmed the importance of beef fatty acid influence on CVD risk markers. However, more studies need to be done in terms of lipoprotein concentration with taking into consideration the genetic and gender differences as well as numerous other factors including participant characteristics, life style in addition to dietary interventions in order to better understand the underlying mechanisms of action involved with CVD.

REFERENCES

1. Chilton RJ. Pathophysiology of coronary heart disease: a brief review. J Am Osteopath Assoc. 2004 Sep;104:S5-8.

2. Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, 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 Jul;16:831-42.

3. Steinberg D, Witztum JL. Lipoproteins, lipoprotein oxidation, and atherogenesis. In: Molecular basis of cardiovascular disease. Chien KR, editor. Philadelphia: Saunders; 1999;458.

4. Goldstein JL, Brown MS. Atherosclerosis: the low-density lipoprotein receptor hypothesis. Metabolism. 1977 Nov;26:1257-75.

5. Gould AL, Rossouw JE, Santanello NC, Heyse JF, Furberg CD. Cholesterol reduction yields clinical benefit: impact of statin trials. Circulation. 1998 Mar 17;97:946-52.

6. Xu J, Kochanek KD, Murphy SL, Tejada-Vera B. Deaths: Final Data for 2007. National vital statistics reports. Vol 53, No 17. Hyattsville, MD: National Center for Health Statistics; 2010.

7. Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation. 1990 Aug;82:495-506.

8. Gambino R, Cassader M, Pagano G, Durazzo M, Musso G. Polymorphism in microsomal triglyceride transfer protein: a link between liver disease and atherogenic postprandial lipid profile in NASH? Hepatology. 2007 May;45:1097-107.

9. Ye J, Li JZ, Liu Y, Li X, Yang T, Ma X, Li Q, Yao Z, Li P. Cideb, an ER- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with apolipoprotein B. Cell Metab. 2009 Feb;9:177-90.

10. Rye KA, Bursill CA, Lambert G, Tabet F, Barter PJ. The metabolism and anti-atherogenic properties of HDL. J Lipid Res. 2009 Apr;50 Suppl:S195-200.

11. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA. 2009 Nov 11;302:1993-2000.

12. Rothblat GH, Phillips MC. High-density lipoprotein heterogeneity and function in reverse cholesterol transport. Curr Opin Lipidol. 2010 Jun;21:229-38.

13. Rosenson RS, Brewer Jr. HB, Chapman MJ, Fazio S, Hussain MM, Kontush A, Krauss RM, Otvos JD, Remaley AT, Schaefer EJ. HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. Clin Chem. 2011 Mar;57:392-410.

14. Abderrahmani A, Niederhauser G, Favre D, Abdelli S, Ferdaoussi M, Yang JY, Regazzi R, Widmann C, Waeber G. Human high-density lipoprotein particles prevent activation of the JNK pathway induced by human oxidised low-density lipoprotein particles in pancreatic beta cells. Diabetologia. 2007 Jun;50:1304-14.

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

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

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

18. Babin PJ, Gibbons GF. The evolution of plasma cholesterol: direct utility or a "spandrel" of hepatic lipid metabolism? Prog Lipid Res. 2009 Mar;48:73-91.

19. Mann CJ, Anderson TA, Read J, Chester SA, Harrison GB, Kochl S, Ritchie PJ, Bradbury P, Hussain FS, et al. The structure of vitellogenin provides a molecular model for the assembly and secretion of atherogenic lipoproteins. J Mol Biol. 1999 Jan 8;285:391-408.

20. White DA, Bennett AJ, Billett MA, Salter AM. The assembly of triacylglycerol-rich lipoproteins: an essential role for the microsomal triacylglycerol transfer protein. Br J Nutr. 1998 Sep;80:219-29.

21. Chirieac DV, Collins HL, Cianci J, Sparks JD, Sparks CE. Altered triglyceride-rich lipoprotein production in Zucker diabetic fatty rats. Am J Physiol Endocrinol Metab. 2004 Jul;287:E42-9.

22. Taghibiglou C, Carpentier A, Van Iderstine SC, Chen B, Rudy D, Aiton A, Lewis GF, Adeli K. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance. Evidence for enhanced lipoprotein assembly, reduced intracellular ApoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. J Biol Chem. 2000 Mar 24;275:8416-25.

23. Dolinsky VW, Douglas DN, Lehner R, Vance DE. Regulation of the enzymes of hepatic microsomal triacylglycerol lipolysis and re-esterification by the glucocorticoid dexamethasone. Biochem J. 2004 Mar 15;378:967-74.

24. Sundaram M, Zhong S, Bou Khalil M, Links PH, Zhao Y, Iqbal J, Hussain MM, Parks RJ, Wang Y, Yao Z. Expression of apolipoprotein C-III in McA-RH7777 cells enhances VLDL assembly and secretion under lipid-rich conditions. J Lipid Res. 2010 Jan;51:150-61.

