Influence of a 6-week cholesterol education program on blood lipids and LDL oxidation
by Kimberly Rae Monahan

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Health and Human Development
Montana State University

Abstract:
To evaluate the effectiveness of two different approaches of dietary education to reduce blood lipids and oxidative stress. METHODS: Volunteers with moderately elevated low-density lipoprotein cholesterol (LDL-C) levels (100mg/dL-159mg/dL) were randomized into either intensive or conventional education groups. The intensive education group (n=12) attended 6 classes and received a nutrition education manual. The conventional group (n=l1) received a nutrition education manual. Three-day weighed diet records, 3-day physical activity records, anthropometric measurements, blood pressure, plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and LDL-C were collected at baseline, 6-weeks and 12 weeks. Oxidized LDL-C was measured via copper-mediated oxidation. RESULTS: At 6-weeks, there was a significant (P=0.05) decrease in TC (-4.96%) and a trend (P=0.05) to decrease LDL-C (-10.37%) for the intensive group. At 12-weeks, there was a significant (P=0.05) decrease in LDL-C for the conventional (-9.55%) and intensive (-10.54%) groups. The intensive group had greater increases in polyunsaturated fat (4.58%E at baseline; 6.67%E at midstudy; 7.08%E at study-end, P<0.05) and greater reductions in saturated fat (9.08%E at baseline; 6.83%E at mid-study, P<0.01); however these reductions returned to baseline values at Study-end (9-.42±2.02 %E, P<0.05). Neither educational method resulted in significant oxidative modifications of LDL-C. CONCLUSIONS: The reduction in LDL cholesterol level achieved after counseling through intensive education is not superior to that achieved by conventional education. The oxidative modification of LDL-C is not directly effected by nutrition education. Intensive nutrition education can result in alterations of dietary fat intake. Dietary education can result in significant reductions in LDL-C, regardless of the method used.

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APPROVAL

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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GLOSSARY

Copper Mediated Oxidation	Isolated LDL oxidized in the presence of copper.
Hypercholesterolemia	High blood cholesterol
Hyperlipidemia	High lipid (fat) levels in the blood.
Initial Absorbance	The initial level of conjugated dienes or oxidation as assessed by the baseline copper absorbance of a sample subtracted from the uncatalyzed control sample
Lag Time	A phase of the LDL-oxidation process, where the oxidative modification is suppressed by endogenous antioxidants.
LDL oxidation	The oxidative modification of low-density lipoproteins.
Propagation Phase	The propagation phase of LDL oxidation begins after the endogenous antioxidants have been consumed.

ACRONYMS

AHA	American Heart Association
ATP III	Adult Treatment Panel III
CAD	Coronary Artery Disease
CVD	Cardiovascular Disease
HDL	High-density Lipoprotein
LDL	Low-density Lipoprotein
MUFA	Monounsaturated Fatty Acid
NCEP	National Cholesterol Education Program
NHLBI	National Heart Lung and Blood Institute
OxLDL	Oxidized Low-density Lipoprotein
PUFA	Polyunsaturated Fatty Acid
SF	Saturated Fat
TC	Total Cholesterol
TF	Total Fat
TG	Triglycerides
TLC	Therapeutic Lifestyle Changes
USPSTF	US Preventive Services Task Force

ABSTRACT

PURPOSE: To evaluate the effectiveness of two different approaches of dietary education to reduce blood lipids and oxidative stress. METHODS: Volunteers with moderately elevated low-density lipoprotein cholesterol (LDL-C) levels (100mg/dL-159mg/dL) were randomized into either intensive or conventional education groups. The intensive education group (n=12) attended 6 classes and received a nutrition education manual. The conventional group (n=11) received a nutrition education manual. Three-day weighed diet records, 3-day physical activity records, anthropometric measurements, blood pressure, plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and LDL-C were collected at baseline, 6-weeks and 12 weeks. Oxidized LDL-C was measured via copper-mediated oxidation. RESULTS: At 6-weeks, there was a significant (P=0.05) decrease in TC (-4.96%) and a trend (P=0.05) to decrease LDL-C (-10.37%) for the intensive group. At 12-weeks, there was a significant (P=0.05) decrease in LDL-C for the conventional (-9.55%) and intensive (-10.54%) groups. The intensive group had greater increases in polyunsaturated fat (4.58%E at baseline; 6.67%E at midstudy; 7.08%E at study-end, P<0.05) and greater reductions in saturated fat (9.08%E at baseline; 6.83%E at mid-study, P<0.01); however these reductions returned to baseline values at study-end (9.42±2.02 %E, P<0.05). Neither educational method resulted in significant oxidative modifications of LDL-C. CONCLUSIONS: The reduction in LDL cholesterol level achieved after counseling through intensive education is not superior to that achieved by conventional education. The oxidative modification of LDL-C is not directly effected by nutrition education. Intensive nutrition education can result in alterations of dietary fat intake. Dietary education can result in significant reductions in LDL-C, regardless of the method used.

CHAPTER 1

INTRODUCTION

The National Heart Lung and Blood Institute (NHLBI), American Heart Association (AHA), and other organizations have mounted a major effort to reduce risk factors for cardiovascular disease (CVD) in the United States. These risk reduction programs emphasize the importance of healthy eating habits, coupled with other healthful lifestyle behaviors, to reduce the risk of CVD. With an average of one death every 33 seconds from CVD in the United States, the increased need for continuing research on improved methods for risk reduction and active promotion of theses programs in community settings is exceedingly apparent (1).

The most important risk factors for atherosclerosis include smoking, hypertension, dyslipidemia (increased concentrations of low-density lipoproteins (LDL) and decreased concentrations of high-density lipoproteins [HDL]), diabetes, aging, and a family history of premature atherosclerosis (2). The U.S. Preventive Services Task Force (USPSTF) recommends intensive behavioral dietary counseling for adult patients with hyperlipidemia and other known risk factors for cardiovascular and diet-related chronic disease (3). The USPSTF recommends that intensive counseling be delivered by primary care clinicians or other specialists, such as nutritionists or dietitians (3).

Intensive nutrition education programs, defined as two or more contacts between advisor and patient per month (4), counseling, and behavioral interventions that reduce dietary fat and cholesterol intake can result in significant improvements in blood lipid levels, therefore reducing the development and progression of coronary artery disease

(CAD) (5). What is less clear is the effect of conventional dietary education, defined as less than one contact between patient and advisor per month (4), on blood lipids and hypercholesterolemia management. The National Cholesterol Education Program's (NCEP) Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults Adult Treatment Panel III (ATP III) (6) recommends dietary therapy as the first line of treatment for individuals with elevated LDL-C (7). ATP III recommends a LDL-C <160 mg/dL as the goal for persons with 0 or 1 CVD risk factor; the primary aim of therapy in this category is to reduce long-term risk. In individuals with a LDL-C between 100mg/dL and 160mg/dL, baseline blood lipids are assessed and persons are started on dietary therapy. The LDL-C response to a cholesterol-lowering diet has been well documented in inpatient and outpatient diet studies (8).

ATP III promotes diet and lifestyle changes as essential strategies for reducing risk for cardiovascular diseases (9). Dietitians are encouraged to adopt these recommendations in treating all patients with elevated blood cholesterol levels and apply their professional experience to the task of blending and tailoring the best dietary approaches for each patient (9). Evidence suggests that intervention programs, taught by dietitians and other health care professionals, which utilize strategies such as two-way-communication as the primary means of managing risk factors, provide a greater benefit to individuals when compared to one-way-communication strategies, such as printed educational materials (10). Two-way communication implies that the two parties in communication have a two-way flow of ideas and information. When communication

exists without the opportunity for the second party to respond or initiate, it is one-way communication (4). Additionally, patients who have received intensive nutrition education, with the objective of lowering serum blood lipids, have been documented as having gained significantly more nutrition knowledge, having greater perceptions of the efficacy of following a cholesterol-lowering diet, consuming a significantly lower percentage of fat, a higher percentage of carbohydrates, and less dietary cholesterol (11). These outcomes are particularly of interest because the objective of nutrition education is not simply to impart knowledge, but also to provide individuals with the knowledge to make wise food choices to aid in reducing serum blood lipids.

The conventional risk factors, such as dyslipidemia, hypertension, diabetes, and smoking, cannot account for all the cases of coronary artery disease. Thus, there are other novel risk factors that appear to be related to premature atherosclerosis. One plausible phenomenon may be the oxidative modification of LDL, rendering the molecule more atherogenic (12). As levels of LDL-C molecules become elevated in the circulation, the opportunity for oxidative modification increases. These modified molecules are then taken up by macrophages inside the arterial wall. It is these cholesterol-laden macrophages that form the start of atherosclerotic plaques (13). In addition, increased oxidation, specifically of the LDL-C molecules, may cause premature atherosclerosis in spite of a lipoprotein profile within reference values (2).

Diet may also play a prominent role in the oxidative modification of LDL-C. Dietary factors such as dietary fat and cholesterol can influence the fatty acid and antioxidant composition of LDL-C, which regulates LDLs susceptibility to oxidation

(14). A significant increase in the capacity of LDL-C to resist oxidation has been associated with behavioral interventions that reduce dietary fat and cholesterol intake and may be associated with a decrease in CAD risk (15).

Statement of Problem

Research comparing the effectiveness and practicality of intensive and conventional approaches to dietary counseling in the area of hyperlipidemia has been mixed (16). We performed a randomized controlled study employing the NCEP ATP III dietary guidelines in subjects with elevated LDL-C. The objective of the present study was to compare the effect, on serum blood lipids and the oxidative modification of LDL, of additional dietary counseling provided through six, one-hour nutrition education classes with conventional nutrition education provided through printed educational materials.

Hypothesis

We hypothesized that when compared to education provided through printed materials, intensive nutrition education, provided through nutrition education classes, would result in greater reductions in serum lipids, greater appropriate changes in macronutrient intake consistent with the NCEP ATP III recommendations, greater use of cardioprotective foods, more favorable awareness regarding appropriate nutrition and high blood cholesterol knowledge, and greater appropriate changes in LDLs susceptibility to *ex-vivo* oxidation:

$$H_O: \mu_{\text{post Intensive}} = \mu_{\text{post conventional}}$$

$$H_A: \mu_{\text{post Intensive}} > \mu_{\text{post Conventional}}$$

where H_A is equal to greater appropriate changes in blood lipids, markers of oxidative stress, use of cardioprotective foods, and nutrition and high blood cholesterol knowledge at study-end.

Delimitations

The inclusion of subjects for this investigation integrated specific fixed limits and boundaries. The first and most significant delimitation is the narrow scope of the inclusion criteria limiting the magnitude of the study population. Subjects were included based on the criteria that their pre-screening calculated LDL-C values were within the range of 100-159 mg/dL. Since any LDL-C above 100 mg/dL has been shown to be atherogenic and high LDL-C (≥160 mg/dL) is considered a potential target for LDL-C–lowering drug therapy, particularly in persons with multiple CHD risk factors we excluded individuals with a LDL-C < 100mg/dL and > 160mg/dL. This inclusion criterion excluded approximately 47% of the screened individuals. In addition, the scope of individuals that our subject recruitment was able to reach was limited to the Montana State University campus and the reading population of the local newspaper. The combined effects of these factors limited the sampling of subjects and ultimately limits the generalizablity of our findings

Limitations

There are limitations which create a potential weakness for this examination. First, although these analyses provide data on intake from foods, they cannot provide information on the specific behavioral changes associated with these reductions in intake. A three day weighed diet record can not accurately predict the specific changes in

behavioral data, (i.e. increased or reduced frequency of consumption and portion size, or substitution with a lower fat alternative) (8). Second, this was an intent-to-treat analysis. Intent to treat analysis is a broad strategy to assess randomized data; it requires that individuals remain in the group to which they were randomized regardless of compliance, crossover to other treatments or withdrawal. In addition this study did not adjust or control for compliance, weight loss, or life style changes beyond recorded dietary, blood lipid results and energy expenditure alterations. Subjects were free living and self selected their foods and represent a cross section of the general population that would be likely to select general clinical care for hyperlipidemia. These subjects received more dietary instructions and support than usually provided in a routine clinical care setting. Additionally, subjects included in the investigation were volunteers and may have had a greater vested interest in reducing blood lipids through behavior modification. Consequently, the amount of LDL-C lowering achieved may represent about the best that can be expected through community-based or intensive clinical programs. Finally, the intervention utilized in this study was relatively short term (6-weeks) and the subsequent follow-up may have not been long enough to detect changes in serum blood lipids in response to dietary modifications.

CHAPTER 2

REVIEW OF LITERATURE

New food-based dietary recommendations issued by the American Heart Association, to reduce risk for CVD, promote a multifaceted approach (17). The 2000 AHA dietary guidelines recommend a variety of foods to target four major goals: achieve a healthy overall diet, achieve a healthy weight, promote desirable lipid levels, and promote desirable blood pressure (18). Specific foods recommended include fruits and vegetables, grain products, fish, lean meat and poultry, fat-free or low-fat dairy products, and legumes. In addition, NCEPs ATP III therapeutic lifestyle changes (TLC) (Table 1) recommend restrictions of SF and dietary cholesterol and therapeutic dietary options to lower LDL-C: the inclusion of plant stanols/sterols (2-3 g/day), soy protein (25-40g/d) and increased viscous (soluble) fiber (5-10 g/day) (6). Additional cardioprotective foods include nuts and legumes. This chapter will review the scientific basis of cholesterol-lowering diets and strategies for their implementation.

