AN ABSTRACT OF THE THESIS OF

Ye-Sun Lee for the degree of Doctor of Philosophy in Nutrition and Food Management presented on September 21, 1999. Title: The Effect of Supplementation with n-9, n-6, and n-3 Fatty Acids on Plasma Lipid, Lipoprotein, Apolipoprotein B Concentrations, LDL Particle Size, and Oxidative Susceptibility of Two LDL Subfractions in Postmenopausal Women

Abstract approved: _____

Rosemary C. Wander

Current dietary recommendations have placed increasing emphasis on dietary fat as an important element to decrease risk of cardiovascular disease (CVD). Although total fat and the fatty acid composition of diets influence the risk of CVD, the optimal amounts of different fatty acids are not well defined, especially if n-6 and n-3 fatty acids are considered. Despite the fact that postmenopausal women are at increased risk of CVD, few studies have investigated the influence of dietary fatty acids on this risk. Therefore, this study was designed to determine the effect of supplementation with different fatty acids on risk factors of CVD in postmenopausal women. Sixteen healthy, postmenopausal women were randomly assigned in a three-period crossover trial to treatments of 15 g/d supplements of oleic acid-rich sunflower oil (TS), linoleic acid-rich

safflower oil (SO), and eicosapentaenoic acid- and docosahexaenoic acid-rich fish oil (FO). Each treatment period lasted 5 weeks followed by a 7-week washout interval. When the women were supplemented with FO compared to supplementation with either TS or SO, the concentration of high density lipoprotein cholesterol tended to increase (p=0.07 and 0.05, respectively) as did the size of the low density lipoprotein (LDL) particle (P=0.03 in both instances) while the concentration of triacylglycerol (p=0.0001 and 0.02, respectively) and apolipoprotein B (apo B) (P=0.005 and P=0.01, respectively) decreased. The concentration , i.e., total cholesterol, cholesterol ester, free cholesterol, phospholipids, α- and γ-tocopherol, of the two LDL subfractions was not influenced by any of the oil supplements but was greater in the large (L) subfraction than the small (S). When the oxidation of the two subfractions was measured by monitoring the formation of conjugated dienes, the lag time was shorter in both fractions after supplementation with FO compared to supplementation with SO (P=0.0001) or TS (P=0.0001) but the effect was greater in the L subfraction. The rate of formation of conjugated dienes, which was slower after FO supplementation than supplementation with either TS (P=0.02) or SO (P=0.001), was faster in the L compared to the S subfraction. When oxidation was measured by monitoring the increase in negative charge on apo B over 23 hr, only the 1 hr time point differed. The increase was greater in the FO-supplemented group than either the TS- or SO-supplemented groups (P=0.001 in both instances). The change was greater in S LDL (P=0.007). These findings demonstrate a greater potential antiatherogenic property of dietary n-3-rich oil than n-6- or n-9-rich ones as indicated by changes to plasma lipids, lipoproteins, apo B, and particle size but the influence of the oxidative susceptibility of L and S subfractions is less conclusive.

The Effect of Supplementation with n-9, n-6, and n-3 Fatty Acids on Plasma Lipid, Lipoprotein, Apolipoprotein B Concentrations, LDL Particle Size, and Oxidative Susceptibility of Two LDL Subfractions in Postmenopausal Women

by

Ye-Sun Lee

A THESIS

Submitted to

Oregon State University

In partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented September 21, 1999
Commencement June 2000

Doctor of Philosophy thesis of Ye-Sun Lee presented on Sept 21, 1999

APPROVED:

Major Professor, representing Nutrition and Food Management

Head of the Department of Nutrition and Food Management

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Ye-Sun Lee, Author

ACKNOWLEDGEMENTS

I would like to sincerely appreciate to my major professor, Dr. Rosemary C. Wander for hĕlping me enlarge my knowledge in my academic area. Her continuous teaching, encouraging, and endless caring made one of my biggest dreams come true. I am forever indebted to her for her support to pursue a fundamental work that will provide professional career.

I would like to thanks to Drs. Ling-Jung Koong, Carolyn Raab, Willbert Gamble, and Maret G. Traber for serving my graduate committee. Their encouragement and advice made possible to get me accomplish this work.

I would like to thank to my fiance, parents, and family for their emotional and financial support. Special thanks to my sister, Ye-Seol Lee for her infinite support during 8 years of study abroad.

Most of all, I would like to deeply thank the God for putting a bright lamp on my feet all the time.

CONTRIBUTION OF AUTHORS

Dr. Rosemary C. Wander advised me throughout the research process and edited the manuscripts extensively. Shi-Hua Du, Jane Higdon, and Tianyiang Wu cooperated in this research. They were involved in screening, recruiting, and feeding subjects.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF APPENDICES

The Effect of Supplementation with n-9, n-6, and n-3 Fatty Acids on Plasma Lipid, Lipoprotein, Apolipoprotein B Concentrations, LDL Particle Size, and Oxidative Susceptibility of Two LDL Subfractions in Postmenopausal Women

CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD) is the number one killer in the United States. More than 950,000 people died due to CVD in 1996 (American Heart Association 1998). More women than men have died due to CVD since 1984. Although CVD occurs at an earlier age in men than women, as women approach the age of menopause, the risk of CVD begins to rise. Postmenopausal women have an enhanced CVD-risk profile compared to premenopausal women. This is thought to be associated with loss of the protective effect of estrogen (Colditz et al. 1987). Although the CVD-risk increases in this population, minimal experimental data have been accumulated to understand why.

Plasma lipid, lipoprotein, and apolipoprotein, and LDL size and cardiovascular disease

The major CVD risk factors in women are dyslipidemia, physical inactivity and excess weight. Among these, plasma total cholesterol, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) are well defined risk factors of CVD (National Cholesterol Education Program 1988). Numerous epidemiological studies have shown that total cholesterol and LDL-C concentrations are

positively and HDL-C concentration negatively related to CVD (Kannel et al. 1971; Miller and Miller 1975; Pearson et al. 1979; Castelli et al. 1986). Elevated plasma triacylglycerol concentration has also been considered to be an independent risk factor for CVD. Although the literature on epidemiological associations between plasma triacylglycerol and CVD have not been completely consistent, the majority of observational studies demonstrate a significant positive relation (Thind and Sandhu 1981; Goldstein et al. 1973; Fager et al 1981; Kameda et al. 1984; Hamsten et al. 1986; Austin 1991; Havel 1994) that may be greater for women than men (Austin et al. 1998). The ratio of total cholesterol/HDL-C and LDL-C/HDL-C are also well established predictors of CVD (Stampfer et al. 1991; NIH Consensus conference 1993; Gaziano et al. 1997). For example, Gaziano et al. (1997) reported that a one unit (i.e., a change from 4.0 to 5.0) increase in total cholesterol/HDL-C and LDL-C/HDL-C ratios were associated with 49% and 75% increases in risk of myocardial infarction, respectively. Recently, the ratio of triacylglycerol to HDL-C has been shown to be a better predictor of myocardial infarction than either of these other two ratios (Gaziano et al. 1997). The concentration of apolipoprotein B (apo B), the major protein in LDL, is also associated with increased risk of CVD (Brunzell et al. 1984; Genest et al. 1991). Case-control studies showed that apo B may be a better marker than LDL-C or HDL-C in discriminating CVD subjects from normal subjects (Sedlis et al. 1986; Durrington et al. 1988).

LDL particle size is also a risk factor for CVD. LDL particles are heterogeneous in size and density. They range from 1.019 to 1.063 g/ml in density and 22.0 to 28.0 nm in size. Subjects with a heritable phenotype characterized by a predominance of smaller, more dense LDL (phenotype B) show a higher risk of CVD relative to individuals with

predominantly larger, more buoyant LDL (phenotype A) (Austin and Krauss 1986; Austin et al. 1988). Phenotype B individuals have a lipoprotein profile more indicative of CVD-risk than phenotype A (Austin et al. 1990).

Visceral adipose tissue and cardiovascular disease

Recent studies have demonstrated that in addition to body mass index (BMI), visceral adipose tissue was significantly associated with risk factors for CVD and non-insulin-dependent diabetes (Fujioka et al. 1987; Despres et al. 1989; Pouliot et al. 1992). Waist circumference and the abdominal sagittal diameter, two measures of abdominal obesity, were significantly correlated with dyslipidemia, higher blood pressure, impaired glucose tolerance, and higher plasma insulin concentrations (Kalkhoff et al. 1983; Despres et al. 1989).

Effects of dietary fatty acids on cardiovascular disease

More than 50 years of studies have proved that the intake of saturated fatty acids increase plasma total cholesterol and LDL-C (Keys 1967; Keys and Parlin 1966; Hegsted et al. 1965). Therefore, the American Heart Association recommends to the US population that they consume less than 30% of energy from fat and less than 10% of energy from saturated fat to reduce the risk of coronary heart disease (Consensus conference 1985). Furthermore, the National Cholesterol Education Program's Adult Treatment Guidelines recommend that polyunsaturated fats should not exceed 10% of energy and MUFA should be between 10 to 15% of total energy (National Cholesterol Education Program 1988). However, MUFA, PUFA, and/or carbohydrates have been

shown to reduce HDL-C concentration (Hjermann et al. 1979; Shepherd et al. 1980; Schaefer et al. 1981). Ideally, diets recommended to decrease total cholesterol and LDL-C concentration should increase or at least maintain HDL-C concentration.

Keys (1970) reported that the CVD incidence in middle-aged men living in Mediterranean countries was lower than expected for their plasma total cholesterol concentrations. The Mediterranean countries' traditional diet provides a high total fat intake (35 to 40% of the total energy) and a high content of olive oil, which is rich in MUFA. This low incidence of CVD could be related to the potentially beneficial effects on the high consumption of olive oil (Keys 1980). However, the results of the effect of MUFA on plasma lipids from intervention studies have not been consistent. Numerous studies showed that when MUFA was substituted for saturated fatty acids and/or carbohydrates in the diet, total cholesterol and LDL-C concentrations were significantly decreased but HDL-C concentration was not changed in healthy human subjects (Grundy 1986; Mensink and Katan 1987; Baggio et al. 1988; Grundy et al. 1988; Colquhoun et al. 1992; Mensink et al. 1989; Berry et al. 1992). On the other hand, Nydahl et al. (1994) found HDL-C concentration, as well as total cholesterol and LDL-C, was significantly decreased with a MUFA-rich diet.

Keys and Parlin (1966) and Hegsted et al. (1965) showed that isocaloric exchange of n-6 PUFA for carbohydrates or saturated fatty acids lowered the plasma total cholesterol concentration in men consuming diets that provided 10 to 40% of calories from fat. Although the n-6 fatty acids have favorable effects on lipoprotein metabolism, recently it has been suggested that possible detrimental effects occur when diets that contain a high concentration of them are consumed. For instance, Hodgson et al. (1993)

and Gordon and Rifkind (1989) showed that n-6 fatty acid-rich diets also decreased HDL-C concentration. In addition, that fatty acids may increase the susceptibility of LDL to oxidative modifications (Esterbauer et al. 1987; Reaven 1994), a proposed risk for CVD. Several studies showed that n-6 fatty acids increase the secretion of insulin, reduce insulin catabolism, and cause impaired insulin action and leading to insulin resistance (Lardinois et al. 1987; Storlien et al. 1987; Reaven and Chen. 1988; DeFronzo. 1992). An excess in the intake of n-6 fatty acids may also interfere with metabolism of n-3 fatty acids. N-3 fatty acids, in addition to their role in heart disease as discussed below, play an important role in the normal development of the retina, brain and clotting mechanism (Neuringer and Connor 1986). They also promote fibrinolysis, decrease platelet aggregation, decrease blood viscosity and decrease blood pressure (Lands 1993).

Epidemiological studies in Greenland Eskimos led to the hypothesis that fish rich in n-3 polyunsaturated fatty acids, eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), were useful for reducing CVD (Bang et al. 1980; Dyerberg et al. 1978). Subsequent epidemiological studies showed a significant inverse relation between fish consumption and the risk of death from CVD (Kromhout et al. 1985; Norell et al. 1986; Bjerregaard and Dyerberg 1988; Iso et al. 1989; Davidson et al. 1993; Kromhout et al. 1995; Burchfiel et al. 1996; Daviglus et al. 1997; Albert et al. 1998; Zhang et al. 1999). However, the results of other studies are inconsistent with these findings (Curb and Reed 1985; Ascherio et al. 1995; Fraser et al. 1992; Morris et al. 1995). Experimental and clinical studies have found that fish and fish oils rich in n-3 fatty acids have beneficial effects on plasma lipid, lipoprotein metabolism and development of CVD (Bang and Dyerberg 1972; Illingworth et al. 1984; Leaf and Weber. 1988; Harris 1989;

Wander et al. 1996). Fish oil feeding has been shown to consistently reduce triacylglycerol level, particularly in hypertriglyceridemic subjects (Nestel et al. 1984; Harris 1989; Wander et al. 1996), but their effect on LDL-C and HDL-C is inconsistent (Nestel et al. 1984; Sullivan et al. 1986).