25. Wang Y, McLeod RS, Yao Z. Normal activity of microsomal triglyceride transfer protein is required for the oleate-induced secretion of very low density lipoproteins containing apolipoprotein B from McA-RH7777 cells. J Biol Chem. 1997 May 9;272:12272-8.

26. Tran K, Sun F, Cui Z, Thorne-Tjomsland G, St Germain C, Lapierre LR, McLeod RS, Jamieson JC, Yao Z. Attenuated secretion of very low density lipoproteins from McA-RH7777 cells treated with eicosapentaenoic acid is associated with impaired utilization of triacylglycerol synthesized via phospholipid remodeling. Biochim Biophys Acta. 2006 Apr;1761:463-73.

27. Ross R, Glomset JA. The pathogenesis of atherosclerosis (second of two parts). N Engl J Med. 1976 Aug 19;295:420-5.

28. Ross R, Glomset JA. The pathogenesis of atherosclerosis (first of two parts). N Engl J Med. 1976 Aug 12;295:369-77.

29. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB, Gudnason V. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004 Apr 1;350:1387-97.

30. Glynn RJ, MacFadyen JG, Ridker PM. Tracking of high-sensitivity C-reactive protein after an initially elevated concentration: the JUPITER Study. Clin Chem. 2009 Feb;55:305-12.

31. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. J Am Coll Cardiol. 2007 May 29;49:2129-38.

32. Chait A, Han CY, Oram JF, Heinecke JW. Thematic review series: The immune system and atherogenesis. Lipoprotein-associated inflammatory proteins: markers or mediators of cardiovascular disease? J Lipid Res. 2005 Mar;46:389-403.

33. Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation. 2003 Nov 11;108:2317-22.

34. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000 Mar 23;342:836-43.

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

36. Mehra MR, Uber PA, Scott RL, Park MH, Milani RV. Effect of immunosuppressive regimen on novel markers of atherothrombosis in heart transplantation: homocysteine, c-reactive protein, and mean platelet volume. Transplant Proc. 2002 Aug;34:1866-8.

37. Prontera C, Martelli N, Evangelista V, D'Urbano E, Manarini S, Recchiuti A, Dragani A, Passeri C, Davi G, Romano M. Homocysteine modulates the CD40/CD40L system. J Am Coll Cardiol. 2007 Jun 5;49:2182-90.

38. Jump DB. N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. Curr Opin Lipidol. 2008 Jun;19:242-7.

39. Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu Rev Nutr. 2005;25:317-40.

40. Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev. 2000 Jan 15;14:121-41.

41. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev. 1999 Oct;20:649-88.

42. Brown JD, Plutzky J. Peroxisome proliferator-activated receptors as transcriptional nodal points and therapeutic targets. Circulation. 2007 Jan 30;115:518-33.

43. Marx N, Duez H, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. Circ Res. 2004 May 14;94:1168-78.

44. Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RR, Xu HE, Turk J, Semenkovich CF. Identification of a physiologically relevant endogenous ligand for PPARalpha in liver. Cell. 2009 Aug 7;138:476-88.

45. Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, Mangelsdorf DJ. Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. Proc Natl Acad Sci U S A. 1999 Jan 5;96:266-71.

46. Repa JJ, Mangelsdorf DJ. The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. Annu Rev Cell Dev Biol. 2000;16:459-81.

47. Geyeregger R, Zeyda M, Stulnig TM. Liver X receptors in cardiovascular and metabolic disease. Cell Mol Life Sci. 2006 Mar;63:524-39.

48. Joseph SB, McKilligin E, Pei L, Watson MA, Collins AR, Laffitte BA, Chen M, Noh G, Goodman J, et al. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. Proc Natl Acad Sci U S A. 2002 May 28;99:7604-9.

49. Rader DJ, Pure E. Lipoproteins, macrophage function, and atherosclerosis: beyond the foam cell? Cell Metab. 2005 Apr;1:223-30.

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

51. Cao G, Liang Y, Broderick CL, Oldham BA, Beyer TP, Schmidt RJ, Zhang Y, Stayrook KR, Suen C, et al. Antidiabetic action of a liver x receptor agonist mediated by inhibition of hepatic gluconeogenesis. J Biol Chem. 2003 Jan 10;278:1131-6.

52. Laffitte BA, Chao LC, Li J, Walczak R, Hummasti S, Joseph SB, Castrillo A, Wilpitz DC, Mangelsdorf DJ, et al. Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. Proc Natl Acad Sci U S A. 2003 Apr 29;100:5419-24.

53. Hannah VC, Ou J, Luong A, Goldstein JL, Brown MS. Unsaturated fatty acids down-regulate srebp isoforms 1a and 1c by two mechanisms in HEK-293 cells. J Biol Chem. 2001 Feb 9;276:4365-72.

54. Yoshikawa T, Shimano H, Yahagi N, Ide T, Amemiya-Kudo M, Matsuzaka T, Nakakuki M, Tomita S, Okazaki H, et al. Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements. J Biol Chem. 2002 Jan 18;277:1705-11.