The Cholesterol-Lowering Diet

Diet is associated with several common adult diseases in Western countries, including CVD, diabetes, and several cancers (19). The ATP III guidelines focus extensively on the nonpharmacologic therapy used to specifically prevent CVD for those individuals with abnormal blood lipids. ATP III redirects the public focus from the high-SF atherogenic diet, obesity, and a sedentary lifestyle to a program of therapeutic lifestyle changes. The TLC diet de-emphasizes total fat and focuses on the types of fat ingested. Initiating TLC begins with reducing intakes of SF and cholesterol to begin

Table 1. Dietary recommendations based on the ATP III TLC diet.

Nutrient	Recommended Intake
Saturated fat[1,2]	<7%
Polyunsaturated fat[2]	Up to 10%
Monounsaturated fat[2]	Up to 20%
Total fat[2]	25%–35%
Carbohydrate[2]	50%–60%
Fiber	20–30 g/d
Viscous (soluble) fiber	5-10g/d
Protein[2]	15%
Cholesterol	<200 mg/d
Plant stanols/sterols	2-3g/d
Soy protein	25–40 g/day when replacing animal food products

[1]Trans fatty acids also raise LDL-C and should be kept at a low intake.
[2]As percent of total calories
Note: Regarding total calories, balance energy intake and expenditure to maintain desirable body weight.

lowering LDL C. Subsequently, and additionally, is the emphasis of the health-promoting aspects of the diet that include, among other things, fish, omega-3 fatty acids, and the addition of viscous fiber and plant stanol/sterol esters to reduce LDL-C beyond that previously seen with the NCEPs previous recommendations, the Step I and II diets. At all stages of TLC, ATP III encourages the referral to registered dietitians or other qualified nutritionists for medical nutrition therapy. The ATP III guidelines can provide guidance to practitioners who wish to prevent and treat atherogenic progression, by lowering LDL-C levels, and improve the overall health of the patient.

The combined effects of the components in the cholesterol-lowering diet can result in substantial TC and LDL-C reductions. Jenkins et al (20) examined the

combined effects of the cholesterol-lowering dietary components on blood lipids in hypercholesterolemic subjects, consuming low-fat diets. Thirteen subjects (7 men 6 post menopausal women) with a baseline LDL-C of 174 ± 7.7mg/dL participated in a 6-week feeding trial which included the combined use of 1g of plant stanols/1,000kcal via enriched margarine, 8.2g of viscous soluble fiber/1,000kcal via oats, barley, and psyllium, and 22.7g of soy protein/1,000kcal via soymilk, soy sausage, soy cold cuts, and soy burgers. Data collection occurred at baseline while subjects were consuming a Step II diet, at weeks 2 and 4, during the 4-week combination diet, and after week-6, during which subjects resumed the Step II diet. The combination diet was compared to the AHA Step II diet. During the combination diet, no significant weight loss was observed. However, when the subjects resumed the Step II diet, there was a significant ($P<0.01$) – 0.20±0.05kg/week weight loss. This may be explained by the significant ($P<0.05$) decrease in energy intake when compared to the combination diet (1,999±118 kcal/d; 1,703±104 kcal/d, for the combination and Step II diets, respectively). Significant reductions were observed in blood lipids during the combination diet. At baseline TC levels were 249.8 ± 8.1mg/dL. After consuming the combination diet, TC was significantly reduced ($P<0.01$) by 22.3% ± 2.0% (193.7 ± 7.7mg/dL). At baseline LDL-C levels were 163.2 ± 4.3mg/dL. With the combination diet, LDL-C levels were significantly reduced ($P<0.01$) by 29.0% ± 2.7% (116.4 ± 6.6mg/dL). After the combination diet therapy phase, TC levels returned to 228 ± 8.5mg/dL while LDL-C levels increased to 146.9 ± 7.7mg/dL. The combined effects of the cholesterol-lowering dietary components, plant sterols, viscous fibers, and vegetable proteins, caused a

subsequent reduction in dietary cholesterol and SF and ultimately reduced TC and LDL-C levels.

Components of a Cholesterol-Lowering Diet

Dietary Fat The type of dietary fat can influence the development and progression of chronic diseases. Additionally, a significant relationship has been demonstrated between the predominant type of fat in the diet and the lipid-laden cellular membrane composition. The best understood risk factor for atherogenesis is serum cholesterol, which can be easily modified by alterations of dietary fatty acids (21).

Fats, such as SF, have been shown to be particularly harmful due to their role in the development of diseases, such as CVD (22, 23). Reductions of dietary fat are associated with changes in plasma TC and LDL-C (22, 23). The Delta-1 Study, (24) demonstrated that reductions in total fat and SF acids in the diet are accompanied with clinically important reductions in TC and LDL-C concentrations. The three diets were examined in this investigation; the AHA Step I diet (55% energy [E%]) from carbohydrate, 15E% protein, 30E% fat, with 9E%, 14E%, 7E% and <1.5E% from SF, MUFA, PUFA, and *trans* fatty acids, respectively), an Average American diet (AAD) (48E% carbohydrate, 15E% protein, 37E% fat, and 16E%, 14E%, 7E% and <1.5E% from SF, MUFA, PUFA, and *trans* fatty acids, respectively) and a low SF (Low-Sat) diet (59E% carbohydrate, 15E% protein, 26E% fat, with 5E%, 14E%, 7E% and <1.5E% from SF, MUFA, PUFA, and *trans* fatty acids, respectively). The Delta-1 Study utilized a randomized crossover design which included three feeding periods. Each subject was randomized to one of six diet sequences (ABC, ACB, BAC, BCA, CAB, or CBA) with

each diet period lasting 8 weeks, and included breaks of 4 to 6 weeks between diet periods. One hundred three healthy, normolipidemic volunteers (57 women, 46 men) were randomized to one of six diet sequences, separated by 4-6 weeks. All meals, except Saturday dinner, were prepared on site with two weekday meals consumed in a supervised cafeteria. The reduction in TC averaged a significant decrease ($P<0.01$) of 5% between the AAD and Step I diet (202.1 ± 2.8mg/dL to 191.0 ± 2.7mg/dL, respectively) and an additional 4% ($P<0.01$) during the Low-Sat diet (183.4 ± 2.7mg/dL). Similarly, LDL-C decreased significantly ($P<0.01$) by 7% between the AAD and the Step I diet (131.4 ± 2.7mg/dL to 122.2 ± 2.6mg/dL) with an additional stepwise reduction of 4% ($P<0.01$) with the Low-Sat diet (116.9 ± 2.6mg/dL). Between the AAD and the Step I diet, TG concentrations significantly increased ($P<0.01$) by 9% (85.1 ± 3.4 to 92.4 ± 3.7mg/dL), but did not change with the consumption of the Low-Sat diet. The reductions in total and LDL-C observed on the Low-Sat diet indicate that reducing total fat and SF, in a 6 week feeding period, can have a significant impact on lipoproteins in normolipidemic individuals. The TLC diet recommends 25-35%, or less, of the day's total calories come from fat; a range similar to the Step I and Low-Sat diets which produced a 7-11% reduction in LDL-C in the previous investigation. Given the beneficial effects of low total and low-SF diet on several biomarkers of CVD risk, it seems prudent to recommend inclusion of these new recommendations into healthy lifestyle and cholesterol-lowering diets, or as a start, in self-selected diets.

Saturated Fat The collective investigations of Ignatowski in 1908 and McGill in 1979 have led to the classic diet-heart hypothesis (25), which postulates the link between

the development of atherosclerosis and the dietary intake of SF and cholesterol (26, 27). Further examinations, such as the Keys Seven Countries correlations between international CHD mortality and SF intake (28) and the investigations of migrant workers adopting the Westernized lifestyles (29), have led to the overwhelming support of the diet-heart hypothesis.

There is a dose-response relationship between SFA and LDL-C, although the mechanisms, whereby saturated fatty acids (SFAs) raise LDL-C levels are not completely understood (30). The most logical evidence for this correlation (see Figure 1) is that SFAs interfere with the LDL-C receptors ability to clear LDL-C particles. Investigations tracking the clearance of the LDL apolipoprotein B-100 have determined that SFA impair the removal of LDL-C from circulation (31).

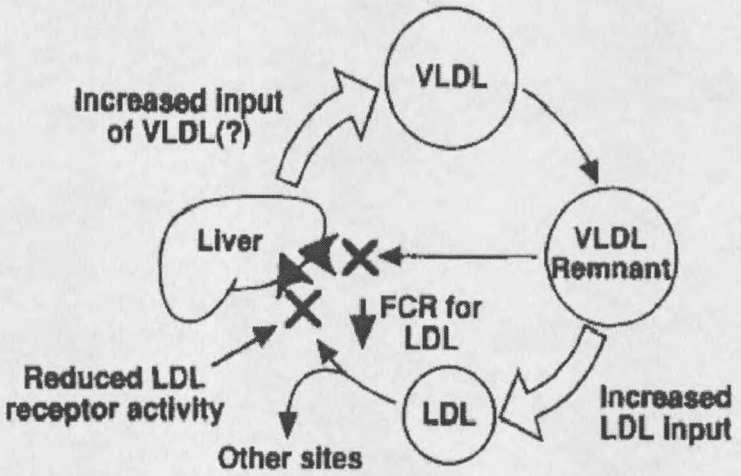

Figure 1. The mechanisms of increased LDL-C levels due to high intakes of SFAs. The major effect of SFAs appears to be a reduced activity of LDL receptors (Reprinted with permission(30)).

Interventions to avoid atherosclerosis might be more successful if launched early in life when eating and life-style patterns are formed. In the STRIP baby trial,

investigators found that decreasing intakes of SF in infants markedly influenced serum lipid values as early as 13 months. The STRIP baby trial was a randomized, prospective trial of more than 1000 healthy children. These children were regularly monitored for nutrient intakes, serum lipid values, growth, and development (32). One thousand sixty-two infants were randomized to intervention and control groups at 7 months of age. The families of the 540 intervention children were counseled to reduce the child's intake of SF and cholesterol and to ensure adequate energy intake. Five hundred twenty-two control children consumed an unrestricted diet. Food records were kept and serum lipids were measured at 5- to 12-month intervals. Intakes of SF and cholesterol were lower in the intervention children than in control children at 13, 24, and 36 months of age. Between 7 and 13 months serum cholesterol and non-high-density-lipoprotein cholesterol concentrations did not change significantly in the intervention group but increased significantly ($P < 0.001$) in the control group. When compared to the control group, the intervention group had lower daily intakes of energy (967.8 ± 189.5 kcal/d; 1040.5 ± 178.1 kcal/d, for the intervention and control groups, respectively, $P < 0.05$) and SF intake (9.3 ± 3.5g/d; 14.5 ± 4.8 g/d, for the intervention and control groups, respectively $P < 0.001$), and intake of polyunsaturated fat (PUFA) was higher (5.8 ± 2.2; 4.4 ±1.4 g/d, for the intervention and control groups, respectively $P < 0.001$). Thus, increases in serum cholesterol and non-HDL-C concentration that occur in infants between the ages of 7 and 13 months can be avoided by individualized diets which reduce SFA intake.

As a class, saturated fatty acids (SFA) pose a potential risk for increasing LDL-C. However, SFA at varying chain lengths, may not affect serum levels of TC and LDL-C

equally. In the prospective cohort Nurses' Health Study, 80,082 women, aged 34–59, completed validated food-frequency questionnaires in 1980. Women were free from known CVD, cancer, hypercholesterolemia, and diabetes (33). During the 14 year of follow-up, 939 documented incident cases of CVD events were reported. In multivariate analyses in which age, smoking, and other covariates were controlled for, intakes of short- to medium-chain SFA (4:0–10:0) were not significantly associated with the risk of CVD. In contrast, intakes of longer-chain SFA (lauric [12:0]; myristic [14:0]; palmitic [16:0]) were each separately associated with a small increase in risk. The multivariate relative risk (RR) for a 1% energy increase from the neutral stearic acid was 1.19 (95% CI: 1.02, 1.37). The ratio of PUFA to SFA was strongly and inversely associated with CHD risk (multivariate RR for a comparison of the highest with the lowest deciles: RR 0.58; 95% CI: 0.41, 0.83; P <0.0001) (33). These data suggest that replacement of long-chain SFA with PUFA will likely reduce the risk of CHD. Intake of the longer chain SFA, lauric, myristic, and palmitic have all been linked to a subsequent rise in LDL-C and TC (34, 22). With the aim of reducing LDL-C, NCEPs TLC diet recommends that all SFAs be reduced to $\leq$ 7% of total energy (6).

Polyunsaturated Fat Two types of PUFAs occur in the diet, linoleic acid (n-6), mainly from plant oils and animal fats, and linolenic acid (n-3) primarily from certain vegetable and fish oils. Controlled clinical trials indicate that the substitution of PUFA for SFA reduces the risk of CVD (30) (see Figure 2). Additionally, prospective and clinical trial data suggest that higher intakes of n-3 fatty acids reduce the risk for coronary events and mortality. The major n-3 fatty acids include: eicosapentaenoic acid

(EPA) and docosahexaenoic acid (DHA). In addition, human feeding trials with increased amounts of n-6 fatty acids in the diet have revealed the increased formation of eicosanoid products, specifically prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins, in larger quantities than those formed from n-3 fatty acids (35).