Although the LDL particle size is thought to be an important contributor to CVD, studies on the effects of different fatty acids on LDL particle size are limited and even existing data have not been consistent.

The effect of dietary fatty acids on oxidative susceptibility of LDL subfractions

The underlying primary cause of most cardiovascular disease is believed to be atherosclerosis, a progressive multifactorial disease of the artery wall (Badimon et al. 1993). It is currently thought that oxidation of LDL by cells in the artery wall leads to a proatherogenic particle that may initiate early lesion formation (Steinberg et al. 1989; Reaven and Witztum 1996). The factors that contribute to the susceptibility of LDL to oxidize have not been clearly defined.

LDL oxidation begins when a reactive radical abstracts a hydrogen atom from a polyunsaturated fatty acid (PUFA) on the LDL particle. Although this process presumably begins in the PUFA of surface phospholipids, it then spreads to involve the cholesterol esters and triacylglycerol in the core of the LDL particles.

Among the factors that may contribute to the oxidative susceptibility of the LDL particle are its composition and concentration of the different PUFA. The fatty acid content of LDL particles has been shown to be easily modified by dietary fatty acids (Reaven and Witztum 1996; Wander et al. 1996). Diets enriched in linoleic acid (18:2n-

6) increased the content of linoleic acid in LDL. In contrast, diets enriched in oleic acid (18:1n-9) produced LDL particles rich in oleic acid (Reaven et al. 1994; Davidson et al. 1997). Fish oil supplementation also increased the content of eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in LDL (Wander et al. 1996). Since dietary fatty acids influence the fatty acid composition of LDL, the amount and type of fat in the diet may affect susceptibility of LDL to oxidative damage. Reaven et al. (1991) reported that oxidative susceptibility of the LDL enriched with oleic acid was significantly lower than that of LDL enriched with linoleic acid ($p=0.0001$). Similar results have been found in other studies (Davidson et al. 1997; Berry et al. 1992; Bonanome et al. 1992).

Results from studies on the oxidative susceptibility of LDL enriched in n-3 fatty acids are less clear. Wander et al. (1996) found that supplementation with fish oil shortened the lag time of the formation of conjugated dienes approximately 18 min ($P<0.001$), suggesting increased susceptibility but slowed oxidation rate 25% ($P<0.001$), suggesting decreased susceptibility. In support of this study Whitman et al. (1994) found that fish oil-enriched LDL had a 30% shorter lag time and a 30% slower oxidation rate but no difference in atherosclerotic lesion development of all major blood vessels. On the other hand Nenster et al. (1992) reported that LDL from fish oil and corn oil supplemented groups showed similar susceptibility to copper catalyzed lipid peroxidation, as indicated by the amount of lipid peroxides and degradation of oxidatively modified LDL in cultured macrophages. In addition, Suzukawa et al. (1995) found that macrophages that were supplemented with fish oil displayed a significantly (P

< 0.001) higher capacity to oxidize LDL than either control cells or cells supplemented with corn oil.

In addition to its composition and different fatty acid concentration, the size of the LDL particle may contribute to its oxidative susceptibility. However, studies on the oxidative susceptibility of LDL subfractions have been incomplete. Previous studies have suggested that small LDL was more susceptible to oxidation than the larger particles (de Graaf et al. 1991; Tribble et al. 1992; Chait et al. 1993; Dejager et al. 1993; Tribble et al. 1994; Tribble et al. 1995a; Tribble et al. 1995b). However, these experiments have not shown clearly the enhanced oxidative susceptibility of small LDL.

For example, Chait et al. (1993) compared oxidative modification in six subfractions of LDL isolated from phenotype A and phenotype B subjects. Although lag time was inversely related ($p < 0.001$) to LDL density in both groups of subjects, the rate of oxidation of LDL did not correlate with LDL subfrations. In addition, Dejager et al. (1993) reported that the oxidative susceptibility of the LDL particle was significantly higher in small LDL; however, they did not report the rate of oxidation.

On the other hand, de Graaf et al. (1991) measured oxidative susceptibility in three LDL subfractions in 11 healthy people (LDL1, d =1.030-1.033; LDL2, d = 1.033-1.044; LDL3, d = 1.040-1.045 g/ml). A significant inverse linear relation was found between LDL size and lag time ($P < 0.001$). The LDL size showed a negative linear relation with the rate of oxidation ($P < 0.001$) and the amount of conjugated dienes formed in the LDL subfractions. However, the range of LDL used in this study was 1.030-1.045 g/ml, which represents only the large LDL fraction.

The composition of LDL subfractions may also be an important factor for its oxidative susceptibility although the information gathered to date is contradictory. Tribble et al. (1995) reported that small, dense LDL particles had more cholesteryl ester and less free cholesterol than large, buoyant ones. They suggested that free cholesterol, which was found predominantly on the surface of the LDL, could be responsible for protecting LDL from lipid oxidation. In support of this theory, Rudel et al. (1985) in their study with monkeys suggested that the increased atherogenecity of LDL particles was associated with the enrichment of these particles with cholesteryl esters. However, in humans de Graaf et al. (1991) reported that large buoyant LDL particles had more cholesteryl ester than smaller ones whereas Dejager et al. (1993) reported that the concentration of cholesteryl ester and free cholesterol was not significantly different between large LDL and small LDL. Previous studies have shown that small LDL contain significantly lower α-tocopherol and ubiquinol-10 concentrations than large (de Graaf et al. 1991; Tribble et al. 1995). The shortened lag time in small LDL is explained in part by a lower content of antioxidants, including ubiquinol-10 and α-tocopherol. However, Dejager et al. (1993) found that the oxidative susceptibility of LDL subfractions in two subfractions was independent of vitamin E content.

The fatty acid content of LDL subfractions may influence its oxidative susceptibility although few studies have investigated this. de Graaf et al. (1991) suggested that small LDL were less resistant to oxidation than large because they have significantly higher concentrations of polyunsaturated fatty acids including 18:2n-6 and 20:4n-6.

Significance of the study

Current dietary recommendations have placed increasing emphasis on decreasing the consumption of dietary fat, in large measure to decrease the risk of CVD, although the optimal ratio of polyunsaturated fat to monounsaturated and saturated fat is not well defined, especially if n-6 versus n-3 fatty acids are considered. To make optimum recommendations about dietary fat, an understanding of the role of different fatty acids on indices of cardiovascular risk factors is important. Although the cardiovascular risk increases in postmenopausal women, few intervention studies have been conducted in this population. We know of no work that compares the oxidative susceptibility of different LDL subfractions enriched with fatty acids that differ in their degree of unsaturation obtained from postmenopausal women. Therefore, this study was designed to investigate the effect of supplementation with n-9, n-6, and n-3 fatty acids on plasma lipid, lipoprotein, and apo B concentrations, and triacylglycerol/HDL-C, LDL-C/HDL-C, LDL particle size, and oxidative susceptibility of the two LDL subfrations in postmenopausal women.

CHAPTER 2

REVIEW OF THE LITERATURE

Cardiovascular disease (CVD) has been the number one killer in the United States. More than 950,000 people died due to CVD in 1996 (American Heart Association 1998). According to the National Health and Nutrition Examination Survey III (1999), 58,800,000 American have one or more types of CVD. Not only are there more deaths associated with CVD than any other disease but there are more expenses associated with it. According to data from the Health Care Financing Administration, $24.6 billion was spent to treat CVD in 1995 (American Heart Association 1998). That amounted to an average $7, 255 per patient discharged from a hospital and represented 32.9 % of all hospitalization expenditures. Medicare beneficiaries whose hospitalization involved a surgical procedure received $16.4 billion in payments. That amounted to $11,746 per discharge.

CVD occurs at an earlier age in men than women; however, as women approach the age of menopause, the risk of CVD begins to rise. This is thought to be associated with the loss of the protective effect of estrogen (Colditz et al. 1987). More females than males have died due to CVD every year since 1984 (American Heart Association 1998). Postmenopausal women have enhanced CVD-risk profile compared to premenopausal women. They have higher levels of triacylglycerol, total cholesterol, very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and a lower level of high-density lipoprotein cholesterol (HDL-C) (Campos et al. 1988;

Hallberg and Svanborg 1967). Although the CVD-risk increases in this population, minimal experimental data have been accumulated to understand why.

Plasma lipid, lipoprotein, and apolipoprotein B concentrations and cardiovascular disease

The major risk factors of CVD in women are believed to be dyslipidemia, physical inactivity and excess weight. Among these, plasma total cholesterol, LDL-C, and HDL-C are important risk factors of CVD (National Cholesterol Education Program 1988). Numerous epidemiological studies have shown that total cholesterol and LDL-C concentrations are positively and HDL-C concentration negatively related to CVD (Kannel et al. 1971; Miller and Miller. 1975; Pearson et al. 1979; Castelli et al. 1986). Castelli and Anderson (1986) reported, using data from the Framingham Offspring Study, that the level of total cholesterol, LDL-C, and HDL-C were excellent predictors of CVD. They found a strong linear association between LDL-C concentration and CVD. In multivariate analysis LDL-C and HDL-C were independently related to CVD risk. The mechanism associated with this risk is thought to be that LDL transports cholesterol from plasma to peripheral tissues resulting in deposits of cholesterol in the tissues while HDL facilitates the uptake of cholesterol from peripheral tissues and its transport to the liver for catabolism and excretion.

Previously, Austin (1989) and Criqui et al. (1993) suggested that the association of plasma triacylglycerol concentration with CVD is complicated due to several factors. Triacylglycerol concentrations are strongly correlated with other lipid parameters. For instance, Albrink et al. (1980) and Davis et al. (1980) reported an inverse correlation between triacylglycerol concentration and HDL. In addition, there is considerable within

individual variability in measured triacylglycerol levels. Jacobs and Barrett-Connor (1982) reported coefficients of variation of about 8% for cholesterol and 25% for triacylglycerol concentrations during Visits 1 and 2 of the Lipid Research Clinics Prevalence Study in 1972-1975.

However, more recent data indicate that elevated plasma triacylglycerol concentration is an independent risk factor for CVD. Although the literature on epidemiological associations between plasma triacylglycerol and CVD is not completely consistent, the majority of observational studies demonstrate a significant univariate relation (Kameda et al. 1984; Thind and Sandhu 1981). These studies showed that elevated triacylglycerol concentration was significantly correlated with CVD. In addition several studies proved triacylglycerol concentration remains a significant predictor of CVD in multivariate statistical analyses after accounting for total cholesterol or LDL-C (Goldstein et al. 1973; Fager et al. 1981; Hamsten et al. 1986; Austin 1991; Havel 1994).

The ratio of total cholesterol/HDL-C and LDL-C/HDL-C are also well established predictors of CVD (Stampfer et al. 1991; NIH Consensus conference 1993; Gaziano et al. 1997). A one unit increase in total cholesterol/HDL-C and LDL-C/HDL-C were associated with 49% and 75% increases in risk of myocardial infarction, respectively (Gaziano et al. 1997). Stampfer et al. (1991) also found that a one unit increase in the LDL-C/HDL-C ratio was associated with a 53% increase in risk of myocardial infarction in the Physicians' Health Study.

Recently, the ratio of triacylglycerol to HDL-C has been shown to be a better predictors of myocardial infarction than either of the two ratios (Gaziano et al. 1997). Gaziano et al. (1997) examined the interrelationships of triacylglycerol concentration,

other lipid parameters, and nonlipid risk factors with risk of myocardial infarction among 340 cases and an equal number of control subjects. They found a significant association of elevated triacylglycerol with risk of myocardial infarction. After adjustment for HDL-C, the relationship remained statistically significant. Furthermore, the ratio of triacylglycerol to HDL was a strong predictor of myocardial infarction.

It has been suggested that plasma apolipoprotein B (apo B), the major protein component of LDL, is also associated with CVD (Genest et al. 1991; Brunzell et al. 1984). Case-control studies showed that apo B may be a better marker than LDL-C and HDL-C in discriminating CVD subjects from normal subjects (Sedlis et al. 1986; Durrington et al. 1988). Sedlis et al. (1986) studied 281 CVD patients to determine whether measurements of apolipoprotein levels were more predictive of the presence of CVD than the corresponding levels of LDL-C, HDL-C, or triacylglycerol. They reported an independent effect of apo B on CVD even after adjustments were made for LDL-C, HDL-C, and triacylglycerol. Durrington et al. (1988) compared middle-aged men who had had a myocardial infarction with a control group by measuring total cholesterol, LDL-C, HDL-C, triacylglycerol, and apo B concentrations, and blood pressure. They found apo B concentration was the best predictor to distinguish patients from controls.