55. Brown MS, Goldstein JL. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc Natl Acad Sci U S A. 1999 Sep 28;96:11041-8.

56. Eberle D, Hegarty B, Bossard P, Ferre P, Foufelle F. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie. 2004 Nov;86:839-48.

57. Horton JD, Bashmakov Y, Shimomura I, Shimano H. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. Proc Natl Acad Sci U S A. 1998 May 26;95:5987-92.

58. Worgall TS, Sturley SL, Seo T, Osborne TF, Deckelbaum RJ. Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. J Biol Chem. 1998 Oct 2;273:25537-40.

59. Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, Goldstein JL. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J Clin Invest. 1996 Oct 1;98:1575-84.

60. Shimano H, Shimomura I, Hammer RE, Herz J, Goldstein JL, Brown MS, Horton JD. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. J Clin Invest. 1997 Oct 15;100:2115-24.

61. Attie AD, Krauss RM, Gray-Keller MP, Brownlie A, Miyazaki M, Kastelein JJ, Lusis AJ, Stalenhoef AF, Stoehr JP, et al. Relationship between stearoyl-CoA desaturase activity and plasma triglycerides in human and mouse hypertriglyceridemia. J Lipid Res. 2002 Nov;43:1899-907.

62. Flowers MT, Ntambi JM. Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism. Curr Opin Lipidol. 2008 Jun;19:248-56.

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

64. Miyazaki M, Kim HJ, Man WC, Ntambi JM. Oleoyl-CoA is the major de novo product of stearoyl-CoA desaturase 1 gene isoform and substrate for the biosynthesis of the Harderian gland 1-alkyl-2,3-diacylglycerol. J Biol Chem. 2001 Oct 19;276:39455-61.

65. Miyazaki M, Man WC, Ntambi JM. Targeted disruption of stearoyl-CoA desaturase1 gene in mice causes atrophy of sebaceous and meibomian glands and depletion of wax esters in the eyelid. J Nutr. 2001 Sep;131:2260-8.

66. Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, Sharma R, Hudgins LC, Ntambi JM, Friedman JM. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science. 2002 Jul 12;297:240-3.

67. Erbay E, Babaev VR, Mayers JR, Makowski L, Charles KN, Snitow ME, Fazio S, Wiest MM, Watkins SM, et al. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. Nat Med. 2009 Dec;15:1383-91.

68. Miyazaki M, Dobrzyn A, Man WC, Chu K, Sampath H, Kim HJ, Ntambi JM. Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and -independent mechanisms. J Biol Chem. 2004 Jun 11;279:25164-71.

69. Gidding SS, Dennison BA, Birch LL, Daniels SR, Gillman MW, Lichtenstein AH, Rattay KT, Steinberger J, Stettler N, et al. Dietary recommendations for children and adolescents: a guide for practitioners: consensus statement from the American Heart Association. Circulation. 2005 Sep 27;112:2061-75.

70. Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr. 1999 Dec;70:1001-8.

71. Kelly FD, Sinclair AJ, Mann NJ, Turner AH, Raffin FL, Blandford MV, Pike MJ. Short-term diets enriched in stearic or palmitic acids do not alter plasma lipids, platelet aggregation or platelet activation status. Eur J Clin Nutr. 2002 Jun;56:490-9.

72. 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 May;77:1146-55.

73. Oosterveer MH, van Dijk TH, Tietge UJ, Boer T, Havinga R, Stellaard F, Groen AK, Kuipers F, Reijngoud DJ. High fat feeding induces hepatic fatty acid elongation in mice. PLoS One. 2009;4:e6066.

74. Sampath H, Miyazaki M, Dobrzyn A, Ntambi JM. Stearoyl-CoA desaturase-1 mediates the pro-lipogenic effects of dietary saturated fat. J Biol Chem. 2007 Jan 26;282:2483-93.

75. Hulshof KF, van Erp-Baart MA, Anttolainen M, Becker W, Church SM, Couet C, Hermann-Kunz E, Kesteloot H, Leth T, et al. Intake of fatty acids in western Europe with emphasis on trans fatty acids: the TRANSFAIR Study. Eur J Clin Nutr. 1999 Feb;53:143-57.

76. Anon R. The diet and all-causes death rate in the Seven Countries Study. Lancet. 1981;2:58-61.

77. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb. 1992 Aug;12:911-9.

78. Reiser R, Probstfield JL, Silvers A, Scott LW, Shorney ML, Wood RD, O'Brien BC, Gotto AM, Jr., Insull W, Jr. Plasma lipid and lipoprotein response of humans to beef fat, coconut oil and safflower oil. Am J Clin Nutr. 1985 Aug;42:190-7.

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

80. Lai CQ, Corella D, Demissie S, Cupples LA, Adiconis X, Zhu Y, Parnell LD, Tucker KL, Ordovas JM. Dietary intake of n-6 fatty acids modulates effect of apolipoprotein A5 gene on plasma fasting triglycerides, remnant lipoprotein concentrations, and lipoprotein particle size: the Framingham Heart Study. Circulation. 2006 May 2;113:2062-70.