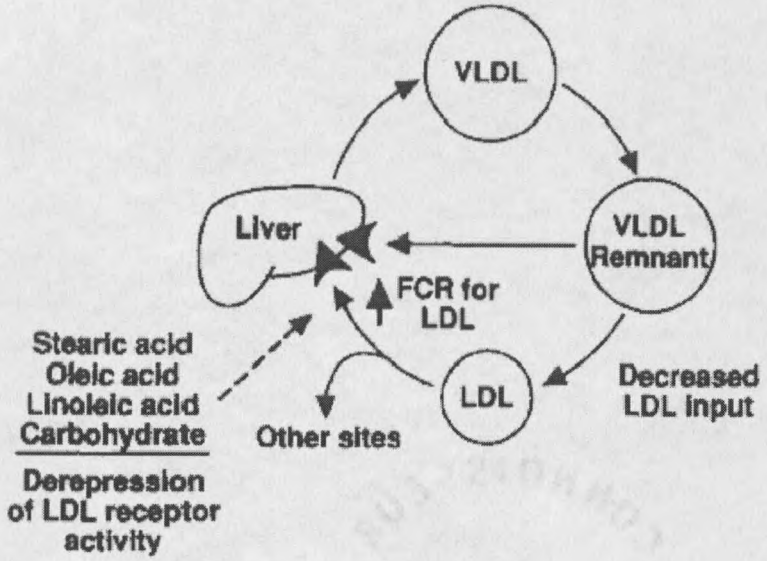

Figure 2. Alternative nutrients for cholesterol-raising SFAs. These include stearic acid, oleic acid, linoleic acid, and carbohydrates. When any of these nutrients replace SFAs in the diet, the level of serum LDL-C falls. The primary mechanism for LDL-C lowering for each nutrient apparently is a release of suppression of SFAs on LDL receptor activity (Reprinted with permission (30)).

The ATP III guidelines recommend the consumption of no more than 10% of total energy in the form of PUFAs. These recommendations are in agreement with Grundy (36) who reviewed the effects of various types of fatty acids on serum cholesterol concentrations. According to his research, linoleic acid (n-6) lowers total and LDL-C concentrations however in cases of PUFA intakes greater than 10%E there is an increased cancer risk as well as an increased susceptibility of LDL to oxidation. Grundy proposed

that diets rich in PUFAs, especially those high in linolenic acid, not make up more than 7% of total caloric intake to avoid complications associated with diets rich in PUFA.

Monounsaturated Fat Monounsaturated fatty acids (MUFA) lower LDL-C levels when compared to SF without reductions in HDL-C or increases in TG levels. In a randomized, double-blind, 5-period crossover study, the effects of a high-MUFA diet on serum lipids and lipoproteins were examined in 22 subjects (37). The study compared the CVD risk profile of an Average American Diet (AAD) (16%E SF, 11%E MUFA, 7%E PUFA) with a Step II diet (7%E SF, 12%E MUFA, 6%E PUFA), a Olive Oil (OO) diet (7%E SF, 21%E MUFA, 6%E PUFA), a Peanut Oil (PO) diet (7%E SF, 17%E MUFA, 9%E PUFA), and a Peanut Butter (PB) diet (8%E SF, 18%E MUFA, 10%E PUFA). The three high-MUFA diets lowered total cholesterol by 10% and LDL-C by 14%. The response was comparable with that observed for the Step II diet. Triglyceride concentrations were 13% lower in subjects consuming the high-MUFA diets and were 11% higher with the Step II diet than with the AAD. The high-MUFA diets did not lower HDL-C, whereas a 4% reduction was observed in the Step II diet compared to the AAD. The results of the present study provide evidence that higher-fat diets that are high in MUFAs and low in SFAs can lower total and LDL cholesterol to a greater degree when compared to an AAD and a Step II diet.

A high-MUFA diet can be an alternative to the recommendations of the Step I diet to favorably affect CVD risk provided it does not exceed the SF recommendations and individual energy needs (38). The high MUFA diet is most widely associated with the Mediterranean diet. In a follow-up trial of the Lyon Diet Heart Study, (39) consecutive

patients who survived a first myocardial infarction, MI, were randomized to either an experimental group (n=144) where they were asked to comply with a Mediterranean-type diet, or a control group (n=83) where they received no dietary advice from the investigators, but were advised to follow a sensible diet by their attending physicians. Eligible patients were <70 y, clinically stable, and had no medical or social conditions that would limit their ability to participate. The rate of cardiac death and nonfatal MI in the experimental group after 46 months (1.24:100 patients per year) is similar to that observed after 27 months (1.32:100). The rate in control subjects was 4.07:100 after 46 months, whereas it was 5.55:100 after 27 months. Following the 46-month investigation, all-cause and cardiovascular mortality, along with recurrent MI and cardiac death, were reduced (P=0.01) with the Mediterranean diet. This investigation confirms the long term protective effect of the Mediterranean diet. In response, to these findings, the ATP III guidelines recommend the use of unsaturated fatty acids, up to 20% of total energy, in the form of MUFAs.

Trans Fatty Acids Trans isomers of fatty acids are formed when liquid vegetable oils are partially hydrogenated to form margarine or shortening (40). The relationship between CVD and *trans* fatty acids has grown out of the finding that the straight configuration of the *trans* isomer is similar to that of SFs. In order to further investigate the relationship between *trans* fatty acid intake and CVD mortality, Willett et al studied the dietary data collected from the Nurses' Health Study (40). Among the 85,095 women followed for 8 years, there were 431 cases of CAD and the mean intake of *trans* fat was 4.0g. When adjusted for age and energy intake, the intake of *trans* fatty acids was

strongly associated with the risk of CAD; the multivariate RR for the highest (5.7g) versus the lowest quintile (2.4g) was 1.50 (95% CI; $P < 0.001$).

Mensink and Katan (41) determined that *trans* fatty acids increase plasma LDL-C levels when exchanged for *cis* unsaturated fatty acids in the diet. Subjects were randomly assigned to receive 10%E as oleic, SF, or *trans* fatty acids. The *trans* diet increased plasma LDL, TG, and lipoprotein (a), and additionally lowered HDL cholesterol levels. Replacement of SFAs and *trans* fatty acids with PUFA or MUFA can result in protective benefits. These strategies can be complementary and have the potential to provide the maximum chronic disease protective benefits of dietary fatty acids.

Dietary Cholesterol The major (physiological) effect of dietary cholesterol is to raise LDL-C levels (30). High intakes of dietary cholesterol can lead to elevated levels of circulating LDL by down-regulating the synthesis of the LDL-receptor (42, 43), and increase the size and composition of the LDL particle (44, 30) (See Figure 3). Investigations examining the response of lipoproteins to a high dietary cholesterol diet have shown mixed results. In the outpatient settings where subjects included eggs daily into their customary diets, no significant increase in mean serum cholesterol was found, nor was there a significant association of dietary cholesterol intake with either serum cholesterol or triglyceride (45, 46). In addition, Vorster et al (47) found that the consumpsion of 3-14 eggs/week consumed by 70 young healthy men, who followed a high SF diet, did not influence CVD risk markers. However, metabolic ward investigations have shown a significant relationship between the intake of dietary cholesterol and elevated LDL-C.

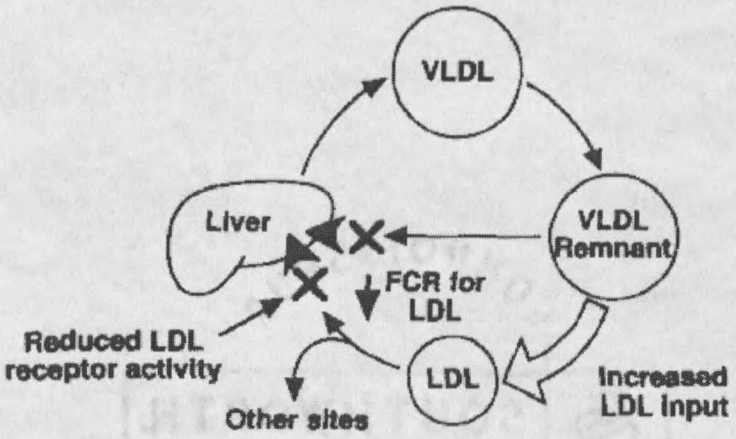

Figure 3. Mechanisms of increase in LDL-C levels with high dietary cholesterol intakes. An increase in hepatic cholesterol content, secondary to excess dietary cholesterol, suppresses synthesis of LDL receptors (Reprinted with permission (30)).

Zanni et al (48) examined 9 healthy women, aged 22-73, and their lipid response to diets with varying dietary cholesterol and SF contents. Four diets, with equivalent macronutrient compositions (14% protein, 31% fat, and 55% carbohydrate), were consumed for 15 days in duration, and were each separated by 3 weeks without diet control. The first diet (corn) was based on corn oil, had a PUFA to SF ratio (P/S) of 2.14, and contained 130 mg of cholesterol. The second diet (corn+) was identical to the first but contained a total of 875 mg of cholesterol. The third diet was based on lard, had a P/S ratio of 0.64, and contained 130 mg of cholesterol. Lastly, the fourth diet (lard+), was identical to the third, but contained 875 mg of cholesterol per day. The main sources of PUFA and SFs were corn oil and lard, respectively, and egg yolk was used for cholesterol supplementation. All diets affected both the number of lipoprotein particles as well as the composition of LDL and HDL. Compared to the corn diet, the high

cholesterol and high SF diet each increased the number of LDL particles by 17% and 9%, respectively, and the cholesterol per lipoprotein by 9%. The combination of SF and cholesterol (corn+) increased the LDL particle number by 18% and decreased particle size, to a small dense molecule, by 24%. Switching from a high SF, high cholesterol diet to a high SF, low cholesterol diet caused an 18% reduction in LDL-C (101.4 ± 32.9 mg/dl; 83.7 ± 30 mg/dl). Switching from a high SF, high cholesterol diet to a high PUFA, high cholesterol diet caused an 11% reduction in LDL-C (101.4 ± 32.9 mg/dl; 88.6 ± 26.1 mg/dl). Switching from a high SF, high cholesterol diet to a high PUFA, low cholesterol diet caused a 28% reduction in LDL-C (101.4 ± 32.9 mg/dl; 72.4 ± 27.2 mg/dl), indicating that a low SF, low cholesterol diet results in the greatest LDL-C lowering response. Therefore, it is reasonable to conclude that diets which utilize corn oil as a primary fat source, which are low in dietary cholesterol and SF, produce greater LDL-C lowering benefit when compared to high dietary cholesterol and high SF diets.

Plant Stanols and Plant Sterols The ATP III guidelines recommend dietary intakes of 2-3g/day of plant stanol or sterol derivatives to reduce LDL-C levels (6). Sterols are an essential component of cell membranes, and both animals and plants produce them (19). Stanols are saturated sterols and are found in less abundance in nature compared to the sterols. In 1954, Best et al (49) recognized the benefit of using plant sterols to lower serum concentrations of cholesterol. These reductions, observen with both plant sterol and stanols, occur by reducing the absorption of cholesterol from the gut by competing for the limited space for cholesterol in the micelle (50). In addition, adding plant stanol-fortified margarine to the diet results in a reduced absorption of cholesterol in the gut,

exogenous and endogenous, by about half (19). This reduced absorption results in lowered serum cholesterol levels. Clinical studies ranging 4-weeks to 1 year have demonstrated that replacement of butter and margarine with these sterol or stanol spreads can reduce LDL-C levels by approximately 15%-20% (51, 52, 53).

Hallikainen and Uusitupa (54) investigated the cholesterol-lowering effects of two forms of a plant stanol-enriched margarine. This parallel, double-blind feeding study involved 55 hypercholesterolemic subjects who were randomized after a 4-week high fat diet (baseline) to either wood stanol ester margarine (Benecol®), a vegetable stanol ester margarine (Take Control®), or a control margarine for 8-weeks. The daily mean intake of stanol esters was 2.31g and 2.16g for the wood and vegetable stanol margarines, respectively. After 8-weeks, TC was significantly reduced ($P<0.001$) 18.3% (253.29 ± 30.16mg/dL to 206.5 ± 29.39mg/dL) in the wood stanol ester group and 15.9% (237.05 ± 31.32mg/dL to 199.15 ± 30.16mg/dL) in the vegetable stanol ester group. Additionally, LDL-C was significantly reduced ($P<0.001$) 23.3% (175.56 ± 27.84mg/dL to 134.57 ± 29.78mg/dL) in the wood stanol ester group and there was a trend ($P=0.072$) for a reduction of 18.8% in the vegetable stanol ester group. These investigations offer insight into the use of plant stanol ester-enriched margarines to effectively lower TC and LDL-C in hypercholesterolemic subjects.

Fiber Fiber intake of 5-10g/day of viscous soluble fiber can reduce LDL-C levels by approximately 3-5% (6). Most viscous fibers (i.e. psyllium, oat bran, guar, and pectin) have hypocholesterolemic effects, decreasing LDL-C without a subsequent rise in TG (55). Decreased bile acid absorption, increased fecal bile acid losses, depleted bile salt

pools and the diversion of cholesterol synthesis to lipoprotein precursors likely are the mechanism for the hypocholesterolemic mechanism of fiber (55). In a meta-analysis, Brown et al. (56) examined 67 controlled trials to determine the effect of fiber intake on cholesterol levels. Approximately 2-10g/d of soluble fiber was associated with a 1.74 mg/dL reduction in TC and a 2.20 mg/dL reduction in LDL-C. Triglycerols and HDL-C were not significantly influenced by consuming soluble fiber.

There is debate as to the degree of cholesterol reduction caused by soluble fibers (56). Kelley et al (57) examined the subsequent reduction in TC and LDL-C after consuming 100 g of oat bran for 4 weeks. Thirteen hypercholesterolemic subjects (7 men, 6 postmenopausal women, TC>200 mg/dL) participating in an adult fitness program substituted their low-fat, low cholesterol diet with recipes containing oat bran. Blood samples were collected at baseline, 2-weeks, and 4-weeks. After 4-weeks, TC was significantly reduced (P <0.05) 8.2% compared to baseline (226.61±10.05 mg/dl to 208.04±9.28 mg/dl) and LDL-C was significantly reduced (P <0.05) 9.9% (155.45±9.67 mg/dl to 139.99±8.12mg/dL). Although this investigation utilized 100g/d of oat bran to achieve maximum reductions, Jenkins et al (20) determined that consuming no more than 8.2g/d of oat bran, used in combination with other soluble fibers, soy, and plant sterols, reduced TC by 22.3% and LDL-C 29.0%. Thus, daily intakes of 100g/d of oat bran produce a 10% reduction in LDL-C however, when used in combination with soy, plant sterols and other soluble fibers, only 8.2g of oat bran are needed to achieve and LDL-C reduction of 29%.