Low-density lipoprotein heterogenity

LDL particles are heterogeneous in size and density. They range from 1.019 to 1.063 g/ml in density and 22.0 to 28.0 nm in size. LDL density linearly decreases with increasing LDL particle diameter (Shen et al, 1981). More than seven distinct LDL subfractions have been identified. LDL-1 occurs in the density range of 1.019 to 1.033

g/ml, LDL-2 and LDL-3 in the range of 1.033 to 1.038 g/ml, LDL-4 and LDL-5 in the 1.038 to 1.050 g/ml fraction, and LDL-6 and LDL-7 in the 1.050 to 1.063 region (Campos et al. 1992; Shen et al. 1981).

Several analytical tools are used in the determination of LDL subclasses. LDL density is measured using density gradient ultracentrifugation (Krauss and Burke. 1982). Particle diameter is frequently measured by nondenaturing gradient polyacrylamide gel electrophoresis (PGGE) (McNamara et al. 1987; Nichols et al. 1986). The molecular weight of LDL is determined by agarose gel chromatography (Rudel et al. 1977).

In the density gradient ultracentrifugation method to determine LDL subclasses, lipoproteins float at a rate dependent on their density, size and shape. With increasing density, peak flotation rates and mean particle diameters decrease progressively. Shen et al. (1981) separated up to six subfractions of LDL by this method.

Nondenaturing gradient polyacrylamide gel electrophoresis and agarose gel chromatography are most often used to define LDL subclasses. In the PGGE method, electrophoresis of whole plasma or LDL is carried out using 2-16% polyacrylamide gradient gels. After the electrophoresis, gels are scanned with a densitometer to determine migration distances of each of the major bands. LDL particle diameters corresponding to each of these bands are calculated from calibration curves determined using standards of known size.

It is also possible to determine the molecular weight of LDL rather than its density or diameter using agarose gel chromatography. The distribution of mass among LDL subclasses in plasma is generally reflected by the particle diameter and density of the predominant LDL species. In the agarose gel chromatography method LDL

molecular weight is calculated by comparing elution volume of standard LDL to that of the unknown sample of LDL. A relative size index, r_I, is determined by dividing the elution volume of the internal standard (^{125}I-LDL) by the elution volume of the sample LDL. Rudel et al. (1977) reported that comparison of r_I to molecular weights measured by ultracentrifuge analysis showed a linear relationship ($r = 0.985$). Molecular weight of LDL can be calculated from the r_I value and the known molecular weight of the standard.

LDL phenotypes and the risk of cardiovascular disease

Austin et al. (1988) identified two distinct lipoprotein phenotypes based on analysis of the LDL subclasses: phenotype A and B. Phenotype A, which occurs in the density range from 1.019 to 1.045 g/ml (25.5-27.5 nm), is characterized by predominantly large, buoyant LDL, while phenotype B, with a density range from 1.045 to1.063 g/ml (24.0-25.5 nm), is characterized by predominantly small, dense LDL.

Subjects with a heritable phenotype characterized by a predominance of smaller, more dense LDL (phenotype B) show a higher risk of CVD relative to individuals with predominantly larger, more buoyant LDL (phenotype A) (Austin and Krauss 1986). Phenotype B subjects have been associated with up to a threefold increased risk of myocardial infarction (Austin et al. 1988). As part of Physician's Health Study (Stampfer et al. 1996) compared LDL particle diameters between controls (n=308) and subjects with a risk of myocardial infarction (n=266). Subjects with a risk of myocardial infarction had significantly smaller LDL particle diameter than controls (25.6 ± 0.1 vs 25.9 ± 0.1 nm, $P<0.001$).

Phenotype B individuals had a lipoprotein profile more indicative of CVD-risk than phenotype A in a community-based study of 301 subjects (Austin et al. 1990). Phenotype A individuals were classified with peak LDL particle diameters of 26.6 nm (n = 208) while phenotype B individuals were classified with peak LDL particle diameters of 24.9 nm (n =93). Phenotype B subjects had higher concentrations of triacylglycerol, apo B, intermediate-density lipoprotein cholesterol (IDL-C), VLDL-C, and lower concentrations of HDL-C (Austin et al. 1990; Lamarche et al. 1997). Increased VLDL-C and IDL-C concentrations are commonly found in CVD patients (Austin et al. 1988; Havel. 1994).

Numerous studies have found a strong negative correlation between plasma triacylglycerol concentration and LDL particle diameters (Stampfer et al. 1996; Austin et al. 1990; Campos et al. 1992). For instance, Stampfer et al. (1996) found that the LDL particle diameter had a high inverse correlation with plasma triacylglycerol concentration ($r= -0.71$, $n= 266$), even stronger than the inverse correlation between HDL-C and plasma triacylglycerol concentrations ($r= -0.60$) (Stampfer et al. 1996). Campos et al. (1992) also reported that decreased LDL particle size was significantly associated with increased triacylglycerol concentration in 1,168 women and 1,172 men as part of the Framingham Offspring Study ($p < 0.0001$).

Although a detailed understanding of the mechanism for this relationship is not established yet, there is evidence that postheparin lipoprotein lipase (LPL) and cholesteryl ester transfer protein (CETP) may be involved. The usual function of plasma lipoprotein lipase is to release fatty acids from chylomicrons and VLDL, causing an influx of fatty acids into adipose tissue.

Karpe et al. (1993) studied postheparin plasma lipase activity in 32 men with coronary atherosclerosis and in 10 age matched-healthy control men. Hypertriglyceridaemic patients had a preponderance of small LDL particles compared with normotriglyceridaemic patients and controls and the LPL activity correlated with the content of large LDL. In support of this study, Campos et al. (1995) found a significant inverse relationship between postheparin lipoprotein lipase activity and plasma levels of triacylglycerol. In addition, increased postheparin lipoprotein lipase activity raised LDL particle size. Krauss (1997) suggested that this may be due to the transfer of surface lipids during chylomicrons and VLDL triacylglycerol hydrolysis. High activity of postheparin lipoprotein lipase increased delivery of triacylglycerol from VLDL and chylomicron not only to adipocytes but also to LDL and HDL. This resulted in producing more triacylglycerol-rich, larger LDL and HDL, as well as enhances clearance of VLDL-C and chylomicron from plasma.

Abbey et al. (1990) suggested that CETP activity may also contribute to the increase in LDL particle size. CETP is involved in the exchange of triacylglycerol from VLDL and chylomicrons for cholesteryl ester in LDL, a function which is stimulated by plasma triacylglycerol (Sakai et al. 1991; Lagrost et al. 1995). Lagrost et al. (1995) found a significant positive relationship between the activity of plasma CEPT and LDL size in normolipidemic subjects.

Association between LDL phenotype B and insulin resistance syndrome

Patients with non-insulin-dependent diabetes (NIDDM) are resistant to insulin-dependent glucose uptake. The body's response to a decrease in insulin-mediated glucose

uptake results in an increase in insulin secretion. As long as a state of compensatory hyperinsulinemia can be maintained, normal glucose uptake can be regulated. Frequently this defect in insulin action and/or the associated hyperinsulinemia will lead to an increase in plasma triacylglycerol, a decrease in HDL-C concentration, and high blood pressure. Although it has long been known that obesity, often measured using body mass index (BMI), is a risk factor for CVD and NIDDM, recent studies have demonstrated that the amount of visceral adipose tissue (central body fat) may also be a risk factor for CVD and NIDDM (Fujioka et al. 1987; Despres et al. 1989; Pouliot et al. 1992). Waist circumference and abdominal sagittal diameter are commonly used methods for measuring visceral adipose tissue. They were both significantly correlated with higher blood pressure, impaired glucose tolerance, and higher plasma insulin concentrations (Kalkhoff et al. 1983; Despres et al. 1989). Pouliot et al. (1994) suggested that waist circumference values above approximately 100 cm, or abdominal sagittal diameter values above 25 cm are most likely to be associated with risk of CVD. Both systolic and diastolic blood pressures are also related to risk of coronary heart disease: the higher the level of pressure, the greater the incidence of coronary heart disease (Castelli and Anderson 1986). This cluster of factors has recently been associated with one syndrome. Reaven (1993) defined syndrome X (insulin resistance syndrome) as being characterized by dyslipidemia and a cluster of other CVD risk factors including obesity, increased central body fat, high blood pressure, and impaired glucose tolerance.

Haffner et al. (1994) and Selby et al. (1993) reported that a predominance of small, dense LDL particles was significantly inversely correlated with body mass index (BMI), waist circumference, systolic blood pressure, and fasting glucose concentration.

In addition, diabetic subjects have smaller, denser LDL than nondiabetic subjects and LDL size is strongly correlated with glucose concentration (Barakat et al. 1990; Campos et al. 1992; Feingold et al. 1992). Feingold et al. (1992) studied LDL subclass phenotypes in normolipidemic men with NIDDM and in age-matched control subjects who had similar lipid levels. There was a twofold increase in the percentage of individuals with the phenotype B in the NIDDM subjects. The phenotype B was associated with higher plasma triacylglycerol levels and a trend of lower HDL-C levels compared with the phenotype A NIDDM subjects. In both control and NIDDM subjects, the clearance of triacylglycerol-rich lipoproteins was slowed in the subjects with the phenotype B compared with those with the phenotype A. Barakat et al. (1990) also found LDL size inversely correlated with plasma insulin levels independent of triacylglycerol levels and BMI in NIDDM patients.

Effects of dietary fatty acids on cardiovascular disease

More than 50 years of studies have proved that increased intake of saturated fatty acids increases plasma total cholesterol and LDL-C (Keys 1967; Keys and Parlin 1966; Hegsted et al. 1965). Therefore, it is recommended by numerous groups that address the influence of diet on health that dietary saturated fatty acids be replaced by monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and/or carbohydrates for the reduction of total cholesterol and LDL-C concentrations to reduce the risk of CVD (Consensus conference 1985). The American Heart Association recommends to the US population that they consume less than 30% of energy from fat and less than 10% of energy from saturated fat to reduce the risk of coronary heart

disease. Furthermore, the National Cholesterol Education Program's Adult Treatment Guidelines recommend that polyunsaturated fats should not exceed 10% of energy and MUFA should be between 10 to 15% of total energy (National Cholesterol Education Program 1988).

Additionally, MUFA, PUFA, and/or carbohydrates have been shown to reduce HDL-C concentration (Shepherd et al. 1980; Hjermann et al. 1979; Schaefer et al. 1981). Ideally, diets recommended to decrease total cholesterol and LDL-C concentration should increase or at least maintain HDL-C concentration. It is not well defined which fatty acids, how much of them, and/or the ratio among them that will best decrease risk factors associated with CVD.

Effect of monounsaturated fatty acids on cardiovascular disease: The distinguishing structural feature of MUFA is the presence of one double bond. The most common one in the human diet is oleic acid. This particular fatty acid has its double bond located nine carbons from the methyl end of the molecule.

In the Seven Countries Study conducted 30 years ago, investigators studied the association between dietary intakes and risk of CVD in 16 populations of middle-aged men residing in seven countries: Finland, the Netherlands, Italy, Yugoslavia, Greece, the United States, and Japan (Keys 1970). This study showed that the CVD incidence in middle-aged men living in Mediterranean countries was lower than expected for their plasma total cholesterol concentrations (Keys 1970). The Mediterranean countries' traditional diet provides a high total fat intake (35 to 40 % of the total energy) and a high content of olive oil, which is rich in MUFA. This low incidence of CVD could be related

to the potentially beneficial effects on the high consumption of olive oil (Keys 1980; de Lorgeril et al 1994), although recent work has indicated that other dietary components are also involved (Renaud and Ruf 1994).

Trichopoulou et al. (1995) investigated in three Greek villages the dietary patterns of 182 men and women all of whom were older than 70 years. This study compared a group which followed the dietary patterns of the traditional Greek diet with a group which deviated from this. The latter group consumed more meat and meat products. In the traditional diet, olive oil dominated the fat intake with total fat exceeding 35% of total energy intake. The risk of death from CVD was lower in those whose diets followed closely to the traditional Greek pattern than those whose diet deviated from it. Trevisan et al. (1990) analyzed consumption of olive oil and risk factors for CVD in a sample of 4903 Italian men and women 20 to 59 years of age. Consumption of olive oil was inversely associated with serum cholesterol and systolic blood pressure, two CVD risk factors.

Numerous intervention studies in healthy human subjects have shown that when MUFA was substituted for saturated fatty acids and/or carbohydrates in the diet, total cholesterol and LDL-C concentration was significantly decreased but HDL-C concentration was not changed (Grundy 1986; Mensink and Katan 1987; Baggio et al. 1988; Grundy et al. 1988; Mensink et al. 1989; Berry et al. 1992; Colquhoun et al. 1992). For example, Gumbiner et al. (1998) found that a MUFA-supplemented group showed a greater decrease in total cholesterol, LDL-C, and apo B as well as triacylglycerol than a CHO-supplemented one. However, the HDL-C concentration was not changed

significantly in the MUFA-supplemented group while it decreased in the CHO-supplemented group.