81. Campos H, Willett WC, Peterson RM, Siles X, Bailey SM, Wilson PW, Posner BM, Ordovas JM, Schaefer EJ. Nutrient intake comparisons between Framingham and rural and Urban Puriscal, Costa Rica. Associations with lipoproteins, apolipoproteins, and low density lipoprotein particle size. Arterioscler Thromb. 1991 Jul-Aug;11:1089-99.

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

83. Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willett WC. Dietary fat intake and the risk of coronary heart disease in women. N Engl J Med. 1997 Nov 20;337:1491-9.

84. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Jr., Kris-Etherton P, Goldberg IJ, et al. AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation. 2000 Oct 31;102:2284-99.

85. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr. 1965 Nov;17:281-95.

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

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

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

89. Berry EM, Eisenberg S, Friedlander Y, Harats D, Kaufmann NA, Norman Y, Stein Y. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins--the Jerusalem Nutrition Study. II. Monounsaturated fatty acids vs carbohydrates. Am J Clin Nutr. 1992 Aug;56:394-403.

90. Mensink RP, Katan MB. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. N Engl J Med. 1989 Aug 17;321:436-41.

91. Bonanome A, Grundy SM. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N Engl J Med. 1988 May 12;318:1244-8.

92. Grande F, Anderson JT, Keys A. Comparison of effects of palmitic and stearic acids in the diet on serum cholesterol in man. Am J Clin Nutr. 1970 Sep;23:1184-93.

93. St John LC, Young CR, Knabe DA, Thompson LD, Schelling GT, Grundy SM, Smith SB. Fatty acid profiles and sensory and carcass traits of tissues from steers and swine fed an elevated monounsaturated fat diet. J Anim Sci. 1987 May;64:1441-7.

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

95. Scott LW KK, Wittels EH, Dunn KJ, Brauchi DJ, Pownall HJ, Herd JA, Smith BR, Savell JW, Papadopoulos LS, Collins SA, Cross HR, Gotto AM. Effects of a lean beef diet and of a chicken and fish diet on lipoprotein profiles. Nutr Metab Cardiovasc Dis. 1991; 1:25-30.

96. 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 Apr;227:266-75.

97. Chan W, Brown J, Church SM, Buss D. Meat products and dishes. London: The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food; 1996.

98. Scollan N, Hocquette J, Nuernberg K, Dannenberger D, Richardson I, Moloney A. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Science. 2006;74:17-33.

99. Howell WH, McNamara DJ, Tosca MA, Smith BT, Gaines JA. Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. Am J Clin Nutr. 1997 Jun;65:1747-64.

100. Wood JD, Enser M. Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. Br J Nutr. 1997 Jul;78 Suppl 1:S49-60.

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

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

103. Ruiz-Gutierrez V, Muriana FJ, Guerrero A, Cert AM, Villar J. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. J Hypertens. 1996 Dec;14:1483-90.

104. Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willett WC. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. BMJ. 1996 Jul 13;313:84-90.

105. Mann N, Pirotta Y, O'Connell S, Li D, Kelly F, Sinclair A. Fatty acid composition of habitual omnivore and vegetarian diets. Lipids. 2006 Jul;41:637-46.

106. Ruxton CH, Reed SC, Simpson MJ, Millington KJ. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J Hum Nutr Diet. 2004 Oct;17:449-59.

107. Li D, Siriamornpun S, Wahlqvist ML, Mann NJ, Sinclair AJ. Lean meat and heart health. Asia Pac J Clin Nutr. 2005;14:113-9.

108. Turpeinen AM, Mutanen M, Aro A, Salminen I, Basu S, Palmquist DL, Griinari JM. Bioconversion of vaccenic acid to conjugated linoleic acid in humans. Am J Clin Nutr. 2002 Sep;76:504-10.

109. French P, Stanton C, Lawless F, O'Riordan EG, Monahan FJ, Caffrey PJ, Moloney AP. Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. J Anim Sci. 2000 Nov;78:2849-55.

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

111. Beam TM, Jenkins TC, Moate PJ, Kohn RA, Palmquist DL. Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. J Dairy Sci. 2000 Nov;83:2564-73.

112. Hargrave-Barnes KM, Azain MJ, Miner JL. Conjugated linoleic acid-induced fat loss dependence on Delta6-desaturase or cyclooxygenase. Obesity (Silver Spring). 2008 Oct;16:2245-52.

113. Lock AL, Corl BA, Barbano DM, Bauman DE, Ip C. The anticarcinogenic effect of trans-11 18:1 is dependent on its conversion to cis-9, trans-11 CLA by delta9-desaturase in rats. J Nutr. 2004 Oct;134:2698-704.

114. Tricon S, Burdge GC, Kew S, Banerjee T, Russell JJ, Grimble RF, Williams CM, Calder PC, Yaqoob P. Effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic acid on immune cell function in healthy humans. Am J Clin Nutr. 2004 Dec;80:1626-33.