Vegetable and fruit fibers have been associated with a reduction in the incidence of CVD. Jenkins et al (58) examined the effects of a high fiber diet, predominantly from fruits and vegetables on lipoproteins. Ten healthy, normolipidemic volunteers were randomized to one of three diet sequences each two weeks in duration. A high fiber vegetable and nut diet (55g/1,000 kcal) was compared to a starch-based diet containing cereals and legumes and a therapeutic low-fat diet. The high fiber vegetable and nut diet produced the greatest reductions in LDL-C (33% ± 4%, P <0.001) and the greatest outputs of fecal bile acid (1.13± 0.03g/d, P =0.002). The lipid reductions occurred within one week of initiating the high fiber vegetable and nut diet and were sustained for the two-week duration of the diet.

Nuts Epidemiologic and clinical studies have shown that nut consumption is associated with favorable plasma lipid profiles and reduced cardiovascular risk (59). These effects may result from their high MUFA content, but nuts contain constituents, other than fatty acids, that might be cardioprotective. Jenkins et al (60) observed that 73 g of almonds, used as snacks in the diets of hyperlipidemic subjects, significantly reduce coronary heart disease risk factors: LDL-C (-9.4 ± 1.9%; P<0.001), the LDL:HDL cholesterol ratio (-12.0±2.1%; P<0.001), and oxidized LDL concentrations (-14.0 ± 3.8%; P<0.001). These reductions were probably in part due to the nonfat (protein and fiber) and MUFA components of the nut. Additional human feeding studies have demonstrated reductions of 8-12% in LDL-C when almonds and walnuts are substituted for more traditional fats (61).

The TLC diet recommends using nuts to meet the MUFA recommendation of up to 20% of total energy (6). The NCEP recommends that diets contain 1 oz of nuts per day to reduce the risk of heart disease. In addition, NCEP suggests that intake of nuts fit within the calorie and fat goal of the hypercholesterolemic patient. Based on the data from the Nurses' Health Study it is estimated that substitution of the fat from 1 ounce of nuts for equivalent energy from carbohydrate in a 2000 kcal diet was associated with a 30% reduction in CHD risk and the substitution of nut fat for SF was associated with 45% reduction in risk (62).

Animal versus Soy Protein Dietary protein, in general, has little effect on serum LDL-C levels or other lipoprotein fractions (6). However, substituting soy protein for animal protein has been reported to lower LDL-C (63). The ATP III panel recommends that total protein intake should not be excessive (average 50 to 100 g/d) and should be reasonably proportional (15% of calories per day) to carbohydrate (50-60% of calories per day) and fat (25-35% of calories per day) intake. Further, selected protein foods should not contribute excess total fat, SF, or cholesterol to the diet (6).

High intakes of soy protein can cause small reductions in LDL-C when it replaces animal protein. Soybeans are naturally high in a group of compounds collectively termed isoflavonic phytoesterogens or isoflavones (64). The three major isoflavones in soybeans are genistin, daidzin, and glycetin (65). Evidence for an independent effect of isoflavonoids on blood cholesterol concentrations has been demonstrated in humans. Findings are inconsistent regarding both the dose and potential benefit of soy protein. Cassidy et al (66) observed significant reductions in TC and LDL-C in young healthy

women with a 45mg dose of isoflavonoids, but did not observe a comparable response with 23mg of isoflavonoids. In contrast, investigations have indicated that 25 g/day of soy protein, in a diet low in SFAs and cholesterol, can have an LDL-C lowering effect of approximately 5% (63).

The mechanism for which soy produces a cholesterol-lowering response is mixed. Dietary soy may enhance bile acid secretion and indirectly cause a subsequent increase in rates of cholesterol excretion (67). In addition, isoflavones may reduce cholesterol via an estrogen-mediated mechanism and produce a similar cholesterol response as in females receiving estrogen replacement therapy (68).

Isoflavones have additionally been linked to reducing the oxidative modification of LDL-C by macrophages (69), enhance the resistance of LDL to modification (70), and increase the antioxidant pool, providing a longer phase of protection for the molecule (71). Feeding rats soy protein, compared with casein, has been reported to lower oxidative stress as measured by thiobarbituric acid-reactive substances (TBARS). Additionally, Kapiotis (69) has observed the ability of genistein to inhibit LDL oxidation *in vitro*.

Carbohydrates A central tenet of most dietary advice relating to lipids and cardiovascular disease risk for the past 40 years has been to reduce fat (and cholesterol) intake (72). However, diets that replace fat with carbohydrate, isocalorically, have been consistently observed to worsen certain elements of the plasma lipid profile (73). There is some concern in consuming high-carbohydrate (>55% of calories from carbohydrates), lower-fat diets. Reduction in total fat coupled with increased carbohydrate, intake can

result in a decrease in HDL-C (24). Ginsberg et al (24), found that patients with normo-triglyceride levels had an increase on the Step I and Low-SF diets compared to the AAD. These changes in plasma triglycerides correlated with the changes in HDL ($r = -.40$, $P < .001$). The mean HDL-C level fell further when patients were changed from the Step I diet to the Low-SF diet. This decrease is most likely due to the increase in carbohydrate intake from 55% to 69% of total calories.

The mechanisms underlying carbohydrate-induced hypertriglyceridemia remain unresolved (74). Hudgins et al. (75) measured the synthesis of new fatty acids (de-novo lipogenesis) by two independent methods in obese and lean volunteers given 10% fat and 75% carbohydrate versus 30% fat and 55% carbohydrate diets. Both diets were relatively high in simple sugars (sugar:starch ratio, 60%:40%). The study utilized a randomized, cross-over design. Serum triglycerides increased significantly ($P<0.001$) on the high-carbohydrate diet to similar degrees in both lean and obese subjects, and the change in triglycerides was highly correlated ($r=0.89$) with the stimulation of hepatic de-novo lipogenesis, as measured by indirect calorimetry. However, there were no relationships between body mass index (BMI), insulin levels, hepatic de-novo lipogenesis or plasma triglyceride concentrations. The authors concluded that high-carbohydrate/low-fat diets that are high in simple sugars stimulate fatty acid synthesis from carbohydrate feedings, and that plasma triglyceride levels increase in proportion to the amount of fatty acid synthesis suggesting a mechanistic link (76). In addition, a few reports suggest that high-carbohydrate diets interfere with the lipolysis of TG-rich lipoproteins, (i.e. the removal of fatty acids from VLDL) (See Figure 4) (30).

Bunyard et al. (77) had similar findings in an investigation of dietary factors associated with the decrease in HDL-C concentration in obese, postmenopausal women placed on a low-fat diet. Adherence to the AHA Step I diet reduced HDL-C (-16%±10%). The only dietary change that predicted decreases in HDL-C concentrations was the increase in the percent of energy from simple sugar ($r=-0.32$, $P<.05$). Therefore, a substitution of simple carbohydrates for dietary fat may lead to a reduction in HDL-C. Bunyard et al. (77) suggested placing emphasis on complex carbohydrates, such as fruit and whole grains, to produce a less drastic HDL-C decrease.

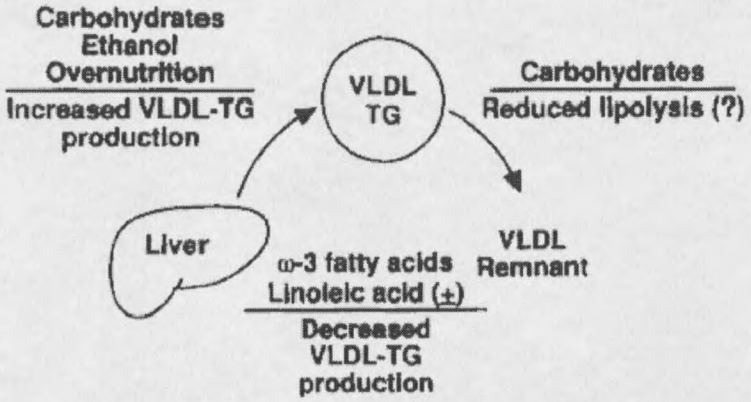

Figure 4. Actions of various nutrients on serum TG levels. The rise in TG on low-fat, high carbohydrate diets may be due, in part, to a reduced synthesis of lipoprotein lipase (Reprinted with permission (30).

Diet and LDL Oxidation

LDL modified by oxidation has been implicated in the development of atherosclerosis (78). Oxidized LDL (OxLDL) is taken up by special receptors that do not recognize unmodified LDL (78). Uptake by these receptors is not down-regulated by internal macrophage cholesterol content and leads to the production of lipid-laden foam

cells (79). The cytotoxicity of the OxLDL has been considered in the promotion of endothelial dysfunction, vascular smooth muscle cell growth and proliferation, and rapid evolution of the fatty streak into a more advanced lesion, therefore increasing the risk of developing CVD (80).

There are primarily four phases in LDL oxidation, *in vitro*. First, initiated by a reactive oxygen species, a hydrogen atom is extracted from a PUFAs double bond causing molecular rearrangement and congegated diene formation (2). Next, the lag phase is characterized by the presence of endogenous and exogenous antioxidants that suppress the oxidation process (2). The period of LDLs resistance to oxidative susceptibility, the lag time, has been inversely correlated with the severity of the coronary atherosclerosis (i.e., as the lag phase is prolonged, the severity of atherosclerosis is less). Third, in the propagation phase, another hydrogen atom is extracted from the PUFAs double bond forming lipid peroxides (2). Finally, in the decomposition phase, double bonds are cleaved forming aldehydes (2).

Dietary fats influence the fatty acid composition of LDL (81) which then regulates LDLs susceptibility to oxidation (82) (see Figure 5). To investigate the effects of long-term-well characterized dietary habits (a strict vegetarian diet, n=11; a high fish intake diet, n=9; and a high SF (milk fat) diet, controls, n=7) on the oxidative modification of LDL, Korpela, et al. (83) examined the LDL of 27 volunteers. The mean intake of SF of the control, vegetarian, and fish diets was 19 ± 1%E, 9±2%E, and 14 ± 2%E, respectively. Using sequential flotation ultracentrifugation, oxidation of LDL was carried out by using copper sulfate as a pro-oxidant. The lag phase of LDL oxidation was

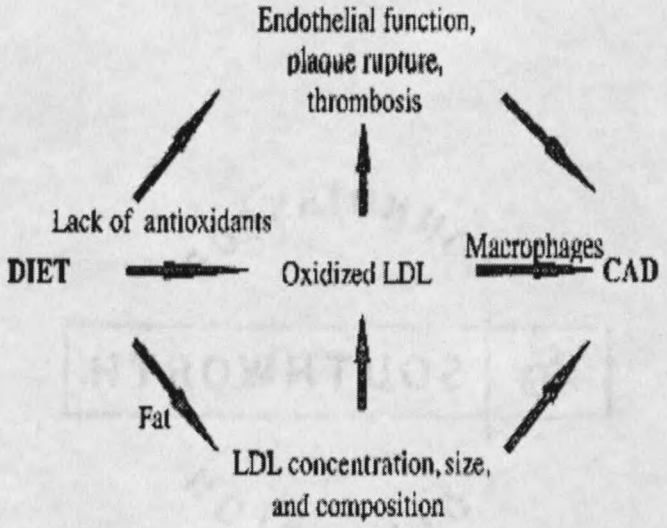

Figure 5. Hypothetical pathways from diet to CAD that involve LDL oxidative modification as an intermediate Step (84).

shortest (116±12min) in the fish group and longest (165± 23min) in the vegetarian group, and the control group was between the first and second groups (129±6min). The oxidation percentage, a measure of the final absorbance of CuSO4 when oxidation is stopped using ethylenediaminetetraacetic acid (EDTA) after 2.5 h, was highest (44%) in the fish group and lowest (22%) in the vegetarian group and intermediate (31%) in the control group. The proportions of EPA and DHA, within the LDL molecule, were highest in the fish and lowest in the vegetarian groups. Linoleic acid was highest among the vegetarians. In the fish group, the vitamin A concentration in serum was higher than in vegetarians and controls. Beta-carotene was lower than in the other groups, but in alpha-tocopherol, or lycopene concentrations there were no statistically significant differences. Long-term dietary habits can be useful in predicting the fatty acid composition of the LDL particle and can influence the susceptibility of LDL to oxidation.

In the fish group with the highest content of omega-3 fatty acids in LDL, the oxidation susceptibility of LDL was highest. In the vegetarian group with less omega-3 fatty acids in LDL, the LDL was more resistant to oxidation.

LDL particles rich in n-6 PUFAs have been shown to be more susceptible to oxidative modification than LDL particles enriched with MUFAs (78, 85, 86, 87). It is now generally accepted that the more PUFAs are present in the diet, the more readily LDL is to undergo oxidative modification (80, 85, 87). In general, a daily intake of PUFAs comprising more than 10% of total energy intake is not recommended (78). Moderate use of MUFA oils, canola oil and olive oil, decreases LDL-C levels and LDL oxidation and appears preferable to use of highly PUFA fats. In one study (88), olive oil decreased LDL oxidation and also decreased macrophage uptake of LDL-C.