Grundy (1986) compared consumption of a diet rich in MUFA (High-MUFA), a diet low in fat (Low-Fat), and a diet high in saturated fatty acids (High-Sat) on CVD risk factors. The High-Sat and High MUFA diets contained 40% of total calories as fat and 43% as carbohydrate. The High-Sat diet contained 25% of calories as saturated fatty acid while the High-MUFA diet contained 28% of calories as MUFA. The Low-Fat diet contained 20% fat of total calories and 63% carbohydrates. Both the High-MUFA and Low-Fat diets lowered plasma total cholesterol by 13% and 15%, respectively and LDL-C by 21% and 15%, respectively, as compared with the High-Sat diet. The Low-Fat diet raised triacylglycerol levels and significantly reduced HDL-C as compared with the High-Sat diet. However, the High-MUFA diet had no effect on levels of triacylglycerol or HDL-C.

Mensink and Katan (1987) studied the effects of two diets, one rich in complex carbohydrates, the other rich in olive oil on serum lipids in human. Total cholesterol levels decreased on average by 17 mg/dl in the carbohydrate group and 18 mg/dl in the olive oil group. HDL-C levels decreased by 7 mg/dl in the carbohydrate group and did not change in the olive oil group. On the other hand, Nydahl et al. (1994) found HDL-C concentration was significantly decreased with a MUFA-rich diet as well as total cholesterol and LDL-C. In this study twenty-six subjects with an average age of 51 years participated in a crossover study during two consecutive 3.5-wk treatment periods. The energy content of monounsaturated fatty acids and polyunsaturated fatty acid were 15% and 4%, respectively, in the MUFA-rich diet.

Effects of n-6 polyunsaturated fatty acids on cardiovascular disease: Using the system of nomenclature traditionally used by lipid nutritionists, PUFA can be divided into two families of fatty acids: the n-6 and the n-3 families. The distinction between these two groups of fatty acids is the location of the double bonds. The n-6 fatty acids have the first double bond six carbon atoms from the terminal methyl group. The position of first double bond of n-3 fatty acids is three carbons from the terminal methyl group.

The major dietary PUFA with the double bond in the n-6 position in the Western diet is linoleic acid (18:2n-6). Linoleic acid is an essential fatty acid. The minimum requirement is recommended at 1% of the energy intake (Soderhjelm et al. 1970).

Keys and Parlin (1966) and Hegsted et al. (1965) showed that isocaloric exchange of PUFA for carbohydrates or saturated fatty acids lowered the plasma total cholesterol concentration in men consuming diets that provided 10 to 40% of calories from fat. They made no distinction between the n-6 and n-3 fatty acids. Nydahl et al. (1994) gave n-6 fatty acid rich-diets to 26 subjects with an average age of 51 years for 3.5 weeks. The mean total cholesterol and LDL-C decreased by 19% and 23%, respectively. However, the HDL-C concentration also was significantly decreased.

Friday et al. (1991) studied the effect of diets containing n-6 fatty acids on plasma lipoprotein metabolism in five subjects with heterozygous familial hypercholesterolemia (FH), as well as five normal controls. The FH subjects reduced their total plasma cholesterol by 26% during the n-6 diet ($P < 0.001$) when compared with values while on a saturated fatty acid diet. In addition, LDL-C was lowered by 29% ($P < 0.001$) and apo B levels dropped by 27% ($P < 0.01$).

Jackson et al. (1984) assessed the effects of varying polyunsaturated/saturated (P/S) fat ratios on the plasma lipoproteins in six normal healthy subjects. The P/S ratio for the diets was 0.4, 1.0, or 2.0. Each diet was consumed for 2 weeks. Mean plasma total cholesterol decreased by approximately 6 and 12% on the P/S = 1.0 or P/S = 2.0 diets compared to the P/S = 0.4 diet, respectively.

Although the n-6 fatty acids are involved in favorable effects on lipoprotein metabolism, recently it has been suggested that possible detrimental effects occur when diets that contain a high PUFA concentration are consumed. As mentioned earlier, Nydahl et al. (1994) found that HDL-C was lowered significantly on n-6 fatty acid-rich diet. Hodgson et al. (1993) and Gordon and Rifkind (1989) also showed an n-6 fatty acid-rich diet decreased HDL-C concentration.

In addition, PUFA may increase the susceptibility of LDL to oxidative modifications, a proposed risk for CVD (Esterbauer et al. 1987; Reaven. 1994). Oxidatively modified LDL is thought to be a major contributor to the development of the atherosclerotic plaque (Steinberg et al 1989; Berliner and Heinecke 1996). Oxidation of LDL presumably begins when a reactive radical abstracts a hydrogen atom from a PUFA on the LDL (Palinski et al. 1989). Incorporation of more PUFA on the LDL particle may increase oxidative susceptibility of LDL. Since the major PUFA in LDL is linoleic acid (Esterbauer et al. 1987; Wander et al. 1996; Wander et al. 1997), studies that have demonstrated increased oxidation in LDL correlated with its PUFA content are usually referring to its concentration of linoleic acid.

Several studies showed that n-6 fatty acids increase the secretion of insulin, reduce insulin catabolism, cause impaired insulin action, and lead to insulin resistance

(Lardinois et al. 1987; Storlien et al. 1987; Reaven and Chen 1988; DeFronzo 1992). Storlien et al. (1987) suggested that diets high in n-6 fatty acids lead to insulin resistance. They reported that replacement of 6% of the n-6 fatty acid with n-3 fatty acids prevented the development of insulin resistance in rats.

An excess n-6 fatty acid intake may interfere with metabolism of n-3 fatty acids. N-3 fatty acids, in addition to their role in heart disease discussed below, play an important role in the normal development of the retina, brain and clotting mechanism (Neuringer and Connor 1986). They also promote fibrinolysis, decrease platelet aggregation, decrease blood viscosity and decrease blood pressure (Lands 1993).

Effects of n-3 fatty acids on cardiovascular disease: The possible beneficial effects of the consumption of fish and fish oil recently have been the focus of intensive investigation. Epidemiological studies in Greenland Eskimos lead to the hypothesis that fish rich in n-3 polyunsaturated fatty acids, eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), were useful for reducing CVD (Bang et al. 1980; Dyerberg et al. 1978). Subsequently several epidemiological studies showed a significant inverse relation between fish consumption and the risk of death from CVD (Kromhout et al. 1985; Norell et al. 1986; Bjerregaard and Dyerberg 1988; Iso et al. 1989; Davidson et al. 1993; Kromhout et al. 1995; Burchfiel et al. 1996; Albert et al. 1998; Daviglus et al. 1997; Zhang et al. 1999).

Kromhout et al. (1995) measured the CVD risk factors in 272 elderly people living in the Netherlands. The dietary history method was used to obtain information on fish consumption in 1971. During the 17 years of follow-up period, 58 people died from

CVD. About 60% of the elderly ate fish and 40% did not eat fish. After accounting for the confounding effects of major CVD-risk factors, results showed an inverse relation between fish consumption and CVD mortality.

The same group (Kromhout et al. 1985) investigated the relation between fish consumption and coronary heart disease in 852 middle-aged men without coronary heart disease in the Netherlands. The amount of fish consumed was collected in 1960 by a dietary history. During 20 years of follow-up, 78 men died from coronary heart disease. An inverse dose-response relation was observed between fish consumption in 1960 and death from coronary heart disease. Mortality from coronary heart disease was more than 50% lower among those who consumed at least 30 g of fish per day than among those who did not eat fish.

Albert et al. (1998) investigated the association between fish consumption and the risk of sudden cardiac death as part of the Physicians' Health Study. A total of 20, 551 US male physicians 40 to 84 years of age and free of myocardial infarction, cerebrovascular disease, and cancer at baseline who completed an abbreviated, semiquantitative food frequency questionnaire on fish consumption were then followed up to 11 years. There were 133 sudden deaths (death within 1 hour of symptom onset) over the entire time of the study. After accounting for coronary risk factors including age, aspirin and taking beta-carotene, dietary fish intake (1 fish meal per week) was associated with a reduced risk of sudden death. For men who consumed fish at least once per week, the multivariate relative risk of sudden death was 0.48 compared with men who consumed fish less than monthly.

Daviglus et al. (1997) investigated the data from the Chicago Western Electric Study to examine the relation between base-line fish consumption and the risk of death from coronary heart disease over a 30-year interval. The 1822 men who participated were 40 to 55 years old and free of cardiovascular disease at base line. Fish consumption, as determined from a detailed dietary history, was stratified (0, 1 to 17, 18 to 34, or more than 35 g per day). Mortality from coronary heart disease was classified as death from myocardial infarction (sudden or nonsudden) or death from other coronary causes. During 30-years of follow-up, there were 430 deaths from coronary heart disease; 293 were due to myocardial infarctions (196 were sudden and 94 were nonsudden). Men who consumed 35 g or more of fish per day as compared with those who consumed none had relative risks of death from coronary heart disease and from sudden or nonsudden myocardial infarction of 0.62 and 0.56, respectively.

Zhang et al. (1999) investigated fish consumption and mortality in individuals who were 45-74 years old from 36 countries from data were obtained from the Food and Agriculture Organization and the World Health Organization. They found an inverse correlation between fish consumption and ischemic heart disease ($P < 0.05$ to < 0.01) mortality in both men and women.

However, the results of other studies are inconsistent with these findings (Curb and Reed 1985; Fraser et al. 1992; Ascherio et al. 1995; Morris et al. 1995). Ascherio et al. (1995) studied 44,895 male health professionals, 40 to 75 years of age, who were free of known cardiovascular disease as part of the Health Professionals Follow-up Study in 1986. The participants completed dietary questionnaires. There were 1543 coronary events in this group during 6 years of follow-up: 264 deaths from coronary disease, 547

nonfatal myocardial infarctions, and 732 coronary-artery bypass or angioplasty procedures. There was no significant associations between dietary fish intake and the risk of coronary disease after accounting for age and several coronary risk factors. The multivariate relative risk of coronary heart disease was 1.12 (95 % confidence interval, 0.96 to 1.31) for men in the top fifth of the group in terms of intake of n-3 fatty acids (median, 0.58 g per day) as compared with the men in the bottom fifth (median, 0.07 g per day). The multivariate relative risk of coronary disease was 1.14 (95 % confidence interval, 0.86 to 1.51) for men who consumed six or more servings of fish per week as compared with those who consumed one serving per month or less.

Morris et al. (1995) did not find any evidence of significant association between dietary intake of fish and any cardiovascular endpoint, including myocardial infarction, stroke, and cardiovascular death in the Physicians' Health Study. This is the same population in which Albert et al. (1998) later found a significant negative relationship between fish consumption and sudden cardiac death fish but not overall cardiovascular endpoint.

These different results may be explained by the limitations of epidemiological studies. These include the difficulty of excluding confounding factors, the methodological limitation of obtaining accurate dietary intake data, the limitation of dietary studies in free-living populations, the consumption of different amounts and species of fish, study duration, and sample size.

Experimental and clinical studies have found that consumption of fish and fish oils rich in n-3 fatty acids including EPA and DHA have beneficial effects on plasma lipid, lipoprotein metabolism and development of CVD (Bang and Dyerberg 1972;

Illingworth et al. 1984; Harris. 1989; Leaf and Weber. 1988; Wander et al. 1996). They have been shown to consistently reduce plasma triacylglycerol concentration, particularly in hypertriglyceridemic subjects (Nestel et al. 1984; Harris 1989) , but their effect on LDL-C and HDL-C is inconsistent (Nestel et al. 1984; Sullivan et al. 1986; Gerhard et al, 1991).

Nestel et al. (1984) studied seven subjects who received up to 30% of daily energy needs from fish oil. Fish oil lowered the plasma triacylglycerol concentration. They also studied the effect of fish oil on the rate of production of the triacylglycerol-transporting VLDL. Compared with a diet similarly enriched with safflower oil (a diet rich in n-6 fatty acids), the fish oil diet lowered VLDL and apo B concentrations. HDL-C was also lowered. This suggests that fish oil reduced primarily the production of VLDL. Wander et al. (1996) also found the fish oil consumption significantly decreased plasma triacylglycerol concentration in forty-eight women taking 15-g supplement of fish oil per day.

Illingworth et al. (1984) found a decrease LDL-C with a diet rich in EPA/DHA. They suggested that this result is caused by reducing the rate of synthesis of apo B (Illingworth et al. 1984). On the other hand, LDL-C and apo B levels tended to increase with fish oil supplementation in familial combined hyperlipidemia subjects in study of (Failor et al. 1988). Gerhard et al. (1991) also found that consumption of 200 g of fish for 18 days raised LDL-C and apo B concentration in 21 normotriglyceridemic males. In part these differences related to the amount of n-3 fatty acids consumed. In those studies in which large amounts of what eaten, e.g., the one by Illingworth et al. (1984) in which the subjects were given 24 g of n-3 fatty acids per day, LDL-C tended to be lower.