115. Dallman PR. Biochemical basis for the manifestations of iron deficiency. Annu Rev Nutr. 1986;6:13-40.

116. Yip R. Iron deficiency: contemporary scientific issues and international programmatic approaches. J Nutr. 1994 Aug;124:1479S-90S.

117. Gibson S, Ashwell M. The association between red and processed meat consumption and iron intakes and status among British adults. Public Health Nutr. 2003 Jun;6:341-50.

118. Cosgrove M, Flynn A, Kiely M. Consumption of red meat, white meat and processed meat in Irish adults in relation to dietary quality. Br J Nutr. 2005 Jun;93:933-42.

119. Johnston J, Prynne CJ, Stephen AM, Wadsworth ME, British Birth C. Haem and non-haem iron intake through 17 years of adult life of a British Birth Cohort. Br J Nutr. 2007 Nov;98:1021-8.

120. Black RE. Zinc for Child Health. Am J Clin Nutr. 1998; 65:409S-516S.

121. Gibson RS. Content and bioavailability of trace elements in vegetarian diets. Am J Clin Nutr. 1994 May;59:1223S-32S.

122. Dagnelie PC, van Staveren WA, Hautvast JG. Stunting and nutrient deficiencies in children on alternative diets. Acta Paediatr Scand Suppl. 1991;374:111-8.

123. Allen LH. Vitamin B12 metabolism and status during pregnancy, lactation and infancy. Adv Exp Med Biol. 1994;352:173-86.

124. Casterline JE, Allen LH, Ruel MT. Vitamin B-12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months postpartum. J Nutr. 1997 Oct;127:1966-72.

125. Scott JM. Folate and vitamin B12. Proc Nutr Soc. 1999 May;58:441-8.

126. Kris-Etherton PM, Etherton TD, Carlson J, Gardner C. Recent discoveries in inclusive food-based approaches and dietary patterns for reduction in risk for cardiovascular disease. Curr Opin Lipidol. 2002 Aug;13:397-407.

127. Baggio G, Pagnan A, Muraca M, Martini S, Opportuno A, Bonanome A, Ambrosio GB, Ferrari S, Guarini P, et al. Olive-oil-enriched diet: effect on serum lipoprotein levels and biliary cholesterol saturation. Am J Clin Nutr. 1988 Jun;47:960-4.

128. Bouwens M, Grootte Bromhaar M, Jansen J, Muller M, Afman LA. Postprandial dietary lipid-specific effects on human peripheral blood mononuclear cell gene expression profiles. Am J Clin Nutr. 2010 Jan;91:208-17.

129. Cheng JF, Zapf J, Takedomi K, Fukushima C, Ogiku T, Zhang SH, Yang G, Sakurai N, Barbosa M, et al. Combination of virtual screening and high throughput gene profiling for identification of novel liver X receptor modulators. J Med Chem. 2008 Apr 10;51:2057-61.

130. Rizzo M, Berneis K. Small, dense low-density-lipoproteins and the metabolic syndrome. Diabetes Metab Res Rev. 2007 Jan;23:14-20.

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

132. Morrison WR, Smith LM. Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boron Fluoride--Methanol. J Lipid Res. 1964 Oct;5:600-8.

133. Troup J. Method for analyzing blood for lipoprotein components. U.S. Pat. Appl. Publ. US 2008038762, 2007.

134. Keys A. Coronary heart disease in seven countries. 1970. Nutrition. 1997 Mar;13:250-2.

135. Kato H, Tillotson J, Nichaman MZ, Rhoads GG, Hamilton HB. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California. Am J Epidemiol. 1973 Jun;97:372-85.

136. Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, Djordjevic BS, Dontas AS, Fidanza F, et al. The diet and 15-year death rate in the seven countries study. Am J Epidemiol. 1986 Dec;124:903-15.

137. Adams TH, Walzem RL, Smith DR, Tseng S, Smith SB. Hamburger high in total, saturated and trans-fatty acids decreases HDL cholesterol and LDL particle diameter, and increases TAG, in mildly hypercholesterolaemic men. Br J Nutr. 2010 Jan;103:91-8.

138. Hayes KC, Khosla P. Dietary fatty acid thresholds and cholesterolemia. FASEB J. 1992 May;6:2600-7.

139. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. Lancet. 1987 Jan 17;1:122-5.

140. Roche HM. Dietary carbohydrates and triacylglycerol metabolism. Proc Nutr Soc. 1999 Feb;58:201-7.

141. Austin MA. Plasma triglyceride as a risk factor for cardiovascular disease. Can J Cardiol. 1998 May;14 Suppl B:14B-7B.

142. Kontush A, Chantepie S, Chapman MJ. Small, dense HDL particles exert potent protection of atherogenic LDL against oxidative stress. Arterioscler Thromb Vasc Biol. 2003 Oct 1;23:1881-8.