Intensive atherosclerosis treatment programs have been shown by Parks et al. (89) to lower the oxidative modification of LDL-C in patients with CAD. This intensive treatment program consisted of exercise, stress management, and the consumption of a diet containing < 10% of total calories from fat. It is important to note the ATP III guidelines recommend an intake of 25-35% of calories from fat. A LDL-C goal was set at 105 mg/dL for the 25 participating subjects. There was no control group used for this study. Subjects were assessed for age, BMI, menopausal status, health history, medication and supplement use, and lipid panel was performed at baseline. The three month treatment program included: exercise at 60-70% of maximum heart rate for 20 min/d, 5 d/week, stress management education including mental imagery and relaxation techniques, and group meals 1 d/week. Subjects were instructed to give up all meat, fish,

chicken, oil, and dairy products and to remove all of these food items from their home. After three months, subjects were reassessed. At baseline and 3-months the following data was reported: carbohydrate intake 248 g/d to 337 g/d, fat intake 39 g/d to 16 g/d and 20.9%E to 8.2 %E for baseline and 3-months, respectively. Parks et al. (89) reported a significant 24% increase in mean lag time and a significant 29% reduction in propagation rate for Ox-LDL concluding that this diet of < 10%E from total fat poses a risk reduction for future CAD-related events. Thus, patients with CAD, who undergo atherosclerosis-reversal therapy, specifically dietary therapy, can reduce LDL oxidative susceptibility

Strategies for Implementing a Cholesterol-Lowering Diet

Individualized dietary therapy is the key to achieving life-long management of a cholesterol-lowering diet (90). In addition both health professionals and patients must be willing to make a long-term commitment to this endeavor. Permanent dietary changes require both time and effort. Whereas some patients are able to modify their food-consumption practices relatively quickly and easily, others may need 6-12 months or even longer (90).

There are two major objectives of patient education and counseling related to nutrition: improving health status and changing dietary behaviors. The primary therapeutic aim of the TLC diet is to reduce long-term risk. Initiation of TLC is recommended for those individuals with 0–1 risk factor whose LDL-C levels are ≥160 mg/dL (see Table 2). The NCEP ATP III guidelines recommend drug therapy when LDL-C levels are ≥190 mg/dL and is determined by clinical judgment at LDL-C levels of 160-189 mg/dL. The ATP III guidelines recommend that LDL-C levels should be

checked after patients have been on the TLC diet for 6 weeks. Referral to registered dietitians or other qualified nutritionists is encouraged during all stages of dietary therapy.

Despite the evidence that secondary prevention and behavior modification reduce mortality in patients with CAD, these types of programs and guidelines, such as ATP III, fail to be completely utilized in the clinical setting. Pearson et al. (91) found only 18% of patients with documented CAD reached the goals set forth by the NCEP Step II guidelines. Davidson (92) found <50% of patients with CAD reached the LDL-C goal of <100 mg/dL. Since LDL- cholesterol levels <100 mg/dL throughout life are associated with a very low risk for CHD in populations, they can be called *optimal* (6) (Table 3). Even when LDL-C concentrations are *near optimal* (100–129 mg/dL), atherogenesis occurs; hence, such levels must also be called *above optimal* (6). At levels that are *borderline high* (130–159 mg/dL), atherogenesis proceeds at a significant rate, whereas at levels that are *high* (160–189 mg/dL) and very high (190 mg/dL) it is markedly accelerated (6).

Counseling directed at behavioral and attitudinal change may produce greater changes than traditional educational approaches to health promotion (93). Complete behavior modifications have been shown to prevent the development and progression of CAD. In the 1980s and 1990s, the Lifestyle Heart Study investigated the idea that aggressive, comprehensive lifestyle changes could alter the clinical and angiographic progression of coronary disease (94) The original trial analyzed both lipid and quantitative angiographic parameters after a 12-month intervention period that included

Table 2. Schematic of risk-reduction therapy options.

ATP III: LDL-C Treatment Cutpoints for Therapy (6)		
Risk Category	Initiate TLC	Consider Drug Therapy
CAD and CAD risk equivalents*	≥100 mg/dL	≥130 mg/dL (100–129 mg/dL: drug optional)†
≥2 risk factors	≥130 mg/dL	10-year risk 10%–20%: ≥130 mg/dL 10-year risk <10%: ≥160 mg/dL
0–1 risk factor	≥160 mg/dL	≥190 mg/dL (160–189 mg/dL: LDL-C–lowering drug optional)

*CAD risk equivalents include: non-coronary forms of clinical atherosclerotic disease and diabetes.
†Some authorities use LDL-C–lowering drugs if TLC does not achieve LDL-C <100 mg/dL; others use drugs to modify HDL-C and TG.

intensive lifestyle changes involving diet (strict vegetarian, with 10% of calories obtained from fat), aerobic exercise, stress management, smoking cessation, and group psychosocial support (95). Comprehensive risk factor reduction resulted in a 37.2% reduction in LDL-C levels, which was associated with a decrease of 91% in the frequency of cardiac angina episodes. Although the study sample was small and the interventional strategy extremely rigorous, the Lifestyle Heart Trial clearly suggested that modifications of lifestyle alone, including diet, smoking, exercise, and stress exposure, in a highly motivated population may yield measurable benefits (95). Additionally, the Lifestyle Heart Trial noted a correlation with the degree of change in atherosclerosis and extent of lifestyle change, suggesting potential for slowing progression of atherosclerosis with modest changes and potential halting or reversal of coronary atherosclerosis through considerable lifestyle modification.

Table 3. Lipoprotein classifications based on the ATP III guidelines.

ATP III: Lipoprotein Classification (6)	
LDL-C (mg/dL)	
<100	Optimal
100–129	Above, near optimal
130–159	Borderline high
160–189	High
≥190	Very high
HDL C (mg/dL)	
<40	Low
≥60	High
TC (mg/dL)	
<200	Desirable

TC= total cholesterol, HDL-C= high density lipoprotein cholesterol, TG= triglyceride, LDL-C= low density lipoprotein cholesterol.

In order to examine the dose response effect of intensive dietary intervention on blood lipids, Walden et al. (96) implemented a six month dietary trial for hypercholesterolemic men and women utilizing the NCEP Step II diet (<30% of calories from total fat and <7% from SF). The beFIT trial, a randomized diet therapy trial, included 409 free-living subjects randomized to either receive immediate counseling (n=217) or delayed counseling (n=192). Dietary counseling consisted of eight weekly educational classes designed to achieve a NCEP Step II diet. The immediate group began classes promptly following inclusion, while the delayed group began approximately six months later. All subjects were screened for blood lipids, anthropometrics, medical history, and dietary analysis, utilizing a 4-day diet record, at 1, 2, 3, and 6-months following initiation of the dietary counseling. Hypercholesterolemia was defined as elevated LDL-C at two screening evaluations, while combined hyperlipidemia was defined as a triglyceride level at or above the age and sex-specific 75th percentile value at

one or both evaluations. Following 6-months of dietary intervention, women and men in both the hypercholesterolemia and combined hyperlipidemia groups significantly lowered ($P<.001$) their intakes of: calories, total, saturated, MUFA, and PUFA fats (% kcal), and dietary cholesterol. After 6 months of dietary intervention, TC and LDL-C were significantly lower in all groups ($P<.001$). The LDL-C reduction was the least in hypercholesterolemic women, intermediate in combined hyperlipidemic women and men, and greatest in hypercholesterolemic men. The amount of change was not different for total or LDL-C when compared between women and men with the same lipid disorder, between women with different lipid disorders, or between men with different lipid disorders. HDL cholesterol was significantly decreased ($P<.001$) in hypercholesterolemic and combined hyperlipidemic women. The reduction was significantly different ($P<.001$) for hypercholesterolemic women, -4.3 mg/dL (-6.4%), compared with both hypercholesterolemic men, -1.0 mg/dL (-1.3%), and combined hyperlipidemic men, -1.4 mg/dL (-2.7%). After 6 months of diet, >50% of each group had LDL-C values in the desirable range based on the NCEP goals (LDL<100mg/dL) and LDL-C reductions of 7.6% to 8.8% after 6 months of dietary intervention were statistically significant. This investigation illustrated teaching the NCEP diet to free-living subjects as a viable strategy for dietary intervention. Additionally, the investigators established that a 6-month dietary intervention could significantly decrease fat and cholesterol intakes along with LDL-C levels. The results of this intervention did not differ among gender, with the exception of HDL, or classification of hypercholesterolemia.

Fonarow et al. (97) developed the Cardiac Hospitalization Atherosclerosis Program (CHAMP) to be implemented before patient discharge. This program development came after a departmental assessment found a lack of physician adherence to national guidelines. This program initiated drug therapy, dietary therapy, and exercise programs in patients with established CAD, and performed an outcome assessment after a two year follow up. After two years, the use of statins increased from 6% to 86% (P <0.01) and there was a significant (P <0.01) increase in patients achieving a LDL-C ≤ 100 mg/dL (6% versus 58%) (97).

In another investigation, hyperlipidemic patients were followed for six months either by a cardiac rehabilitation nurse, or by the patients' cardiologist. Senaratne et al. (98) concluded that a planned strategy of management for hyperlipidemia with a cardiac rehabilitation nurse assigned to obtain and follow lipid profiles and initiate therapy is more effective in controlling hyperlipidemia than leaving the management to the cardiologist. Patients TC, LDL-C, and the percent of patients on drug therapy were significantly better in the intervention group (P= 0.01).

Intervention provided by specialized cardiac case managers can significantly increase physicians' adherence to the NCEP ATP III guidelines. Lacy et al. (99) investigated the percentage of patients with a LDL-C ≥ 130 mg/dL who were admitted to the hospital with diagnosed CAD. With the initiation of the intervention, the use of drug therapy increased from 17% pre-intervention to 82% during intervention (P<0.01).

All health professionals should play a key role in developing and implementing nutrition care plans for patients who require dietary therapy for elevated LDL-C levels.

Specifically, registered dietitians (RDs) must assume a major role in implementing the ATP III guidelines. The role of the RD in LDL-lowing therapy includes: performing an in depth assessment of current diet and dietary habits, developing an individualized and intensive care plan, teaching patients the principles of the ATP III diet, closely monitoring the effectiveness of the dietary therapy plan, and serving as a resource to patients regarding implementation of the therapeutic diet (100). The ATP III guidelines recommend that at any and every stage of dietary therapy, effective dietary modification will be facilitated by consultation with a registered dietitian or other qualified nutritionist for medical nutrition therapy (6). Medical nutrition therapy involves the assessment of nutritional status and the assignment of diet, counseling, and/or specialized nutrition therapies to treat an illness or condition (101).

Patients who adhere to medical nutrition therapy and adopt other appropriate components of lifestyle change may prevent or delay the need for pharmacotherapy (102). The management of hyperlipidemia requires collaborative efforts between health care professionals from multiples disciplines, including dietetics professionals. This multidisciplinary approach can help patients achieve optimum LDL-C-lowering through a combination of medical nutrition therapy and other components of lifestyle change.

CHAPTER 3

METHODS

Subjects and Design

A total sample population (n=51) age 20 years and older was drawn from the Montana State University-Bozeman campus and the Bozeman, Montana community for initial screening. All subjects read and signed an Institutional Review Board approved informed consent document (APPENDIX B). Subjects were interviewed by means of a structured questionnaire (APPENDIX C) for demographic data, history of chronic diseases, lifestyle habits, use of medications, and family history. In addition, all subjects were screened for fasting plasma concentrations of TC, HDL-C, TG and LDL-C.

Inclusion criteria conformed to the NCEP criteria for dietary treatment: LDL-C levels between 100mg/dL and 159mg/dL (6). For this particular range of LDL-C, ATP III recommends assessment of baseline blood lipids and the initiation of dietary therapy. Since any LDL-C above 100 mg/dL appears to be atherogenic and high LDL-C ($\geq$160 mg/dL) is considered a potential target for LDL-C–lowering drug therapy, particularly in persons with multiple CHD risk factors we excluded individuals with a LDL-C < 100mg/dL and > 160mg/dL. Dietary therapy appears to have the greatest impact on LDL-C when baseline values fall between 100mg/dL and 159mg/dL (6).

Potential subjects were excluded based on the following criteria: a diagnosis of normolipidemia upon pre-screening as defined by a plasma LDL < 100mg/dL; or a diagnosis of hyperlipidemia upon pre-screening as defined by a plasma LDL $\geq$

160mg/dL; a serum TG level > 400mg/dL; self reported CVD or any unstable cardiovascular condition; uncontrolled hypertension determined by a blood pressure >140/90 uncontrolled with medication; self-reported diagnosis of diabetes or insulin resistance; pregnancy within the previous 12 months; chronic renal or liver disease; and treatment of hyperlipidemia with lipid-lowering drugs.

Female participants taking estrogen, in the form of birth control and hormone replacement therapy (HRT) were included in the pre-screening process. Currently, the data regarding the effects of estrogen on the oxidation of LDL-C are mixed (103). Santanam et al (104) acknowledged that estradiol, in the form of HRT does not inhibit the oxidation of LDL by copper. Bureau et al (105) established HRT's potential to reduce LDL-C but determined no affect on oxidizability or oxidation of LDL.

All participants were allowed to continue complimentary and alternative supplement use such as antioxidants, multivitamins, functional foods, and other forms of non-pharmaceutical therapy. Based on a similar preliminary study in our lab, a very high proportion (65%) of older women interested in research study participation were currently taking some form complementary or alternative medicine. This proportion may even be an underestimate as woman initially taking these supplements were not included in the test population.

Research Protocol

Subjects who met the inclusion criteria (n=24) were assembled at an orientation class, during which the program director provided information regarding the study and guidance on performing a 3-day weighed diet record and a Bouchard (106) physical

activity record. At this session, the subjects were randomly assigned using a random number generator (Random Number Generator Pro®, Issaquah, WA) to receive counseling by means of intensive cholesterol education (N=12) or conventional cholesterol education (N=12)

All participants completed 3-day weighed diet (APPENDIX G) and physical activity records (APPENDIX H) including two regular weekdays and one weekend day. After completion of the 3-day records, all subjects were scheduled for a pre-intervention screening session during which the program director reevaluated the subject's risk factors, performed a brief physical examination of height, weight, and blood pressure, and performed a pre-intervention fasted blood collection. Each participant was tested for baseline knowledge using the Cholesterol IQ Quiz (APPENDIX I) (107).