However, in those studies in which diets contained more reasonable amounts, LDL-C is either increased or unchanged. This could be because fish and fish oil when consumed in large quantities also provides a significant decrease in the consumption of saturated fatty acids.

The influence of dietary fatty acids on LDL particle size

The fatty acid composition of the diet may also have important effects on LDL size. However, studies on the influence of dietary fatty acids on LDL particle size in humans are limited. Almost no work has been done to compare the influence of n-9 and n-6 fatty acids on LDL particle size. In addition, the effect of n-3 fatty acids on LDL size in humans has not been consistent.

Two studies have shown that the consumption of n-3 fatty acids increased LDL size. Suzukawa et al. (1995) examined the influence of n-3 fatty acids on LDL size. This study was a double-blind, crossover design. Forty-two subjects were randomly assigned to two groups. Each group received a test supplement for a 6-week intervention phase. In each 6-week intervention phase, subjects took four 1 g capsules per day containing either fish oil (providing a total of 3.4 g of n-3 fatty acids) or corn oil (providing 2.3 g linoleate and 1.1 g oleate). LDL particle size was determined by 2 to 16% PGGE as described by McNamara et al. (1987). LDL size was significantly increased with the use of fish oil supplement but not with corn oil as compared to baseline (fish oil 12.58 ± 0.34, corn oil 12.49 ± 0.34, baseline 12.42 ± 0.35). The values of LDL size from this study were not comparable to values reported by any others using the method of McNamara et al.

(1987). However, it is difficult to know why this occurred because the authors did not report calibrating standards, methods, or unit of LDL size.

Contacos et al. (1993) compared the effects of fish oil, a 3-hydroxy-3-methylglutaryl- coenzyme A reductase inhibitor (pravastatin), and a pravastatin placebo on plasma lipids and lipoproteins in patients with hyperlipidemia. After a 6-week initial run-in phase, 32 patients were randomly assigned to three groups which received either fish oil (3 g of n-3 fatty acids/d), n = 10, pravastatin 40 mg/d, n = 10, or pravastatin placebo for 6 weeks. In the fish oil group LDL diameter significantly increased from 25.0 to 25.9 nm ($P < 0.05$). There were no changes in the placebo and pravastatin groups.

On the other hand, two studies have shown that the consumption of n-3 fatty acid did not change LDL size. Sorensen et al. (1998) studied the influence of consumption of n-3 fatty acid-enriched margarine on LDL size in forty-seven healthy men with a low habitual intake of fish. The study was a two-period randomized design. During a 3-week baseline period, all subjects consumed 30 g/day of sunflower oil margarine. After the baseline period they were assigned to consume either the sunflower oil or fish oil margarine, 30 g/day, for the following 4-week intervention period. Fish oil margarine contained 4 g of n-3 fatty acids per 30 g. The procedure for measuring LDL particle size was adapted from the method as described by McNamara et al. (1987). In contrast to the study by Suzukawa et al. (1995), LDL size was not significantly different between the groups before or after intervention (26.7 ± 0.2 nm baseline, 26.7 ± 0.2 nm sunflower oil, 26.8 ± 0.2 nm, fish oil).

Nenseter et al. (1992) also studied the effects of the consumption of ethyl esters of n- 3 fatty acids on LDL size in humans. The subjects received supplements of six

capsules daily, each capsule containing 1 g of either highly concentrated n- 3 fatty acids (85% EPA and DHA) (n = 12) or corn oil (56% linoleic and 26% oleic acid) (n = 11). After 4 months of oil supplementation, LDL size was measured by electron microscopy. No detectable differences in the diameter of n-3 fatty acid-enriched LDL particles versus control LDL were observed (24.5 ± 0.2 vs 25.0 ± 0.2 nm).

Limited data are available to evaluate the effect of other fatty acids on LDL size. Carmena et al. (1996) compared LDL size after feeding n-9 and n-6 fatty acids in eighteen healthy males. During the baseline period, all subjects consumed an olive oil-rich diet. After the baseline plasma lipid determination, subjects consumed n-6 fatty acid rich-sunflower oil diets (10% to 15% of daily energy for 3 weeks). At the end of this period, blood was drawn and subjects were placed for another three weeks on the n-9 fatty acid rich-olive oil diet, in which they consumed 62 g/day of olive oil, 22% of daily energy which is approximately two times greater than the amount which would be consumed on a 2500 Kcal diet which contained 10% MUFA. The size of LDL in serum samples was measured using PGGE. LDL size was larger after the sunflower oil diet than after the olive oil diet. The relative mobility was 0.39 ± 0.06 on the sunflower oil diet compared to 0.42 ± 0.07 on the olive oil diet. However, the amount of MUFA consumed is exceptionally large, thus this study may not reflect the outcome of a reasonable dietary intervention.

Dreon et al. (1998) studied the effect of saturated fatty acids on LDL size. One hundred-five men were randomly assigned to a low-fat (24% of energy as total fat, 6% of energy as saturated fat) and a high-fat (46% energy as total fat, 10% of energy as saturated fat) diet for 6 weeks each in a crossover design. Subjects were free-living and

were trained how to select the experimental diets by registered dietitians. To verify dietary intakes, dietary records on the subjects following each experimental diet were collected at the end of the sixth week of each diet. At the end of each six-week intervention period, blood samples were drawn. LDL particle size was measured using PGGE. High saturated fat intake increased concentration of larger LDL particles compared to low saturated fat intake (26.5 ± 0.1 vs 25.9 ± 0.1 nm).

Monkeys have frequently been used as a model to study the influence of different fatty acids on LDL particle size. Rather than using PGGE, agraose gel chromatograpy has been used to measure molecular weight as a way to look at LDL particle size. N-3 fatty acid feeding decreased LDL particle size as compared to saturated fatty acid feeding. The effects of n-9 and n-6 fatty acids on LDL particle size of monkeys were not consistent.

Parks and Bullock (1987) studied twenty-four adult male African green monkeys (*Ceropithecus aethiops*). The animals were fed diets containing 42 % of the calories as fat with 11 g of lard or fish oil/100g of diet for 8 months. LDL molecular weight was quantitated after being separated by agarose gel chromatography using the method of the Rudel et al. (1977). The fish oil group had significantly smaller LDL than the lard oil group (2.91 vs 3.43 g/μmol).

Linga et al. (1993) compared the effect of the consumption of fish oil and lard on LDL size in cynomolgus monkeys. Ten male cynomolgus monkeys were assigned to two experimental groups for two 15-week, crossover periods with a 5-week washout period. The diets contained 40 % of calories as fat. Half of the fat calories were derived from fish oil or lard. The molecular weight of LDL was determined by the method of Rudel et

al. (1977). Again LDL size after consumption of fish oil was reduced as compared with that of lard (3.58 ± 0.14 vs 4.76 ± 0.39 g/μmol).

Rudel et al. (1995) studied the effects of fatty acids on LDL particle size in forty-four adult male African green monkeys. The animals were divided into three experimental groups after being matched for similar plasma cholesterol concentrations in response to a baseline diet that was enriched with saturated fat. After the baseline diet period and a 10-week washout period, each group was fed for about 5 years the experimental diets which contained 35% of kcal as fat. The diets differed only in fatty acid composition. They contained saturated, n-9, or n-6 fatty acid-enriched oils. LDL particle size was measured as LDL molecular weight as described by Rudel et al. (1977). LDL molecular weight was significantly larger in the saturated and n-9 fatty acid fed groups than in the n-6 fatty acid-fed group (3.68 ± 0.08, 3.70 ± 0.11, and 3.37 ± 0.13 g/μmol; saturated fatty acid group, n-9 fatty acid group, and n-6 fatty acid group, respectively).

Wolfe et al. (1994) compared African green monkeys (n = 108) consuming diets enriched with n-6 polyunsaturated fat with animals consuming diets enriched with saturated fat from birth until young adulthood. The diets contained approximately 40% of calories from fat, 40% from carbohydrates, and 20% from protein. LDL particle size was determined as described by Rudel et al. (1977). There was no difference in LDL size with n-6 polyunsaturated fat versus saturated fat diets (2.93 ± 0.09 vs 2.94 ± 0.05 g/μmol).

It is difficult to compare the results in monkeys to those in humans on LDL particle size. All of the studies in monkeys have been conducted at Wake Forrest

University's laboratory in which investigators do collaborative projects. In addition, the methodology used in measuring LDL size was different from those used to measure LDL size in humans. The data from humans and monkeys collectively suggest that the influence of different fatty acids on LDL particle size is poorly understood.

Oxidation of LDL

The underlying primary cause of most cardiovascular disease is believed to be atherosclerosis, a progressive multifactorial disease of the artery wall (Badimon et al. 1993). One mechanism proposed to explain the development of atherosclerosis is the lipid hypothesis. This theory suggests that in the early stages of atherosclerosis lipid deposits are formed in the subendothelial space of the arterial wall due to increased concentration of plasma LDL, endothelial dysfunction permeability leading to a recruitment of monocytes into the arterial wall, and an imbalance of cholesterol influx and efflux (Steinberg et al. 1989). This theory was expanded to the oxidative modification hypothesis of atherosclerosis (Steinberg et al. 1989). This theory holds that LDL is oxidized by cells in the subendothelial space (endothelial cells, smooth muscle cells, and macrophages) making a proatherogenic particle that may help initiate early lesion formation (Steinberg et al. 1989; Reaven and Witztum 1996).

The oxidative modification of LDL is thought to occur in two stages. The first stage occurs before monocytes are recruited into the artery wall and results in the oxidation of lipids in LDL within the subendothelial space with little change in its major protein, apo B. This can cause LDL that is minimally oxidized. When LDL is minimally oxidized, it is capable of stimulating cell expression of a variety of cytokines and

adhesion molecules that may enhance monocyte chemotaxis, transmigration, adhesion, and stimulation of monocyte differentiation into macrophage as well as vascular smooth muscle cell proliferation and synthesis of extracellular matrix (Navab et al. 1991; Badimon et al. 1993; Berliner et al. 1995).

The second stage begins when monocytes are recruited to the lesion and converted into macrophages. Macrophages have enormous oxidative capacity. In this second stage, the LDL lipids are further oxidized but the protein portion of LDL (apo B) is also modified, leading to a loss of recognition of the LDL particle by the LDL receptor and a shift to recognition by the scavenger receptors on macrophages developing atherosclerotic plaque (Sparrow et al. 1989; Brown and Goldstein 1990). This shift in receptor recognition leads to cellular uptake of the LDL by receptors that are not regulated by the cholesterol content of the cell. The result is a massive accumulation of cholesterol. Such cholesterol-loaded cells have a foamy cytoplasm and have been called foam cells. They are the hallmark of the arterial fatty streak. The factors that contribute to the susceptibility of LDL to oxidize have not been clearly defined yet. However, understanding the determinants of LDL susceptibility to oxidize is essential for developing therapeutic strategies to inhibit this process.

Oxidation begins when a reactive radical abstracts a hydrogen atom from a polyunsaturated fatty acid (PUFA) on the LDL particle. Although this process presumably begins in the PUFA of surface phospholipids, it then spreads to involve the cholesterol esters and triacylglycerol in the core of the LDL particles.

In vitro, oxidation of LDL can be mediated by cells or transition metal ions such as iron and copper-ions (Steinbrecher et al. 1987; Esterbauer et al. 1988). During the

generation of oxidatively modified LDL, PUFA undergo peroxidative decomposition, yielding conjugated diene hydroperoxides and reactive aldehydes, some of which form covalent bonds with apo B (Palinski et al. 1989). Often, in vitro oxidation of the LDL particle is monitored at 234 nm absorbance by measuring the continuous production of conjugated dienes (Esterbauer et al. 1989). This abosorption develops in LDL during oxidation through the formation of conjugated fatty acid hydroperoxides (-CH=CH-CH=CH-CHOOH-), the primary products of lipid peroxidation from PUFA. With this method three phase of oxidation can be obtained. The first is the lag time during which time there is almost no production of conjugated dienes because antioxidants in the LDL are oxidized in preference to PUFA. The second phase is the rate of production of conjugated dienes, usually referred to as the rate of oxidation of LDL, and is characterized by a rapid increase in diene formation. The third is the maximum production of conjugated dienes. Many studies that have measured oxidative susceptibility in this way have reported only changes in lag time produced by dietary intervention. However, the rate at which the PUFA form conjugated dienes as well as the maximum amount of them formed also contributes information valuable to understanding this process.

Oxidation of LDL can be also measured by evaluation of net negative charge on apo B. The oxidation of lipids in LDL proceeds by a free radical-mediated mechanism that increases the negative charge on apo B (Noriko and Niki 1987).