143. Lecerf JM. Fatty acids and cardiovascular disease. Nutr Rev. 2009 May;67:273-83.

144. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr. 2006 Jun;83:1505S-19S.

145. Calder PC. Immunomodulation by omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids. 2007 Dec;77:327-35.

146. Breslow JL. n-3 fatty acids and cardiovascular disease. Am J Clin Nutr. 2006 Jun;83:1477S-82S.

147. Martins de Lima T, Gorjao R, Hatanaka E, Cury-Boaventura MF, Portioli Silva EP, Procopio J, Curi R. Mechanisms by which fatty acids regulate leucocyte function. Clin Sci (Lond). 2007 Jul;113:65-77.

148. Tiwari RL, Singh V, Barthwal MK. Macrophages: an elusive yet emerging therapeutic target of atherosclerosis. Med Res Rev. 2008 Jul;28:483-544.

149. Miyazaki M, Ntambi JM. Role of stearoyl-coenzyme A desaturase in lipid metabolism. Prostaglandins Leukot Essent Fatty Acids. 2003 Feb;68:113-21.

150. May SG, Sturdivant CA, Lunt DK, Miller RK, B. SS. Comparison of sensory characteristics and fatty acid composition between Wagyu crossbred and Angus steers. Meat Sci. 1993;35:389-98.

151. Taniguchi M, Utsugi T, Oyama K, Mannen H, Kobayashi M, Tanabe Y, Ogino A, Tsuji S. Genotype of stearoyl-coA desaturase is associated with fatty acid composition in Japanese Black cattle. Mamm Genome. 2004 Feb;15:142-8.

152. Smith SB, Gill CA, Lunt DK, Brooks MA. Regulation of fat and fatty acid composition in beef cattle. Asian-Australasian J Anim Sci. 2009;22:1225-33.

153. Hayden MR, Clee SM, Brooks-Wilson A, Genest J, Jr., Attie A, Kastelein JJ. Cholesterol efflux regulatory protein, Tangier disease and familial high-density lipoprotein deficiency. Curr Opin Lipidol. 2000 Apr;11:117-22.

154. Hovingh GK, Van Wijland MJ, Brownlie A, Bisoendial RJ, Hayden MR, Kastelein JJ, Groen AK. The role of the ABCA1 transporter and cholesterol efflux in familial hypoalphalipoproteinemia. J Lipid Res. 2003 Jun;44:1251-5.

155. Mott S, Yu L, Marcil M, Boucher B, Rondeau C, Genest J, Jr. Decreased cellular cholesterol efflux is a common cause of familial hypoalphalipoproteinemia: role of the ABCA1 gene mutations. Atherosclerosis. 2000 Oct;152:457-68.

156. Klucken J, Buchler C, Orso E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, Kapinsky M, Diederich W, Drobnik W, et al. ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci U S A. 2000 Jan 18;97:817-22.

157. Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet. 1999 Aug;22:347-51.

158. Costet P, Luo Y, Wang N, Tall AR. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. J Biol Chem. 2000 Sep 8;275:28240-5.

159. Venkateswaran A, Repa JJ, Lobaccaro JM, Bronson A, Mangelsdorf DJ, Edwards PA. Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. A transcriptional role for specific oxysterols. J Biol Chem. 2000 May 12;275:14700-7.

160. Zhu Y, Qi C, Jain S, Rao MS, Reddy JK. Isolation and characterization of PBP, a protein that interacts with peroxisome proliferator-activated receptor. J Biol Chem. 1997 Oct 10;272:25500-6.

161. Ge K, Guermah M, Yuan CX, Ito M, Wallberg AE, Spiegelman BM, Roeder RG. Transcription coactivator TRAP220 is required for PPAR gamma 2-stimulated adipogenesis. Nature. 2002 May 30;417:563-7.

162. Infante RE, Abi-Mosleh L, Radhakrishnan A, Dale JD, Brown MS, Goldstein JL. Purified NPC1 protein. I. Binding of cholesterol and oxysterols to a 1278-amino acid membrane protein. J Biol Chem. 2008 Jan 11;283:1052-63.

163. Innerarity TL. Structural biology. LDL receptor's beta-propeller displaces LDL. Science. 2002 Dec 20;298:2337-9.

164. Brown MS, Dana SE, Goldstein JL. Cholesterol ester formation in cultured human fibroblasts. Stimulation by oxygenated sterols. J Biol Chem. 1975 May 25;250:4025-7.

165. Brown MS, Goldstein JL. Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. J Lipid Res. 2009 Apr;50 Suppl:S15-27.

166. Bouwens M, van de Rest O, Dellschaft N, Bromhaar MG, de Groot LC, Geleijnse JM, Muller M, Afman LA. Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. Am J Clin Nutr. 2009 Aug;90:415-24.

167. Konstantinidou V, Khymenets O, Covas MI, de la Torre R, Munoz-Aguayo D, Anglada R, Farre M, Fito M. Time course of changes in the expression of insulin sensitivity-related genes after an acute load of virgin olive oil. OMICS. 2009 Oct;13:431-8.