The conventional group received a cholesterol education manual (APPENDIX J) which included general information about hypercholesterolemia, guidelines for healthy nutrition, as well as some specific dietary recommendations and recipes obtained from the AHA and the NCEPs ATP III guidelines. This group was instructed to read the manual at a self guided, self paced rate. No further education was provided for this group.

The intensive group attended 6-one hour cholesterol education sessions (defined as "intervention phase") designed to simplify the dietary modifications conforming to the NCEP ATP III diet and provide more specific dietary recommendations. These counseling sessions were executed by the program director, nutrition graduate students, and dietitians specializing in nutrition education. Emphasis was placed on awareness of

the cholesterol-heart disease connection, fats and oils, reading nutrition fact labels, heart healthy shopping, portion size control, incorporating soy foods, adding plant stanols or sterol spreads, choosing low-fat options, increasing soluble fiber intake, selecting lean meats, poultry and fish, and increasing the intake of fruits and vegetables. In addition, the intensive group received the cholesterol education manual.

Post-intervention and follow-up measurements were performed in both groups. Fasted blood samples were collected at 6 weeks (defined as "post-intervention") and 12 weeks ("follow-up"). In addition, all subjects completed a 3-day weighed diet and physical activity record, a medical status questionnaire (APPENDIX E, F), a Cholesterol IQ Test, and a brief physical examination of height, weight, and blood pressure.

The education provided through the 6-week educational classes and the cholesterol education manual employed the principles of Evidence-based Medicine. Evidence-based Medicine involves integrating multiple clinical expertises with the best available external clinical evidence from systematic research (108). The one-way and two-way communication education models were developed through a conscientious, explicit and judicious use of current research, specifically utilizing the NCEP ATP III guidelines (6).

Laboratory Analysis

Blood samples were collected after an overnight fast of at least 12 hours from the anicubital vein into one 6-mL heparin and two 6-mL K_3-EDTA Vacutainer® collection tubes (Greiner Vacuette Evacuated Blood Collection Tubes, Fisher Scientific, Pittsburgh,

PA) using standard venipuncture techniques. Samples were separated within 10 minutes of collection using a refrigerated 21000R Marathon centrifuge (Fisher Scientific, Pittsburgh, PA) at 2500 (g force) and 16°C for 10 minutes. All samples were then placed into 1.5-mL microcentrifuge tubes (Phoenix Research Products Hayward, CA) using standard pipeting techniques, and stored at –80 °C until analysis. Samples obtained for LDL oxidation were stored with a 20μL solution prepared from 1.8g sucrose (Sigma-Aldrich, St. Louis, MO) dissolved in 500μL of deionized water. Kleinveld et al (109) reported storage of plasma samples by this method reduced the incidence of oxidizibility. For each analysis, all samples for an individual were analyzed in the same assay run.

Plasma TC, HDL-C, and TG concentrations were measured by standard laboratory techniques using a Kodak Ektachem analyzer (Vitros DT60, Rochester, NY) and the procedure described by Lie et al (110). The LDL-C was calculated as follows: (LDL-C) = (TC) – [HDL-C + (TG/5)], as described by Friedewald et al (111). Fasting plasma glucose was determined, to later observe subjects for metabolic syndrome. Each sample was run in duplicate, from heparinized plasma using a Sigma-Aldrich glucose enzymatic assay, (product number 115A-IKT), (Sigma-Aldrich, St. Louis, MO) modified for a 96-well plate analysis. Analyses were rerun when the coefficient of variance (CV) exceeded 10%. The average CV for a single analytical intrassay run for glucose was 5.4%, while the between-run interassay was 3.2%.

LDL-Oxidation The following procedure is currently used to perform the kinetic analysis of lipid peroxidation in the Montana State University Nutrition Research Laboratory. Due to the lower densities of plasma lipoproteins, relative to the other

plasma proteins, sequential flotation ultracentrifugation has been the principle method used for their isolation and classification (112). Thawed plasma samples for baseline, post-intervention and follow-up visits were isolated via sequential density ultracentrifugation with sodium chloride (.09% and 16.7%, respectively) (112). To adjust the density of the plasma to 1.006 g/mL, 0.5 mL of a 0.9% NaCl solution was combined with 0.5 mL of plasma and transferred to a 1-mL thick-walled, polyallomer tube (Beckman Centrifuge Tubes) allowing plasma and saline to mix. The plasma-0.9% NaCl mixture was then placed in a benchtop ultracentrifuge (Optima TLX) and a fixed-angle rotor (TLA 120.2) (Beckman Instruments, Palo Alto, CA) at 16 °C for 2.5 hours at 10,000 RPM.

After the initial ultracentrifugation of the plasma, the tubes were transected at 0.5 mL from the base using a graduated slicer (Beckman Instruments, Palo Alto, CA). The upper VLDL contents were discarded leaving 0.5mL of the HDL/LDL sample. The remaining solution in the lower section was transferred via a 9" glass Pasteur pipette (Fisher Scientific, Pittsburgh, PA) to a new polyallomer tube. The final volume and density of the sample was adjusted to 1.063 g/mL using 500μL of a 16.7% NaCl solution. Subsequent to centrifugation at 16 °C for 2.5 hours at 10,000 RPM, each tube was transected 0.05mL from its base leaving the upper 0.05 ml of the isolated LDL contents for collection.

Following isolation, the isolated LDL was desalted and made EDTA free by gel-filtration in Econo-Pac 10DG columns (Bio-Rad, Richmond, CA) with a sodium phosphate buffer solution using the method of Puhl et al (113).

Protein concentrations of the LDL filtrate recovered from the column were then determined using a Pierce BCA Protein Assay Kit (Pierce, Rockford, IL) and the methods of Smith et al (114) modified for measurement using a μQuant Universal microplate spectrophotometer (Bio-Tek Instruments, Inc. Winooski, VT.). The protein concentration was used to normalize LDL-C concentrations to 0.10 mg protein/ml for oxidation assay.

Oxidation was determined as the production of hydroperoxides with conjugated double bonds (conjugated dienes) by continuously monitoring the change in absorbance at 234 nm as described by Esterbauer et al. (115). The LDL preparations were diluted with PBS to contain 0.10 mg protein/ml. Oxidation was started by adding 10 μl of Cupric Chloride Dihydrate solution ($CuSO_4$) to 1.0 ml of the adjusted LDL solution into the wells of a Costar 96 well UV flat bottom plate. All samples were prepared in duplicate. For these measurements, we used a μQuant Universal microplate spectrophotometer (Bio-Tek Instruments, Inc. Winooski, VT.), kept at 37°C. The absorbance was automatically recorded at 10-minute intervals for 8 hours. The spectrophotometer was connected to a computer for data collection and analysis. Several measurements were obtained from the absorbance versus time curves. The resulting diene verses time profile provided lag phase, propagation rate, and maximum diene concentration for each subject.

To minimize the effect of method variation on the results, all samples from an individual study subject were analyzed simultaneously. In addition, one control sample was always processed with the subject samples. The control sample was drawn from one healthy subject, treated and stored identically to the subjects. The average CV for a

single analytical intrassay run for OxLDL was 4.3%, while the between-run interassay was 5.5%.

Dietary Analysis

The evaluation of the baseline, post-intervention, and follow-up dietary intake was performed using the average daily intake of the individual nutrients from the 3-day dietary records of all subjects. Nutrient composition of daily intake was computed using the computer program, Nutritionist Pro™ Version 1.2 (First DataBank Inc., San Bruno, California).

Physical Activity Analysis

The Bouchard Three-Day Activity Record (106) was utilized to determine average daily energy expenditure at baseline, post-intervention, and follow-up. Subjects recorded their physical activity intensity from the one of nine intensity categories every 15 minutes on a data form. Each category is assigned an energy expenditure value per kg/15 minutes from which daily energy expenditure for physical activity was calculated. The three days of activity monitored included 2 regular weekdays and 1 weekend day. In a reliability study, an interclass coefficient yielded 0.96 (106).

Anthropometrics

Subjects were assessed for height and weight for calculation body mass index (BMI). Body weight was measured on a balance beam scale, with shoes and bulky clothing removed. The scale was zeroed each time before use by putting the beam weight on zero and assessing the beam scale balance. Height was measured using a stadiometer. Subjects were asked to remove shoes, stand with heels together, with back straight, and

heels, buttocks, shoulders and head touching the back of the stadiometer. BMI was calculated using the Quetelet index or kg/m^2 (116).

Blood pressure

Blood pressure was measured using a sphygmomanometer and a stethoscope. All subjects were fitted with the proper cuff size and assessed on the left arm at all three visits unless extenuating circumstances arose.

Statistical Methods

All data were analyzed using SPSS version 10.0 (SPSS Inc., Chicago, Illinois). A two-way between-subjects repeated measures analysis of variance was used to determine significance using education group assignment as the independent between-subjects variable, the time points as the within-subjects variable, and the demographic, diet composition, blood lipid and LDL oxidation measurements as the dependent variables. The main effect (education and time) and interaction between the factors (education x time) were tested using the multivariate criterion of Wilks's lambda (λ), where 1=the strongest possible relation and 0= no relation between a repeated measure and the dependent variable. Post hoc paired *t*-tests were used following a significant time main effect. To ensure that the overall risk remained 0.05 the post hoc analyses were controlled for Type I errors across the three tests at the 0.05 level using the Holm's sequential Bonferroni procedure by using the equation α/n, where α is equal to 0.05 and n is equal to the number of comparisons. In addition, post hoc paired *t*-tests were used following a significant education x time interaction. Post hoc independent sample *t*-tests were used following a significant group interaction. A tetrad comparison, a comparison

involving four means, was employed using the independent samples *t*-tests following a significant group interaction to determine the mean difference between education groups over the three time points. This was accomplished by subtracting the mean values of the intensive group from the mean values of the conventional group, and then comparing the mean differences for each time point. Treatment effects were considered significant at a value of $P<0.05$. A Wilcoxon test was utilized to compare differences in subject awareness between groups at baseline and follow-up. The computation of the value of the Wilcoxon test involves a) computing the difference scores, b) ranking the absolute values of the difference scores, and then c) finding the mean rank for all the cases with negative difference scores and the mean rank for all cases with positive difference scores.

In order to provide and unbiased assessment of this investigation's efficacy (118), an intention-to-treat analysis was strictly applied. Subjects remained in their given groups and data from subjects who withdrew from the investigation were included in the statistical analysis. In addition, missing data was appropriately handled. Missing data was handled via a method termed user-missing values. User-missing values are numeric values which are defined as missing for the SPSS statistical package. The number 9,999 was chosen to stand for missing values, since that value did not already exists in the data set. User-missing values were utilized for unattainable lipoprotein measures and values beyond 2 standard deviations.

CHAPTER 4

RESULTS

Fifty-one subjects were screened, and 24 subjects (47%) were excluded for a lipoprotein profile that did not meet the inclusion criteria, one subject was excluded for the use of contraindicated medications, and one subject was excluded for pregnancy. One subject, randomized to the conventional group, withdrew prior to baseline measurements. The following analyses include 23 subjects with completed lipid and dietary data at baseline, post-intervention and follow-up.

Baseline Subject Characteristics

Subjects randomized to the intensive (n=12) and conventional (n=11) groups were similar in age, anthropometric measures, diastolic BP, and demographic characteristics (Table 4). At baseline, systolic BP differed significantly ($P<0.05$) between intensive (123.17 ± 10.43 mm/Hg) and conventional (111.27 ± 13.92 mm/Hg) groups. The majority of subjects were white ($n=22$), none of the subjects were current smokers and only one subject was a former smoker. Approximately 74% ($n=14$) of the women were postmenopausal. Of those postmenopausal women, four were using HRT. Approximately 22% ($n=5$) of all subjects self-reported a family history of early CVD, defined as a cardiovascular event in a primary relative before the age of 55 for men and 65 for women. Roughly 52% ($n=12$) of all subjects self-reported a family history of diabetes mellitus (see Table 4).

Table 4. Baseline subject characteristics, medical history, medication and supplement use data.

Variable	Intensive Group (n = 12)		Conventional Group (n = 11)	
Age (years)	53.67 ± 11.76		51.09 ± 4.28	
Body mass index (kg/m²)	24.66 ± 2.89		24.85 ± 3.83	
Systolic BP (mm/Hg)	123.17 ±10.43		111.27 ± 13.92*	
Diastolic BP (mm/Hg)	76.67 ± 5.21		72.36 ± 8.57	
	n	%	*n*	%
Male sex	3	25	3	27
Postmenopausal women	7	58	7	58
Family history of CAD	8	68	8	73
Family history of early CAD	1	8	4	36
Family history of Diabetes Mellitus	5	42	7	64
Medication and supplement use				
Vitamin supplements	10	83	5	45
Aspirin	2	17	2	18
Estrogen therapy/HRT	2	17	2	18

Values =mean ± standard deviation. *P<0.05.

Supplement and Medication Use

Fifteen of the 23 subjects used some form of vitamin supplement before entering the program. For these subjects vitamin usage remained constant throughout the 12-week study period, with the exception of one subject from the intensive group who discontinued the use of fish oil supplements and one subject from the conventional group who began using fish oil supplements. Specifically, 11 of the 23 subjects took a multivitamin supplement, 7 used a daily calcium supplement, 4 used a daily vitamin C supplement, 4 subjects used vitamin E, 3 used a B-complex vitamin, 2 used glucosamine, 1 subject used garlic, and 1 subject used gingko biloba supplements (see Table 4). In addition to dietary supplements, subjects were allowed to continue the use of

medications. Subjects self reported the use of aspirin (n=3), antidepressants (n=4), proton pump inhibitors (n=2), antihypertensive medications (n=1), nonsteroidal antiinflammatory agents (n=1), antiepilepsy medications (n=1), antihistaminic medications (n=1), osteoporosis prevention medications (n=1), and antihypothyroidism medication (n=1) (Table 4).