Various antioxidants protect from the oxidative modification of LDL. Niki et al. (1993) suggested that water-soluble antioxidants such as vitamin C and uric acid act as the first defense to protect from initiating LDL oxidation. Lipophilic antioxidants in LDL

such as vitamin E and ubiquinol-10 scavenge reactive radical attacking from outside and also within the LDL. The length of the lag time is directly proportional to the amount of antioxidants (Niki et al. 1993; Thomas et al. 1994). Thomas et al. (1994) studied the association between PUFA (n-6 or n-3)-enriched LDL and α-tocopherol. The lag time was linearly related to the amount of α-tocopherol while the rate of oxidation was linearly dependent upon the concentration of PUFA.

The role of fatty acids on oxidative susceptibility of LDL

Among the factors that may contribute to the oxidative susceptibility of the LDL particle are its different lipid molecules and concentration of the different PUFA. The fatty acid content of LDL particles has been shown to be easily modified by dietary fatty acids (Reaven and Witztum 1996; Wander et al. 1996). Diets enriched in linoleic acid (18:2n-6) increased the content of linoleic acid in LDL. In contrast, diets enriched in oleic acid (18:1n-9) produced LDL particles rich in oleic acid (Reaven et al. 1994; Davidson et al. 1997). Diets enriched in fish oil increased the content of EPA/DHA in LDL (Wander et al 1996). Since dietary fatty acids influence the fatty acid composition of LDL, the amount and type of fat in the diet may affect susceptibility of LDL to oxidative damage. Esterbauer et al. (1987) found that of the PUFA degraded in LDL obtained from humans consuming undefined diets, 85% was linoleate while only 15% was arachidonate. Since linoleic acid constituted nearly 90% of the polyunsaturated fatty acids in these LDL, it was the major substrate of LDL oxidation.

Reaven et al. (1991) compared oxidative susceptibility of the LDL isolated from subjects consuming an oleate-enriched diet with that of LDL from subjects on a linoleate-

enriched diet. Lag time was two times longer in the LDL from the oleate-supplemented group than linoleate supplemented group (p=0.0001). Production of conjugated dienes was significantly lower in the LDL from the oleate-supplemented group than linoleate supplemented group (p=0.0001). In addition, LDL from the oleate-supplemented group was less degraded by macrophages than LDL from the linoleate-supplemented group (p=0.001). Similar results have been found in other studies (Berry et al. 1992; Bonanome et al. 1992; Davidson et al. 1997).

Oxidative susceptibility of LDL enriched in n-3 fatty acids is less clear. Wander et al. (1996) found that supplementation with fish oil shortened the lag time approximately 18 min (P<0.001) suggesting increased oxidation, slowed oxidation rate 25% (P<0.001) suggesting decreased oxidation, and did not significantly affect the maximum production of conjugated dienes. Whitman et al. (1994) investigated LDL oxidation in female Yucatan miniature swine fed with either fish oil or a control oil (a mixture of corn oil, safflower oil, and beef tallow). They found that fish oil supplemented LDL had a 30% shorter lag time and a 30% slower oxidation rate but no difference in atherosclerotic lesion development of all major blood vessels. Thomas et al. (1994) measured rates of oxidation of LDL isolated from cynomolgus monkeys and found that linoleate-enriched diets gave significantly higher rates of oxidation than either saturated, monounsaturated or EPA/DHA-enriched LDL.

On the other hand Nenster et al. (1992) reported that LDL from the fish oil and corn oil fed groups showed similar susceptibility to copper-catalyzed lipid peroxidation, as indicated by the amount of lipid peroxides and degradation of oxidatively modified LDL in cultured macrophages. In addition, Suzukawa et al. (1995) reported that

macrophages that were supplemented with fish oil displayed a significantly ($P < 0.001$) higher capacity to oxidize LDL than either control cells or cells supplemented with corn oil.

The role of LDL density on its oxidative susceptibility

In addition to its composition and PUFA concentration, the size of the LDL particle may contribute to its oxidative susceptibility. Previous studies have suggested that small LDL was more susceptible to oxidation than the larger particles (de Graaf et al. 1991; Tribble et al. 1992; Chait et al. 1993; Dejager et al. 1993; Tribble et al. 1994; Tribble et al. 1995a; Tribble et al. 1995b). However, these experiments have not shown clearly the enhanced oxidative susceptibility of small LDL.

For example, Chait et al. (1993) compared oxidative modification in six subfractions of LDL isolated from phenotype A and phenotype B subjects. Although lag time was inversely related ($p < 0.001$) to LDL density in both groups of subjects, the rate of oxidation of LDL did not correlate with LDL subfrations.

Dejager et al. (1993) studied the oxidative susceptibility of LDL particle subfractions in 9 hyperlipidemic subjects. Five LDL subfractions were isolated by density gradient ultracentrifugation in the density range 1.019-1.063 g/ml: LDL-1, 1.019-1.023 g/ml; LDL-2, 1.023-1.029 g/ml; LDL-3, 1.029-1.039 g/ml; LDL-4, 1.039-1.050 g/ml; LDL-5, 1.050-1.063 g/ml. The LDL profile was skewed towards the dense subfractions (LDL- 4 and LDL-5) in all patients. They found a reduction ($P < 0.01$) in lag time in LDL-5; however, they did not report the rate of oxidation.

On the other hand, de Graaf et al. (1991) measured oxidative susceptibility in three LDL subfractions in 11 healthy people (LDL1, d =1.030-1.033; LDL2, d = 1.033-1.044; LDL3, d = 1.040-1.045 g/ml). A significant inverse linear relation was found between LDL size and lag time ($P < 0.001$). The LDL size showed a negative linear relation with the rate of oxidation ($P < 0.001$) and the amount of conjugated dienes formed in the LDL subfractions. However, the density range of LDL was 1.030-1.045 g/ml in this study. They used only the large LDL fraction.

The role of composition of LDL on its oxidative susceptibility

The concentration of different lipid classes of LDL subfractions may also be an important factor for its oxidative susceptibility although the information gathered to date is contradictory. Tribble et al. (1995) reported that small, dense LDL particles had more cholesteryl ester and less free cholesterol than large, buoyant ones. They suggested that free cholesterol, which was found predominantly on the surface of the LDL, could be responsible for protecting the larger particles from lipid oxidation. In support of this theory, Rudel et al. (1985) in their study with monkeys suggested that the increased atherogenecity of LDL particles was associated with the enrichment of these particles with cholesteryl esters. However, in humans de Graaf et al. (1991) reported that large buoyant LDL particles had more cholesteryl ester than smaller ones whereas Dejager et al. (1993) reported that the concentration of cholesteryl ester and free cholesterol was not significantly different between large LDL and small LDL. Collectively, these data indicate that the influence of the composition of LDL on its oxidative susceptibility requires further investigation.

The shortened lag time in small LDL is explained in part by a lower content of antioxidants, including ubiquinol-10 and α-tocopherol. Previous studies have shown that small LDL contain significantly lower α-tocopherol and ubiquinol-10 concentrations than large (de Graaf et al. 1991; Tribble et al. 1995; Tribble et al. 1994). In addition, Tribble et al. (1995) suggested that the course of oxidative depletion of α-tocopherol in large LDL subfractions appears to involve not only differences in the content of α-tocopherol but also differences in the rate of utilization of this antioxidant. They observed greater rates of depletion of α-tocopherol in dense compared to large LDL. However, Dejager et al. (1993) found that the oxidative susceptibility of LDL in two LDL subfractions was independent of vitamin E content.

The fatty acid contents of LDL subfractions may influence its oxidative susceptibility although few studies have investigated this. de Graaf et al. (1991) suggested that small LDL was less resistant to oxidation than large because they have significantly higher concentrations of polyunsaturated fatty acids including 18:2n-6 and 20:4n-6. In this study the fatty acid content of LDL was expressed relative to the mg of vitamin E. The content of vitamin E was significantly lower in small than large LDL. The difference in the fatty acids content between small and large LDL was not clear in this study since they calculated the difference based on vitamin E concentration.

CHAPTER 3

THE EFFECT OF SUPPLEMENTATION WITH N-9, N-6, AND N-3 FATTY ACIDS ON PLASMA LIPID, LIPOPROTEIN, AND APOLIPOPROTEIN B CONCENTRATIONS AND LDL PARTICLE SIZE IN POSTMENOPAUSAL WOMEN

Ye-Sun Lee
Shi-Hua Du
Jane V. Higdon
Tianyiang Wu
Rosemary C. Wander

Plan to submit to *American Journal of Clinical nutrition*

Oregon State University
Department of Nutrition of Food Management
Corvallis, Oregon

Abstract

Background: Current dietary recommendations have placed increasing emphasis on decreasing the consumption of dietary fat as an important element to decrease risk of cardiovascular disease (CVD). A number of different studies have demonstrated that in addition to total fat, the fatty acid composition of diets influence the risk of CVD. However, the optimal ratio of polyunsaturated fat to monounsaturated and saturated fat is not well defined, especially if n-6 and n-3 fatty acids are considered. Although postmenopausal women have an enhanced CVD-risk profile compared to premenopausal women, minimal experimental data have been accumulated to understand why.

Objective: This study was designed to determine the effect of supplementation with different fatty acids on the risk factors of CVD in postmenopausal women.

Design: Sixteen healthy, postmenopausal women were randomly assigned in a three period crossover trial with three treatments. They were given 15 g/d supplements of oleic acid-rich sunflower oil (TS, n-9), linoleic acid-rich safflower oil (SO, n-6), or fish oil (FO, n-3). Each period lasted 5 weeks followed by a 7-week washout interval.

Results: Total cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations were not significantly different in the TS-, SO-, and FO- supplemented groups. However, high density lipoprotein cholesterol (HDL-C) concentration tended to increase in the FO-supplemented group compared to the TS-supplemented group ($P = 0.07$) and was significantly higher in the FO-supplemented group compared to the SO supplemented one ($P = 0.05$). A significant decrease in plasma triacylglycerol concentration was also observed in the FO-supplemented group when compared to the TS- ($P = 0.0001$) and SO- ($P = 0.0175$) supplemented groups. Fish oil supplementation

lowered the ratio of plasma triacylglycerol to HDL-C significantly compared to the TS- (0.0001) and SO- (0.02) supplemented groups. The concentration of apolipoprotein B (apo B) was decreased in the FO- supplemented group compared to that in the TS- (P = 0.005) and SO- (P = 0.01) supplemented groups. The LDL particle size was significantly larger in the FO-supplemented group compared to the TS-supplemented group (P = 0.03). **Conclusions:** The results of this study showed that the FO supplementation increased the LDL particle size, reduced the concentration of plasma triacylglycerol and apo B concentration and the ratio of triacylglycerol to HDL-C compared to the TS and SO supplementation. These findings demonstrate a greater potential antiatherogenic property of dietary fish oil than either oleic acid-rich or linoleic acid-rich oil in postmenopausal women.

Introduction

Cardiovascular disease (CVD) is the number one killer in the United States. Although CVD occurs at an earlier age in men than it does in women, as women approach the age of menopause, the risk of CVD begins to rise. Plasma total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) are well-defined risk factors of CVD in both genders (1,2). Numerous epidemiological studies have shown that total cholesterol and LDL-C concentrations are positively and HDL-C concentration negatively related to CVD (3-6). Although the literature on epidemiological associations between plasma triacylglycerol and CVD has

not been completely consistent, the majority of observational studies demonstrate a significant positive relationship (7-13) that may be greater for women than men (14).

The ratio of total cholesterol/HDL-C and LDL-C/HDL-C are also well-established predictors of CVD (1,2,15). For example, Gaziano et al. (15) reported that a one unit increase in total cholesterol/HDL-C and LDL-C/HDL-C were associated with 49% and 75% increases in risk of myocardial infarction, respectively. Recently, the ratio of triacylglycerol to HDL-C has been shown to be a better predictor of myocardial infarction than either of them (15).

Case-control studies have also shown that apolipoprotein (apo B), the major protein in LDL, is associated with increased risk of CVD (16,17), as is the size of the LDL particle. Subjects with a heritable phenotype characterized by a predominance of smaller, more dense LDL (phenotype B) show a higher risk of CVD relative to individuals with predominantly larger, more buoyant LDL (phenotype A) (18,19). Phenotype B subjects had higher concentration of triacylglycerol, apo B, intermediate-density lipoprotein cholesterol (IDL-C), VLDL-C, and lower concentrations of HDL-C (26,27).

Recent studies have demonstrated that in addition to body mass index (BMI), the amount of visceral adipose tissue was significantly associated with risk factors for CVD (28-30). Waist circumference and abdominal sagittal diameter, two measures of abdominal obesity, were significantly correlated with dyslipidemia, higher blood pressure, impaired glucose tolerance, and higher plasma insulin concentrations (21,22).