168. Covas MI, Nyyssonen K, Poulsen HE, Kaikkonen J, Zunft HJ, Kiesewetter H, Gaddi A, de la Torre R, Mursu J, et al. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. Ann Intern Med. 2006 Sep 5;145:333-41.

169. Enoch HG, Catala A, Strittmatter P. Mechanism of rat liver microsomal stearyl-CoA desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. J Biol Chem. 1976 Aug 25;251:5095-103.

170. Ducluzeau PH, Perretti N, Laville M, Andreelli F, Vega N, Riou JP, Vidal H. Regulation by insulin of gene expression in human skeletal muscle and adipose tissue. Evidence for specific defects in type 2 diabetes. Diabetes. 2001 May;50:1134-42.

171. Sewter C, Berger D, Considine RV, Medina G, Rochford J, Ciaraldi T, Henry R, Dohm L, Flier JS, et al. Human obesity and type 2 diabetes are associated with alterations in SREBP1 isoform expression that are reproduced ex vivo by tumor necrosis factor-alpha. Diabetes. 2002 Apr;51:1035-41.

172. Kolehmainen M, Vidal H, Alhava E, Uusitupa MI. Sterol regulatory element binding protein 1c (SREBP-1c) expression in human obesity. Obes Res. 2001 Nov;9:706-12.

173. Yang X, Jansson PA, Nagaev I, Jack MM, Carvalho E, Sunnerhagen KS, Cam MC, Cushman SW, Smith U. Evidence of impaired adipogenesis in insulin resistance. Biochem Biophys Res Commun. 2004 May 14;317:1045-51.

174. Uehara Y, Miura S, von Eckardstein A, Abe S, Fujii A, Matsuo Y, Rust S, Lorkowski S, Assmann G, et al. Unsaturated fatty acids suppress the expression of the ATP-binding cassette transporter G1 (ABCG1) and ABCA1 genes via an LXR/RXR responsive element. Atherosclerosis. 2007 Mar;191:11-21.

175. Uehara Y, Engel T, Li Z, Goepfert C, Rust S, Zhou X, Langer C, Schachtrup C, Wiekowski J, et al. Polyunsaturated fatty acids and acetoacetate downregulate the expression of the ATP-binding cassette transporter A1. Diabetes. 2002 Oct;51:2922-8.

176. Pilote L, Dasgupta K, Guru V, Humphries KH, McGrath J, Norris C, Rabi D, Tremblay J, Alamian A, et al. A comprehensive view of sex-specific issues related to cardiovascular disease. CMAJ. 2007 Mar 13;176:S1-44.

177. Grady D, Chaput L, Kristof M. Results of systematic review of research on diagnosis and treatment of coronary heart disease in women. Evid Rep Technol Assess (Summ). 2003 May:1-4.

178. Wenger NK, Speroff L, Packard B. Cardiovascular health and disease in women. N Engl J Med. 1993 Jul 22;329:247-56.

179. Shaw LJ, Bairey Merz CN, Pepine CJ, Reis SE, Bittner V, Kelsey SF, Olson M, Johnson BD, Mankad S, et al. Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: Part I: gender differences in traditional and novel risk factors, symptom evaluation, and gender-optimized diagnostic strategies. J Am Coll Cardiol. 2006 Feb 7;47:S4-S20.

180. American Heart Association. Heart Disease and Stroke Statistics: 2004 Update. Dallas: AHA; 2005.

181. Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am Heart J. 1986 Feb;111:383-90.

182. Manolio TA, Pearson TA, Wenger NK, Barrett-Connor E, Payne GH, Harlan WR. Cholesterol and heart disease in older persons and women. Review of an NHLBI workshop. Ann Epidemiol. 1992 Jan-Mar;2:161-76.

183. DeVon HA, Ryan CJ, Ochs AL, Shapiro M. Symptoms across the continuum of acute coronary syndromes: differences between women and men. Am J Crit Care. 2008 Jan;17:14-24.

184. Isles CG, Hole DJ, Hawthorne VM, Lever AF. Relation between coronary risk and coronary mortality in women of the Renfrew and Paisley survey: comparison with men. Lancet. 1992 Mar 21;339:702-6.

185. Miller VM, Mulvagh SL. Sex steroids and endothelial function: translating basic science to clinical practice. Trends Pharmacol Sci. 2007 Jun;28:263-70.

186. Chow RW, Handelsman DJ, Ng MK. Minireview: rapid actions of sex steroids in the endothelium. Endocrinology. 2010 Jun;151:2411-22.

187. Reis SE, Holubkov R, Conrad Smith AJ, Kelsey SF, Sharaf BL, Reichek N, Rogers WJ, Merz CN, Sopko G, et al. Coronary microvascular dysfunction is highly prevalent in women with chest pain in the absence of coronary artery disease: results from the NHLBI WISE study. Am Heart J. 2001 May;141:735-41.