Dietary Intake

The two groups reported similar energy expenditure and dietary macronutrient intakes of calories: total fat, approximately 30%E and SF, 9% kcal for the intensive and conventional groups; dietary cholesterol, 169 and 253 mg/d and fiber, 21 and 23 g/d for the intensive and conventional group, respectively (see Table 5). At pre-intervention, alpha linolenic acid (n-3) intake was the only baseline dietary observation which displayed a trend (P= 0.09) for significant difference in the intensive group as compared to the conventional group (0.42 ± 0.31 g; 0.73 ± 0.51 g, respectively).

Blood Lipid, Glucose, and Oxidation

Prior to intervention, subjects randomized to the intensive and conventional groups were similar in blood lipid values and LDL oxidation characteristics (see Table 6). At baseline, plasma glucose concentrations differed significantly (P<0.05) between intensive (99.12 ± 15.97 mg/dL) and conventional (114.96 ± 14.52 mg/dL) groups. Additionally, at baseline, there was a trend (P= 0.08) for a significant difference for propagation rate values between intensive (8.46E-03 ± 2.08E-03 abs/min) and conventional (1.05E-02 ± 3.17E-3 abs/min) groups (see Table 7).

Table 5. Comparison of energy expenditure and nutrient intake at baseline between treatment groups.

Characteristic	Intensive Group (n = 12)	Conventional Group (n = 11)	F^2	P Value
Energy expenditure:				
Kcal	3185.58±720.99	3073.92±755.95	0.52	0.34
Dietary intake:				
Kcal	2027.66±612.83	2240.10±682.64	0.50	0.44
Carbohydrate[1]	53.25 ± 12.73	49.73 ± 7.67	1.85	0.44
Protein[1]	17.33 ± 3.50	15.27 ± 4.05	0.45	0.21
Total Fat[1]	29.92 ± 8.14	30.45 ± 5.42	1.42	0.86
Saturated Fat[1]	9.08 ± 2.97	8.73 ± 2.72	0.01	0.77
PUFA[1]	4.58 ± 2.07	5.45 ± 2.54	0.46	0.38
n-6(g)	4.13 ± 3.19	7.76 ± 6.73	2.45	0.11
n-3(g)	0.42 ± 0.31	0.73 ± 30.51	1.98	0.09[a]
MUFA[1]	8.33 ± 2.93	9.82 ± 2.48	1.06	0.21
n-9(g)	10.50 ± 6.43	14.68 ± 6.03	0.03	0.12
Cholesterol (mg)	168.81 ± 113.02	252.59 ± 148.17	0.54	0.14
Fiber (g)	21.36 ± 9.22	23.06 ± 14.50	0.07	0.74

Values =mean ± standard deviation. *P<0.05, [a] P=0.09 between groups.
Kcal=kilocalories, MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acids.
[1] Percent of total Kcal, [2] F statistic for group differences from analysis of variance

Table 6. Comparison of blood lipids, and glucose at baseline between treatment groups.

Characteristic	Intensive Group (n = 12)	Conventional Group (n = 11)	F^2	P Value
Blood Lipids:				
TC[1]	233.58 ± 33.21	252.40 ± 30.41	0.54	0.17
LDL-C[1]	149.87 ± 38.26	161.58 ± 22.23	0.87	0.71
HDL-C[1]	62.75 ± 19.62	62.00 ± 14.64	0.61	0.64
TG[1]	104.83 ± 40.87	132.09 ± 61.17	2.19	0.33
Glucose[1]	99.12 ± 15.97	114.96 ± 14.52	0.17	0.02*

Values =mean ± standard deviation. *P<0.05, [a] P=0.09 compared to Intensive group.
TC= total cholesterol, HDL-C= high density lipoprotein cholesterol, TG= triglyceride, LDL-C= low density lipoprotein cholesterol.
[1] = mg/dL, [2] F statistic for group differences from analysis of variance

Table 7. Comparison of LDLs susceptibility to *ex-vivo* oxidation at baseline between treatment groups.

Characteristic	Intensive Group (n = 12)	Conventional Group (n = 11)	F^2	P Value
Lag time (min)	62.91 ± 14.47	65.48 ± 14.75	0.01	0.68
Propagation rate[1]	8.46E-03 ± 2.08E-03	1.05E-02 ± 3.17E-03	0.74	0.08 [a]
Initial absorbance[1]	1.27E-01 ± 8.80E-02	1.23E-01 ± 6.84E-02	0.01	0.90

Values =mean ± standard deviation. *P<0.05, [a] P=0.08 between groups.
[1] = abs/min, [2] F statistic for group differences from analysis of variance

Analysis of Variance Results

Weight and BMI

Weight (kg) and BMI (kg/m²) were compared using a repeated measures ANOVA on both factors (educational method, time points) to determine the effect of the educational approaches throughout the 12-week assessments. The multivariate tests for weight and BMI indicated no significant differences between the main effects (education and time) or in the interaction between the factors (education and time) (see Table 8).

Blood Lipids and Glucose

A repeated measures ANOVA was performed on both factors (educational method, time points) to determine the effect of the educational approaches on blood lipids and plasma glucose throughout the 12-week assessments. No significant differences between the main effects (education and time) or the interaction between the factors (education and time) existed for TG or glucose. A significant group main effect was determined for TC, a trend for a significant interaction effect was confirmed for HDL-C, and a trend for a significant time effect was established for LDL-C. Figures 6, 7, 8, and 9 depict the

change in blood lipid values represented as a percent between baseline and post-intervention, post-intervention and study-end for each group.

Total Cholesterol The test of education main effect for TC indicated a significant group effect ($F_{2, 20} = 6.29$, $P<0.05$) (see Table 8). To evaluate the differences between the two education methods for each time point an independent samples *t*-test was conducted. For TC, the post-intervention comparison demonstrated a significant ($P<0.05$) difference between the intensive and conventional education groups (222.00±22.83mg/dl versus 252.27±31.89mg/dL, respectively) ($F= 0.80$, $t_{11}= -2.64$) (see Table 8).

To examine the change in TC values through out the investigation the values were examined as a percent change from each time point. The intensive group reduced mean TC values 4.96% at post intervention compared to baseline (see Figure 6). At the

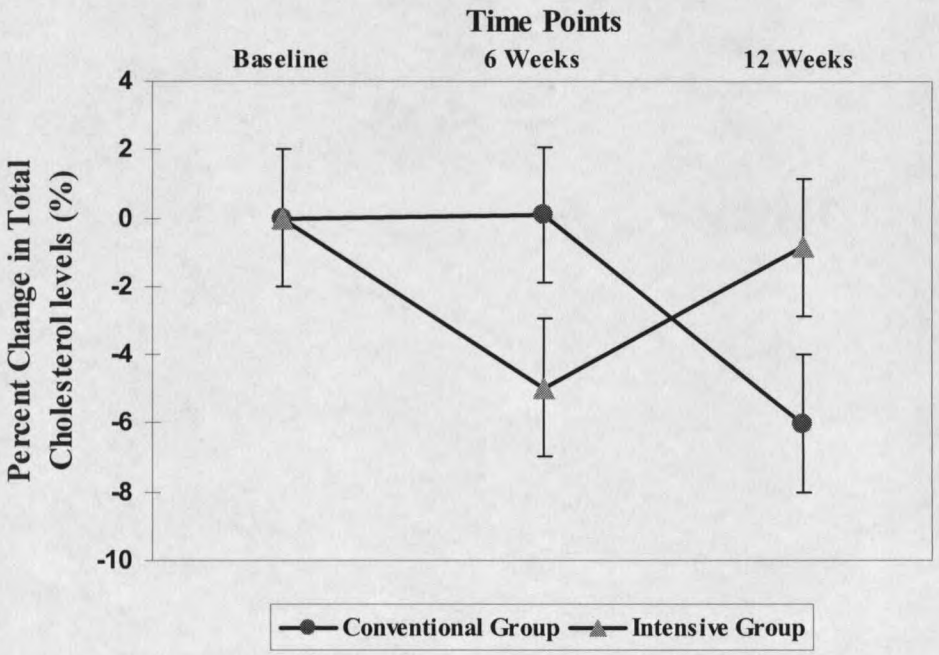

Figure 6. The change in TC, as a percent, between treatment groups.

follow-up analysis, TC was reduced by an additional 0.86%. These findings were not consistent with a Step-wise reduction but rather, continuance of the estimated 5% reduction seen in TC after the intervention. In contrast, the conventional group did not experience a considerable change in TC at post-intervention (0.09%). Although, at follow-up the conventional group saw a reduction of 6.02% in TC; this was an approximate 2% larger reduction when compared to the intensive group at post-intervention.

High-density Lipoprotein Cholesterol The multivariate tests for HDL indicated a trend for a significant interaction effect ($F_{2, 20}$= 2.98, P=0.08, APPENDIX K). In order to evaluate the trend for the significant interaction (education type and time) effect, a tetrad comparison was performed. Differences in mean HDL values between the two education groups displayed a trend for a significant difference between post-intervention and follow-up, (t_{10} = 2.22, P=0.05). Although the differences in mean HDL-C values were lower for the intensive education group at baseline, the difference decreased at post-intervention (see Table 8). At follow-up there was a trend for a significant difference in HDL-C concentrations in the conventional group.

To examine the change in HDL-C values throughout the investigation, the values were examined as a percent change from each time point (see Figure 7). In the intensive group, HDL was reduced 0.94% at post intervention compared to baseline. At the follow-up analysis, HDL was reduced by 1.41%. These findings are consistent with a negative step-wise reduction although this reduction was quite small. In contrast, the conventional group experienced a considerable negative change in HDL at post-

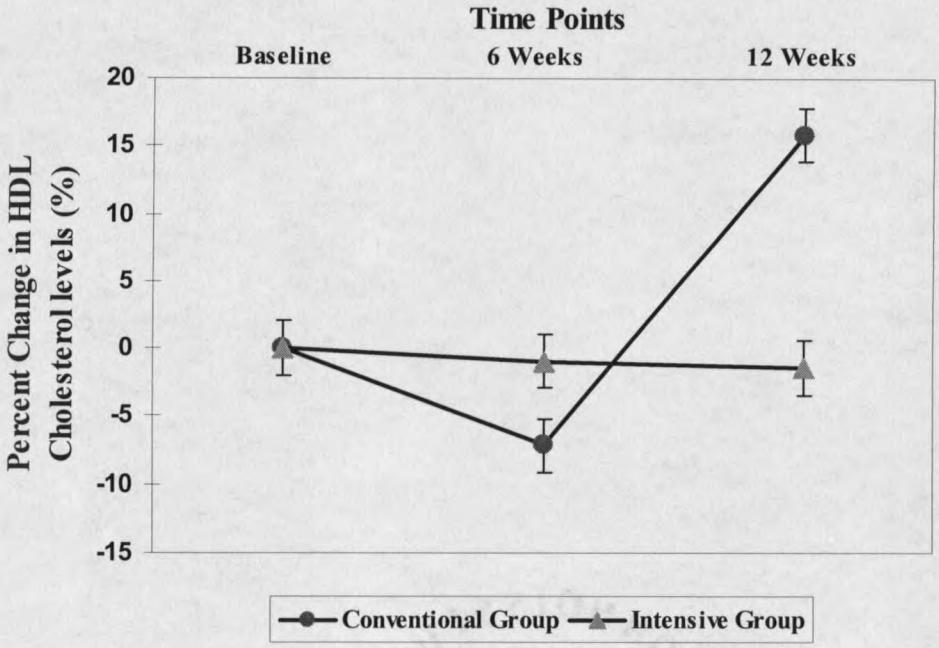

Figure 7. The change in HDL-C, as a percent, between treatment groups.

intervention (-7.19%). Although, at follow-up, the conventional group saw a increase of 15.82% in HDL, a return to baseline followed by an additional ≈ 7.6% increase when compared to the intensive group at post-intervention.

Triglycerides For the intensive group, TG was reduced -14.60% at post intervention compared to baseline. At the follow-up analysis, TG values increased 14.88%, re-establishing baseline values. In contrast, the conventional group did not experience a considerable change in TG at post-intervention (-0.62%). Although, at follow-up, the conventional group saw a reduction of -10.80% in TG; this was greater reduction when compared to the intensive group, whose values returned to baseline at follow-up.

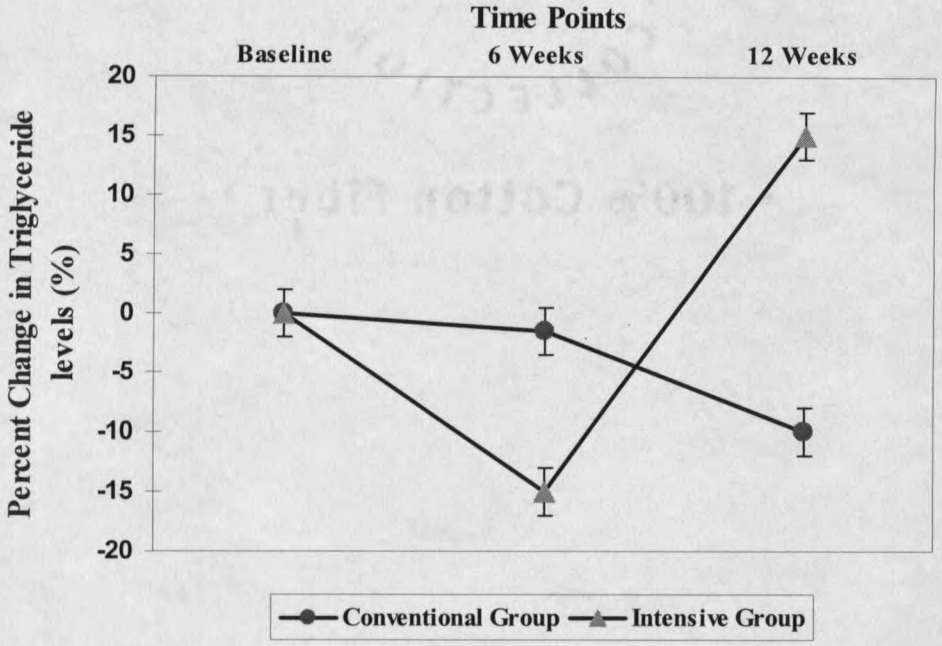

Figure 8. The change in TG, as a percent, between treatment groups.