Current dietary recommendations have placed increasing emphasis on the consumption of dietary fat as an important element to decrease risk of CVD, although the

optimal ratio of polyunsaturated fat to monounsaturated and saturated fat is not well defined, especially if n-6 and n-3 fatty acids are considered. The effect of monounsaturated fatty acids (MUFA) on risk of CVD has not been consistent. In the Seven Countries Study conducted 30 years ago, investigators attributed the decreased risk of CVD that occurred in middle-aged men living in Mediterranean to, at least in part, the high content of olive oil, rich in MUFA, in the diets (23). Since then numerous studies have shown that when MUFA were substituted for saturated fatty and/or carbohydrates in the diet, total cholesterol and LDL-C concentrations were significantly decreased but HDL-C concentration was not changed in healthy human subjects (24-30). On the other hand, Nydahl et al. (31) found HDL-C concentration was also significantly decreased with a MUFA-rich diet as well as total cholesterol and LDL-C.

Although n-6 fatty acids have favorable effects on lipoprotein metabolism, recently it has been suggested that their consumption may cause detrimental effects. For instance, Hedgson et al. (32) and Gordon et al. (33) showed that n-6 fatty acid-rich diets also decreased HDL-C concentration. In addition, these fatty acids may increase the susceptibility of LDL to oxidative modifications (34,35), a proposed risk factor for CVD. Several studies have shown that n-6 fatty acids increase the secretion of insulin, reduce insulin catabolism, and cause impaired insulin action leading to insulin resistance (36-39). Excess intake of n-6 fatty acids may also interfere with metabolism of n-3 fatty acids.

Epidemiological studies in Greenland Eskimos led to the hypothesis that fish rich in n-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), were useful for reducing CVD (40,41).

Subsequent epidemiological studies showed a significant inverse relation between fish consumption and the risk of death from CVD (42-51). However, the results of other studies are inconsistent with these findings (52-55). Experimental and clinical studies have found that fish and fish oils rich in n-3 fatty acids have beneficial effects on plasma lipid, lipoprotein metabolism and risk of development of CVD (56-60). Fish and fish oil consumption have been shown to consistently reduce triacylglycerol level, particularly in hypertriglyceridemic subjects (56,57,61), but the effect on LDL-C and HDL-C is inconsistent (61,62).

To make optimal recommendations about dietary fat, an understanding of the role of different fatty acids on indices of cardiovascular risk must be known. Although cardiovascular risk increases in postmenopausal women, few intervention studies have been conducted in this population. Therefore, this study was designed to investigate the effect of supplementation with n-9, n-6, and n-3 fatty acids on plasma lipid, lipoprotein, apo B concentrations, ratio of triacylglycerol to HDL-C and LDL-C to HDL-C, and LDL particle size.

Subjects and Methods

Subject recruitment, screening, and selection: Sixteen subjects were recruited from the Oregon State University campus and surrounding community. They were healthy, nonsmoking, normolipidemic postmenopausal females, aged 53-72 years who were on hormone replacement therapy (HRT). Subjects were recruited primarily by advertising in local newspapers and posting flyers around campus. The first interview with potential

participants was made by telephone contact. The telephone screening form is shown in **Appendix 1**. Potential subjects had to meet the following criteria. They had to be postmenopausal, their menstrual cycles ceased for at least six months or they used HRT for a comparable period of time. Their body weight was between 50 and 85 percentiles of their mean weight range for their age (63), they had been at a stable weight for the past year, and they were willing to maintain this weight during this study. Individuals who were on chronic medication, such as corticosteroids, thyroid hormones, lipid lowering drugs, or high blood pressure drugs, were not included in the study. Nutritional supplements were not permitted except calcium (1500 mg/day including diet and supplement). The women had to also be free of heart disease or strokes, cancer, hypertension, renal or liver disease, bleeding disorders, active ulcer or gallbladder disease, or diabetes. Women with unusual dietary habits, i.e., macrobiotic diets or insufficient energy intake, were excluded.

Once a potential participant was identified, she visited the Metabolic Feeding Unit in the Department of Nutrition and Food Management at Oregon State University for the second part of the screening procedure. The purpose of the study and their contributions to it were explained to them and informed consent was signed (**Appendix 2**). Approval to work with human subjects was obtained from the Oregon State University's Human Subjects Committee.

At this second meeting the subjects were further evaluated by measuring several variables. First, approximately 20 ml of blood was drawn from the potential subject. Blood was sent to the local hospital for a Chemscreen and complete blood count (CBC). Blood pressure was measured using a mercury column sphygnomanometer, following the

technique recommended by the American Heart Association, by the same individual (64). The cuff bladder was inflated to 20 mm Hg above the systolic pressure previously determined by palpation. As the pressure in the bladder was released the level of appearance of the Korotkoff sounds was recorded as the systolic pressure and the level at which no further sounds were audible were recorded as the diastolic pressure. Blood pressure was measured at least once on each arm.

Height and weight were measured in light clothing without shoes. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). BMI is a global measure of adiposity. In addition, the regional pattern of fat deposition was obtained by measuring waist and abdominal circumference and abdominal sagittal diameter. Waist and abdominal circumference were measured using a measuring tape. Waist circumference was measured at the midpoint between the top of the iliac crest and the lowest rib. Abdominal circumference was measured at the level of the umbilicus. The measurement was made right after the waist circumference so that the tape remained around the subject's body. The abdominal sagittal diameter was measured using a sliding beam caliper. The subject was placed on an examination table in the supine position with both arms relaxed by her sides and both legs fully extended. The iliac crest on the left and right sides were palpated and a small horizontal mark was made in the midline of the anterior abdomen at the level of the iliac crests. The lower arm was inserted underneath the small of the subject's back. The arms of the caliper were perpendicular to the subject's body. The upper arm of the caliper was slid down until it was 2 cm directly above the anterior abdominal mark. The caliper's shaft was checked to ensure that it was vertical. The caliper arm was moved down so that it was touching, but not compressing,

the abdomen. At the end of a normal exhalation, the sagittal diameter was read from the scale on the caliper to the nearest 0.1 cm. This process was repeated three times. The waist circumference and abdominal sagittal diameter was used an index for visceral adipose tissue.

The subjects completed a medical history (**Appendix 3**). The type of hormone replacement therapy they used was established. The lipid profile (total cholesterol, triacylglycerol, HDL cholesterol, and LDL cholesterol) was measured at the Oregon State University Lipid Laboratory using methods discussed later. The lipid profile of each potential subject was compared to the mean values for individuals of similar age, gender, and race from the U.S. population (65). Those whose values were greater than the 75th percentile were not included in the study. They were asked to keep a 3-day diet record. Records were kept for two week days and one weekend day. The records were mailed back to the Lipid Laboratory in stamped, self-addressed envelopes provided to them. The diets were analyzed using Food Processor Plus 7.0 (ESHA Research, Salem, OR). Subjects who completed this study were paid.

Experimental Design: A single-blind, three-treatment, three-period, randomized, crossover trial was used. Sixteen subjects began the study. The treatments were 15 g daily supplements of oleic acid-rich sunflower oil, a good source of n-9 fatty acids (TS), linoleic acid-rich safflower oil, a good source of n-6 fatty acids (SO), or EPA/DHA-rich fish oil, a good source of n-3 fatty acids (FO). The order in which the subjects received the supplements was randomly assigned in a balanced design. There were six treatment-sequence patterns as shown in **Table 3.1**. By the end of the study each subject had

received all three treatments. Each of the three treatment periods lasted five weeks followed by a 7-week washout period to limit any carry over effects. The total study period for each subject was 29 weeks.

TABLE 3.1
Treatment-sequence patterns of the three dietary oils given to postmenopausal women

Subject ID	Group[1]	Sequence[2]	Period 1	Period 2	Period3
2	Red	1	TS	SO	FO
16	Red	6	SO	TS	FO
7	Red	5	FO	SO	TS
5	Red	4	TS	FO	SO
11	Blue	2	FO	TS	FO
14	Blue	3	SO	FO	TS
1	Blue	4	TS	FO	SO
4	Blue	5	FO	SO	TS
8	Green	1	TS	SO	FO
6	Green	2	FO	TS	SO
9	Green	5	FO	SO	TS
15	Green	6	SO	TS	FO
3	Yellow	2	FO	TS	SO
12	Yellow	3	SO	FO	TS
10	Yellow	4	TS	FO	SO
13	Yellow	6	SO	TS	FO

[1] Each group consisted of 4 subjects.
[2]Sequence was the order in which the subjects received the supplements. The subjects were randomly assigned in a balanced design.

Subjects: The subjects were described as belonging to a Red, Blue, Green, or Yellow group. This grouping merely indicated on what days the subjects came to the Metabolic Feeding Unit. Each group had four subjects. This was done because the ultracentrifuge could handle samples from only 4 subjects per day. The women in these groups came to

the Metabolic Feeding Unit different weeks. The week when women from each of the groups came to the laboratory is given in **Appendix 4.**

During the study the participants visited the Metabolic Feeding Unit on Monday and Wednesday on the first and sixth weeks of each period. Blood samples were drawn, empty supplement bottles were taken, any leftover capsules were counted, new supplements were given if appropriate, and breakfast was provided. A typical breakfast menu was a low fat diet which included fresh baked bread, bagels, granola, fresh fruit, orange juice, milk, yogurt and coffee. In addition, their weight and blood pressure were measured during each visit. A profile of their current physical activity was collected and analyzed at each period by the method of Mayer et al. (66). A copy of the form used to assess physical activity is given in the **Appendix 5**. Subjects were also asked to visit the Metabolic Feeding Unit on Monday of the third week of each period. During this visit empty supplement bottles were taken, new supplements were given and breakfast was provided. This visit was made to encourage subjects' involvement in the study thus improving compliance.

Subjects were asked to keep 3-day dietary records once during each period. These were analyzed using Food Processor. These records were used to establish that the subjects did not modify their diets significantly over the course of study.

Administration of Supplements : The TS was obtained in bulk from Humpco, Memphis, TN and the SO was obtained in bulk from Arista Industries, Darien,CO. The TS and SO were encapsulated into size 00 hard gelatin capsules at the Professional Compounding Pharmacy, Corvallis, OR. Fifteen grams of oil were equally distributed into 20 capsules

for each day. One-third of the oil was encapsulated before period 1 began; one-third between periods 1 and 2; and one-third between periods 2 and 3. The FO was obtained in 1g soft gel capsules from the National Institute of Health's (NIH) Fish Oil Test Material Program. The capsules were packed into small individual 2" by 4" plastic bags (Cole Parmer, Vernon Hills, IL) to provide the subjects with a convenient way to manage their supplements. Five to seven bags of capsules containing the oils were put into white opaque bottle. Subjects were instructed to keep the capsules in the bag in the bottle under refrigeration, removing only the packages needed for a day's dose. This was done to minimize oxidation of the oils.

The participants consumed 15g of oil /day. Each oil provided large amount of fatty acids that differed in the degree of unsaturation. The fifteen gram supplement of TS supplied 12.3 g of oleic acid, that of SO 10.5 g of linoleic acid, and that of FO 2.0 g of EPA and 1.4 g of DHA. The fatty acid profile of the plasma was measured as well as the disappearance of the capsules was monitored to determine compliance of the subjects. This intake of fish oil previously found to increase the plasma concentration of the EPA and DHA while not providing a fat intake that was above usual values (56). Davidson et al. (67) had also shown that an intake of oleic acid when provided in the diet in amounts similar to that provided by 15 g oil would increase plasma oleic acid concentration. The fatty acid profile of the oils was measured by gas chromatography as previously described by Song and Wander (68) and is given in **Table 3.2**. Heptadecanoic acid (Nu-Chek Prep, Elysian, MN) was added as an internal standard.

TABLE 3.2
Fatty acid profile of oils given to postmenopausal women as dietary supplement

	TS	SO	FO[1]
		mg/g oil	
14:0	ND[1]	1.6	79.1
15:0	ND	ND	4.5
16:0	32.6	69.1	144
18:0	35.0	24.3	26.3
20:0	3.1	3.4	4.3
22:0	9.9	2.0	1.3
23:0	ND	ND	ND
24:0	3.1	1.3	ND
[2]ΣSFA	83.7	102	260
14:1	ND	ND	0.1
16:1n-7	1.0	1.4	89.4
18:1n-9t	ND	ND	ND
18:1n-9c	818	123	50.6
18:1n-7	ND	9.9	24.4
20:1n-9	2.4	1.8	7.7
24:1	ND	1.2	2.7
[3]ΣMUFA	821	136	175
18:2n-6tt	ND	ND	ND
18:2n-6cc	38.5	697	14.3
18:3n-3	1.1	1.2	9.7
18:4n-3	ND	ND	24.1
20:2n-6	ND	ND	1.4
20:3n-6	ND	ND	2.4
20:4n-6	ND	ND	7.7
20:5n-3	ND	1.1	131
22:5n-3	ND	ND	23.4
22:6n-3	ND	ND	96.1
[4]ΣPUFA	39.6	699	304

[1]Analysis was performed by National Institute of Health, Fish Oil Test Material Program.
[1]ND is not detectable.
[2]ΣSFA is 14:0+15:0+16:0+18:0+20:0+21:0+22:0+23:0+24:0
[3]ΣMUFA is 14:1+16:1n-7+18:1n-9*t* +18:1n-9*c* +18:1n-7+20:1n-9+24:1
[4]ΣPUFA is 18:2n-6*tt* +18:2n-6*cc*+18:3n-3+18:4n-3+20:2n-6+20:3n-6+ 20:4n-6+20:5n3+22:5n-3+22:6n-3

Each fish oil capsule contained 1 mg/g α-tocopherol, 1 mg/g γ-tocopherol, and 0.02% tertiary butylhydroquinone (TBHQ). TBHQ was added as an antioxidant. It is metabolized almost completely within a few days after ingestion and excreted in the urine. It does not function as an antioxidant in vivo since it is not stored in tissue (69). The α-tocopherol, γ-tocopherol and TBHQ content of the TS and SO were matched to that of the NIH fish oil before they were encapsulated. Tenox 20A (20 % TBHQ), Vitamin E-5-676 (72 mg/g α-tocopherol), and Tenox GT-1(300 to 325 mg/g γ-tocopherol + 75 mg/g α-tocopherol + 75 mg/g δ-tocopherol) were generously provided by Eastman Chemical Company (Kingsport, TN). The concentration of α-tocopherol, γ-tocopherol, and δ-tocopherol in the oils before and after these additions are shown in **Table 3.3**.