188. Jacobs AK. Coronary revascularization in women in 2003: sex revisited. Circulation. 2003 Jan 28;107:375-7.

189. Williams MR, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K, Komesaroff PA. Variations in endothelial function and arterial compliance during the menstrual cycle. J Clin Endocrinol Metab. 2001 Nov;86:5389-95.

190. English JL, Jacobs LO, Green G, Andrews TC. Effect of the menstrual cycle on endothelium-dependent vasodilation of the brachial artery in normal young women. Am J Cardiol. 1998 Jul 15;82:256-8.

191. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H, Ouchi Y. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. Circulation. 1995 Dec 15;92:3431-5.

192. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol. 1994 Aug;24:471-6.

193. Halligan SC, Murtagh B, Lennon RJ, Pumper GM, Mathew V, Higano ST, Lerman A. Effect of long-term hormone replacement therapy on coronary endothelial function in postmenopausal women. Mayo Clin Proc. 2004 Dec;79:1514-20.

194. Sader MA, Celermajer DS. Endothelial function, vascular reactivity and gender differences in the cardiovascular system. Cardiovasc Res. 2002 Feb 15;53:597-604.

195. Thompson J, Khalil RA. Gender differences in the regulation of vascular tone. Clin Exp Pharmacol Physiol. 2003 Jan-Feb;30:1-15.

196. Perez-Lopez FR, Chedraui P, Gilbert JJ, Perez-Roncero G. Cardiovascular risk in menopausal women and prevalent related co-morbid conditions: facing the post-Women's Health Initiative era. Fertil Steril. 2009 Oct;92:1171-86.

197. Fatourechi V. Subclinical hypothyroidism: an update for primary care physicians. Mayo Clin Proc. 2009;84:65-71.

198. Perez-Lopez FR, Larrad-Mur L, Kallen A, Chedraui P, Taylor HS. Gender differences in cardiovascular disease: hormonal and biochemical influences. Reprod Sci. 2010 Jun;17:511-31.

199. Barrett-Conner E, Bush TL. Estrogens and coronary heart disease in women. JAMA. 1991;265:1861-7.

200. Reckelhoff JF. Gender differences in the regulation of blood pressure. Hypertension. 2001 May;37:1199-208.

201. Ho JE, Paultre F, Mosca L. The gender gap in coronary heart disease mortality: is there a difference between blacks and whites? J Womens Health (Larchmt). 2005 Mar;14:117-27.

202. Tillett WS, Francis T. Serological Reactions in Pneumonia with a Non-Protein Somatic Fraction of Pneumococcus. J Exp Med. 1930 Sep 30;52:561-71.

203. Page-Wilson G, Goulart AC, Rexrode KM. Interrelation between sex hormones and plasma sex hormone-binding globulin and hemoglobin A1c in healthy postmenopausal women. Metab Syndr Relat Disord. 2009 Jun;7:249-54.

204. Manolakou P, Angelopoulou R, Bakoyiannis C, Bastounis E. The effects of endogenous and exogenous androgens on cardiovascular disease risk factors and progression. Reprod Biol Endocrinol. 2009;7:44.

205. Pan WH, Cedres LB, Liu K, Dyer A, Schoenberger JA, Shekelle RB, Stamler R, Smith D, Collette P, Stamler J. Relationship of clinical diabetes and asymptomatic hyperglycemia to risk of coronary heart disease mortality in men and women. Am J Epidemiol. 1986 Mar;123:504-16.

206. Fraser GE. Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. Am J Clin Nutr. 1999 Sep;70:532S-8S.

207. Snowdon DA, Phillips RL, Fraser GE. Meat consumption and fatal ischemic heart disease. Prev Med. 1984 Sep;13:490-500.

208. Micha R, Wallace SK, Mozaffarian D. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. Circulation. 2010 Jun 1;121:2271-83.

209. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, et al. Summary of American Heart Association Diet and Lifestyle Recommendations revision 2006. Arterioscler Thromb Vasc Biol. 2006 Oct;26:2186-91.

VITA

Ghazal Ghahramany

2471 TAMU,
Department of Animal Science,
College of Agriculture and Life Sciences,
College Station, Texas 77843

(979) 845-3998
ghazal@tamu.edu

Education

Texas A&M University	Shiraz University,
College Station, TX	Shiraz, Iran
Ph.D. in Nutrition	B.S. in Biology
May 2012	December 2006

Work Experience

Graduate Research Assistant	January 2011- August 2011
Graduate Research Assistant	January 2009- May 2010
Graduate Teaching Assistant, Nutrition 489	Fall 2008
Graduate Teaching Assistant, Nutrition 470	Fall 2008
Graduate Research Assistant	January 2008- August 2008

Fellowship Awards

William C. Gilbert Foundation, Graduate Scholarship (2010)

William W. Allen Foundation, Graduate Scholarship (2009)

William W. Allen Foundation, Graduate Scholarship (2008)

William C. Gilbert Foundation, Graduate Scholarship (2007)

The typist for this dissertation was Ghazal Ghahramany.