Low-density Lipoprotein Cholesterol The multivariate tests indicated a trend for a significant time main effect for LDL ($F_{2, 20} = 3.18$, P=0.06). A post hoc paired-samples t-test was performed to determine at which time point the significance was revealed. The analysis identified a significant (P<0.05) pair-wise comparison among the means for post-intervention and follow-up LDL ($t_{11} = 2.36$), and a trend (P=0.05) for significance between the means for baseline and follow-up LDL, ($t_{10} = 2.18$) (see Table 8).

To examine the change in lipid values through out the investigation, the values were examined as a percent change from each time point (see Figure 9). For the intensive group, LDL was reduced 10.37% at post intervention compared to baseline. At the follow-up analysis, LDL was reduced by 0.18%. These findings were not consistent with a Step-wise reduction but rather, continuance of the estimated 10% reduction seen in

Table 8. Comparison of weight, BMI, blood lipids, and glucose between treatment groups.

Measure	Baseline	Post-intervention	Follow-up	*F*-statistic[3]
N				
Intensive	12	12	12	
Conventional	11	11	11	
Weight(kg)				
	72.96±13.31	72.63±12.49	72.69±13.39	0.13
	71.12±14.64	70.64±14.15	70.35±14.58	
BMI(kg/m²)				
	24.66±2.89	24.58±2.72	24.58±2.99	0.01
	24.85±3.83	24.68±3.63	24.58±3.89	
TC[1]				
Intensive	233.58±33.21	222.00±22.83	220.08±25.58	6.30
Conventional	252.05±28.87	252.27±31.89*[e]	237.09±37.07	
HDL-C[1]				
Intensive	58.73±14.49	58.18±11.55	57.36±16.67†[d]	0.46
Conventional	62.00±14.64	57.45±13.82	66.64±16.00†[d]	
TG[1]				
Intensive[2]	110.18±38.21	94.09±38.34	108.09±51.48	1.30
Conventional	132.09±61.17	131.27±77.20	117.09±63.45	
LDL-C[1]				
Intensive[2]	159.15±21.77	142.64±27.39†[a]	142.38±24.18*[b]	2.20
Conventional	162.57±21.35	168.56±32.32†[a]	147.04±33.75*[b]	
Glucose[1]				
Intensive	99.12±15.97	101.73±17.88	103.10±20.15	1.48
Conventional	114.96±14.52*	104.07±10.46	101.68±16.01	

Values =mean ± standard deviation. *P<0.05, ** P<0.01, [a] Time effect compared to baseline, [b] Time effect compared to post-intervention, [c] Education x Time interaction compared to baseline, [d] Education x Time interaction compared to post-intervention, [e] Education effect compared to Intensive group. †P=0.05.

TC= total cholesterol, HDL-C= high density lipoprotein cholesterol, TG= triglyceride, LDL-C= low density lipoprotein cholesterol.

[1] = mg/dL, [2] = Values for LDL and HDL at baseline for the Intensive group were determined using n=11, [3] F statistic for group differences from analysis of variance.

LDL after the intervention. In contrast, the conventional group did not experience a considerable change in TC at post-intervention, and in fact experienced a 3.68% increase.

Although, at follow-up, the conventional group saw a reduction of -12.77% in LDL; this was an approximate 2% larger reduction when compared to the intensive group at post-intervention.

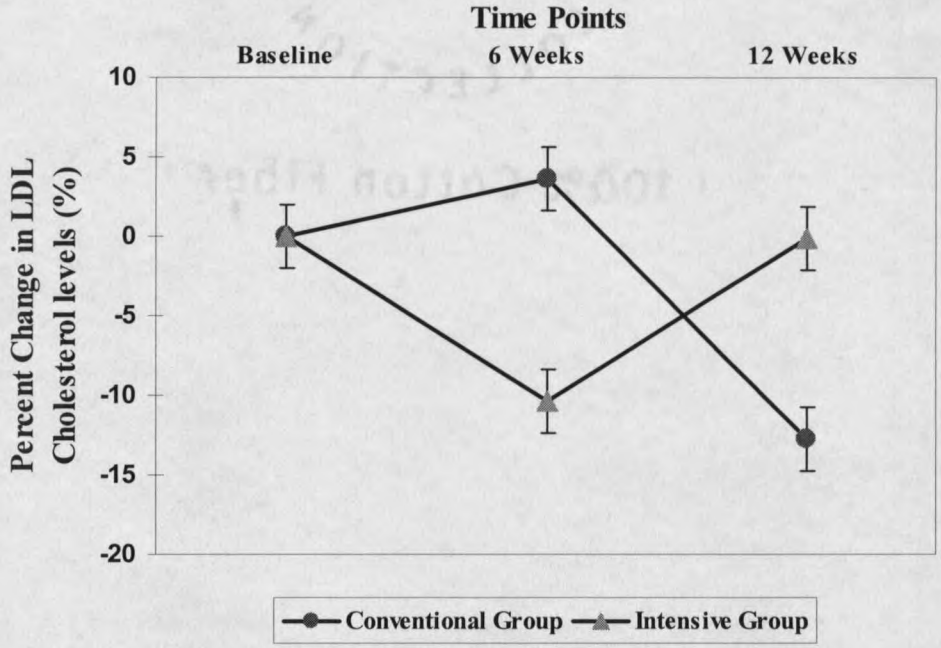

Figure 9. The change in LDL-C, as a percent, between treatment groups.

Dietary Intake

To compare the effect of the nutrition education on total daily energy expenditure and intake and macronutrient intake, at baseline, post-intervention, and follow-up between groups, a two-way between subjects ANOVA was performed (Table 9). No significant differences exist between the main effects (education and time) or in the interaction between the factors (education and time) for energy expenditure and the dietary parameters for energy intake from protein, carbohydrate, MUFA dietary cholesterol, dietary fiber, and vitamin C and E. Significant results were seen in the

dietary parameters for energy intake, total fat, SF, oleic acid, PUFA, linoleic acid and alpha linolenic acid.

Energy Intake The multivariate tests indicated a significant time main effect for energy intake ($F_{2, 20} = 4.24$, $P<0.05$). A post hoc paired-samples *t*-test was performed to determine at which time point the significance was revealed. The analysis identified a significant ($P<0.05$) pair-wise comparison among the means for baseline and post-intervention for energy intake ($t_{11}= 2.48$) (see Table 9).

Total Fat The multivariate tests indicated a significant education x time interaction effect for percent of total energy from fat intake ($F_{2, 20} = 4.20$, $P<0.05$, APPENDIX K). In order to evaluate the significant education x time interaction effect, a tetrad comparison was performed to evaluate whether the mean difference between the two educational methods for total energy from fat intake were the same between any two time periods. Differences in mean total energy from fat intake between the two education groups displayed a significant difference between post-intervention and follow-up ($t_{11}= -3.46$, $P<0.01$).

Table 9 shows that although the difference in mean total energy from fat intake was lower for the intensive education group at baseline and at post-intervention, there was a significant difference at follow-up with the intensive method of education resulting in a greater total energy from fat intake.

Saturated Fat The multivariate tests indicated a significant time main effect for percent of total energy from SF intake ($F_{2, 20} = 3.84$, $P<0.05$, APPENDIX K), and a

Table 9. Comparison of energy expenditure and nutrient intake between treatment groups based on 3-day physical activity and weighted diet records.

Diet	Baseline	Post-intervention	Follow-up	*F*-statistic[3]
N				
Intensive	12	12	12	
Conventional	11	11	11	
Energy Expenditure[1]				
Intensive	3185.58±720.99	3073.92±755.95	2945.25±802.52	2.76
Conventional	2889.73±818.36	2742.27±663.01	2890.55±802.87	
Energy Intake[1]				
Intensive	2027.66±612.83	1846.48±616.95*[a]	1950.94±888.64	0.01
Conventional	2240.10±682.64	1732.52±668.56*[a]	1930.25±699.53	
Protein[2]				
Intensive	17.33±3.50	18.33±2.74	18.08±3.66	1.56
Conventional	15.27±4.05	16.18±4.47	16.73±5.92	
Carbohydrate[2]				
Intensive	53.25±12.73	53.17±9.52	47.00±12.08	0.01
Conventional	49.73±7.67	51.27±8.25	51.45±10.74	
Total Fat[2]				
Intensive	29.92±8.14	28.17±7.48	32.67±5.40**[d]	0.00
Conventional	30.45±5.43	31.36±8.24	29.00±7.39**[d]	
Saturated Fat[2]				
Intensive	9.08±2.97	6.83±2.25**[c]	9.42±2.02*[b] *[d]	0.28
Conventional	8.73±2.72	9.09±2.91**[c]	9.09±4.23*[b] *[d]	
MUFA[2]				
Intensive	8.33±2.93	8.75±2.70	9.75±3.47	0.01
Conventional	9.82±2.48	8.91±3.91	8.36±3.04	
PUFA[2]				
Intensive	4.58±2.07	6.67±2.67*[a]	7.08±1.88*[b] *[c] †[d]	0.08
Conventional	5.45±2.54	7.00±3.44*[a]	5.18±2.14*[b] *[c] †[d]	
Cholesterol(mg)				
Intensive	168.81±113.02	163.96±96.77	211.64±175.25	1.22
Conventional	252.59±148.17	221.91±132.15	205.61±118.24	
Dietary Fiber(g)				
Intensive	21.35±9.22	28.32±13.34	21.92±8.69	0.15
Conventional	23.06±14.50	21.25±12.77	21.94±14.22	

Values =mean ± standard deviation. *$P<0.05$, ** $P<0.01$, [a] Time effect compared to baseline, [b] Time effect compared to post-intervention, [c] Education x Time interaction compared to baseline, [d] Education x Time interaction compared to post-intervention, [e] Education effect compared to Intensive group.
†$P=0.07$
Kcal=kilocalories, MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acids.
[1]= Kcal, [2]= Percent of total Kcal, [3] *F* statistic for group differences from analysis of variance.

Table 10. Measures of Antioxidant Vitamins C and E Intake between treatment groups.

Diet	Baseline	Post-intervention	Follow-up	*F*-statistic[1]
N				
Intensive	12	12	12	
Conventional	11	11	11	
Vitamin C				
(mg)	142.65±116.13	151.46±57.87	107.40±54.28	2.55
Intensive	128.65±68.30	108.01±70.37	132.81±88.65	
Conventional				
Vitamin E				
(mg)	7.94±5.98	8.04±5.19	6.56±6.73	0.84
Intensive	5.65±3.89	7.18±25.74	8.36±8.12	
Conventional				

Values =mean ± standard deviation.
[1] *F* statistic for group differences from analysis of variance.

significant education x time interaction effect for percent of total energy from SF intake ($F_{2,20} = 5.12$, $P<0.05$). A paired-samples *t*-test was performed to determine at which time point the significance was revealed. The analysis identified a significant ($P<0.05$) pair-wise comparison among the means for post-intervention and follow-up SF intake ($t_{11}= -2.29$). In order to evaluate the significant education x time interaction effect, a tetrad comparison was performed to evaluate whether the mean difference between the two educational methods for percent of total energy from SF intake were the same between any two time periods. Differences in mean percent of total energy from SF intake between the two education groups displayed a significant difference between baseline and post-intervention ($t_{11} = -3.24$, $P<0.01$), and a significant difference between post-intervention and follow up ($t_{11} = -2.64$, $P<0.05$). Table 9 shows that although the difference in mean percent of total energy from SF intake was lower for the conventional education group at baseline, the difference reversed significantly at post-intervention and

total energy from SF intake was lower in the intensive education group. This change again reversed itself at follow- up with the intensive method of education resulting in a greater percent of total energy from SF intake.

Oleic Fatty Acid As shown in Table 11, the multivariate tests indicated a trend for a significant education x time interaction effect for the intake from oleic acid ($F_{2, 20}$ = 3.32, P=0.06). In order to evaluate the significant education x time interaction effect, a tetrad comparison was performed to evaluate whether the mean difference between the two educational methods for oleic acid intake were the same between any two time periods. Differences in mean percent of oleic acid intake between the two education groups displayed a significant difference between baseline and follow-up (t_{11} = -2.77, P<0.05).

Polyunsaturated Fat The multivariate tests indicated a significant time main effect for percent of total energy from PUFA intake ($F_{2, 20}$ = 4.48, P<0.05), and a significant education x time interaction effect for percent of total energy from PUFA intake ($F_{2, 20}$ = 4.73, P<0.01, APPENDIX K). A paired-samples t-test was performed to determine at which time point the significance was revealed. The analysis identified a significant (P<0.05) pair-wise comparison among the means for baseline and post-intervention and additionally, baseline and follow-up PUFA intake (t_{11}= -2.86 and t_{11}= -2.48, respectively)

In order to evaluate the significant education x time interaction effect, a tetrad comparison was performed to evaluate whether the mean difference between the two educational methods for percent of total energy from PUFA intake were the same between any two time periods. Differences in mean percent of total energy from PUFA