TABLE 3.3
Vitamin E concentration of the TS and SO before it was matched to that the of FO

	TS		SO		FO
	Before[1]	After[2]	Before	After	
α-Tocopherol(mg/g oil)	0.71	1.20	0.60	1.12	1.23
γ-Tochopherol(mg/g oil)	ND[3]	1.34	ND	1.36	1.31
δ-Tocopherol (mg/g oil)	ND	0.45	ND	0.53	0.54
α-TE (mg/g oil)[4]		1.34		1.26	1.37
α-TE /day (mg/g oil x 15)		20.1		18.9	20.6
IU/day[5]		29.9		28.2	30.6

[1]Before refers to before the addition of antioxidants to match that found in the FO.
[2]After refers to the amount present after the addition of antioxidant.
[3]ND refers to not detectable.
[4]α-TE = RRR-α-tocopherol equivalents obtained by summing mg α-tochopherol, 0.1 mg x γ-tochopherol, and 0.01 mg x δ-tocopherol.
[5]IU = 0.67 x RRR-α-tocopherol.

Oxidation of oils: Peroxide value, a measure of lipid peroxidation and p-anisidine value, an indication of the aldehyde content of fats, were measured before each period to track oxidation of oil by methods used previously in our laboratory (56,70).

Blood sampling and plasma preparation: About 30 to 40 ml of blood was collected after an overnight fast of $\geq$ 10 hours into tubes containing Na_2EDTA (1.5 mg/ml plasma). Plasma was separated by low-speed centrifugation at 600 x g for 15 min at 4°C (TJ-6 Centrifuge, Beckman, Palo Alto, CA). Aliquots were made for each assay and stored at -80°C until processed.

Plasma Assays: The plasma fatty acid profile was measured by gas chromatography as previously described by Song and Wander (68). Plasma cholesterol concentration was measured enzymatically by a modification of the method of Allain et al. (71) (Sigma Kit, Cat.No. 352-100, St. Louis, MO). Triacylglycerol concentration was determined by a modification of the method of McGowan et al. (72) (Sigma Kit, Cat.No. 336-10). The cholesterol concentration of the HDL fraction was measured by the enzymatic method in samples after the precipitation of the LDL fractions with phosphotungstic acid in conjunction with $MgCl_2$ (73) (Sigma Kit, Cat. No. 354-LA). LDL concentration was calculated using the Friedewald formula: LDL cholesterol = Total cholesterol - HDL cholesterol - triacylglycerol/5 (mg/dl) (74). The cholesterol assay met the National Cholesterol Education Program's performance criteria for accuracy and precision. Plasma apo B concentrations were analyzed by an immunoturbidometric as described previously (75) (Sigma Kit, Cat. No. 357A).

LDL size: The procedure for measuring LDL particle size was adapted from the methods described by McNamara et al. (76) and Nichols et al. (77). Plasma from each subject that had been frozen and stored at -80 °C was rapidly thawed. LDL size was measured by non-denaturing gradient gel electrophoresis using 2.5-16% polyacrylamide gradient gels (Isolab, Arkon, OH). A chamber of the appropriate size was not available commercially so one was assembled from readily available parts. Three µl of plasma was loaded onto a gel. Gels were run at pH 8.4 ± 0.1 in a Tris-borate buffer solution. Pre-electrophoresis was performed at 125 volts for 30 minutes before the samples were applied. After application of samples the gels were run for 24 hours at 125 volts. Gels were stained using protein-staining dye, 0.01% R-250 blue (Sigma Diagnostics) for 2 hours. After staining they were soaked in the destaining solution overnight. They were scanned with the Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA) to determine migration distances of each of the major bands. LDL particle diameters corresponding to each of these bands were calculated from calibration curves using standards of known size. Two lyophilized LDL standards (diameters of 25.1 nm and 27.8 nm) were kindly provided by Dr. R. Krauss from the Donnor Laboratory at the University of Berkley in California. In addition, latex beads (38. 0 nm) (Dow Chemical Company, Indianapolis, IN) and high molecular weight electrophoresis calibration kit (thyroglobulin,17.0 nm, apoferritin 12.2 nm, and catalase 10.4 nm; Parmacia, NJ) were used. However, the latex beads and the high molecular weight electrophoresis calibration kit were excluded from the calibration of LDL size since their size was outside the appropriate range. The detailed procedure for measuring LDL particle size is given in **Appendix 6**.

Statistical analysis: Summary statistics were calculated (mean and standard error). Normal distribution and homogeneity of variance were checked before data analysis. The balanced crossover design of this study allowed examination for carryover effects. No carryover was demonstrated for any variable. The effects of each supplement on the variables of interest were compared using one-way analysis of variance (ANOVA) with between subjects sources of variation including sequence of treatment and individual subjects nested within sequence and within subjects sources of variation including period and treatment (78). The calculations were performed using general linear model (GLM) of Statistical Analysis System (SAS) software (version 6.12; SAS Institute, Cary NC). Pair-wise comparisons were made using Fisher's least significant difference test to determine significant differences among treatment (78). P-values ≤ 0.05 were reported as significant. The difference between initial and final values was used for apo B concentrations to compare treatment effects since variations in initial values were demonstrated.

The strength of the relationships between LDL particle size and BMI, blood pressure, abdominal circumference, sagittal diameter, and waist circumference were measured by Pearson correlation coefficients in the subjects before supplementation occurred. P-values ≤ 0.05 were reported as significant.

Results

Subjects: Fifteen subjects completed this study. Overall, compliance to the study was excellent as indicated by the change in the plasma fatty acid profile and disappearance of the oil supplementation capsules. No side effects were reported from the supplementation. All subjects were in good physical condition at the time of the study as indicated from the medical records, Chemscreen, and complete blood count.

The initial characteristics of the subjects are given in **Table 3.4**. According to their BMI the subjects were marginally overweight since the upper limit of a healthy weight BMI is 24.9 kg/m^2 (79). The usual values for abdominal sagittal diameter range from 14 to 35 cm (80). Waist circumferences greater than 100 cm or abdominal sagittal diameter values greater than 25 cm are associated with CVD-risk factors (81). Abdominal circumference values estimated from regression equation by Lemieux et al. (82) greater than 186 cm are associated with CVD-risk. The mean of waist circumference, abdominal saggital diameter, and abdominal circumference were 80 cm, 19 cm, and 94 cm, respectively. These measures of visceral adiposity did not suggest that the subjeccts were at an increased risk of CVD.

The average value of plasma total cholesterol of the subjects was 1 % higher than the desirable level according to the guideline issued by the U.S. National Cholesterol Education Program Adult Treatment Panel II (65) (<200 mg/dl, 5.2 mmol/L). However, the average LDL and HDL cholesterol of the subjects met or surpassed the desirable level (<130 mg/dl, 3.4 mmol/L, ≥ 60 mg/dl, 1.6 mmol/L, respectively). The average plasma triacylglycerol concentration also met the desirable level (<200 mg/dl, 2.3 mmol/L). The

average LDL size was 26.1 ± 0.6 nm. Fourteen subjects were LDL phenotype A (> 25.5 nm) and one subject was phenotype B (< 25.5 nm).

TABLE 3.4
Characteristic of postmenopausal women before supplementation of dietary oils

	Mean ± SD (n = 15)
Age (y)	57.9 ± 5.8
BMI (kg/m^2)	25.9 ± 3.5
Plasma cholesterol (mg/dl)	202 ± 21
HDL cholesterol (mg/dl)	60 ± 15
LDL cholesterol (mg/dl)	115 ± 24
Plasma triacylglycerol (mg/dl)	132 ± 41
Glucose (mg/dl)	88.9 ± 7.6
Systolic blood pressure (mmHg)	129 ± 10
Diastolic blood pressure (mmHg)	76 ± 8
Hemoglobin (g/dL)	13.2 ± 0.7
Hematocrit (%)	40.2 ± 2.4
LDL size (nm)	26.1 ± 0.6
Waist circumference (cm)	80 ± 10
Abdominal circumference (cm)	94 ± 11
Sagittal diameter (cm)	19 ± 2

Blood pressure, blood glucose, and hemoglobin and hematocrit levels were within normal ranges. The alcohol consumption ranged from 0 to 16 drinks/wk and the mean was 2.3 drinks/wk. Most of subjects used 0.3 to 1.25 mg of conjugated equine estrogen for their hormone replacement therapy. Eleven subjects used 0.625, two subjects used 0.3, and one subject used 1 mg of conjugated equine estrogen. Only one subject used 1 mg of estradiol for her hormone replacement therapy. Not all subjects used combined HRT. Of those who did six subjects used 2.5 and one used 5 mg. The average leisure

physical activity level was 2.5 hours/wk. The physical activity level did not change during the study as indicated by the records from the three periods.

The correlation coefficients between LDL particle size and initial characteristics of the subjects were calculated. There was no significant correlation between LDL particle size and BMI, blood pressure, abdominal circumference, sagittal diameter, waist circumference, total cholesterol, LDL-C or apo B as shown in **Table 3. 5**. However, LDL particle size was significantly inversely related with plasma triacylglycerol concentration. HDL-C concentration tended to be directly related to LDL particle size.

TABLE 3.5
Correlation coefficients between LDL particle size and characteristics of subjects before dietary oil supplementation

	LDL particle size	
	R	P
BMI	-0.18	0.5168
SBP	-0.23	0.4182
DBP	-0.19	0.4910
Abdominal circumference	0.18	0.5262
Sagittal diameter	-0.29	0.2939
Waist circumference	-0.23	0.4030
Total Cholesterol	0.27	0.3513
LDL-C	0.14	0.6076
HDL-C	0.46	0.0887
Triacylglycerol	-0.57	0.0266
Apolipoprotein B	-0.30	0.2762

Diet and supplements: The average value of the 3-day diet records calculated at each of the three periods for selected nutrients are reported in **Table 3.6**. The intake of carbohydrate, protein, total fat, saturated fat, and cholesterol was within the recommendations of the Dietary Guidelines (83). The consumption of fat was fairly

equally distributed between three classes of fats: 10 ± 5% saturated, 9 ± 5% monounsaturated, and 10 ± 3% polyunsaturated fatty acid of energy. This met the suggestions from the National Cholesterol Education Program's Adult Treatment Guidelines (<10% saturated fatty acids, 10% PUFA, and 10 to 15% MUFA of total energy) (84). For those nutrient for which the RDA remains the proper standard, a few (vitamins D, magnesium, and zinc) were lower than the recommended amount but within two thirds of their value. For folic acid the average intake was lower than the newly recommended Dietary Reference Intake (DRI) but above the previous RDA of 180 ug. All other nutrients met or exceeded the recommended amounts. The fifteen gram oil supplement provided an additional 565 kJ (135 Kcal) each day. The subjects gained an average of 0.6 kg over the 29 weeks of the study.

TABLE 3.6
Composition of diets of postmenopausal women averaged from 3-day dietary records calculated at each of the three periods.

	Intake Mean ± SE	Recommended[1]
Kj	7375 ± 1895	7959
% Cabohydrates	55 ± 8	55
% Protein	16 ± 3	15
% Fat	29 ± 6	< 30
Saturated fat (g)	19 ± 8	-
MUFA (g)	18 ± 8	-
PUFA (g)	9 ± 5	-
EPA, DHA (mg)	0, 0.2	-
Cholesterol (mg)	178 ± 89	< 300
Fiber (g)	20 ± 8	19.7
Vitamin E (α-TE, mg)	6.4 ± 3.1	8
Vitamin C (mg)	140 ± 83	60
Vitamin A (RE)	1210 ± 936	800
Thiamin (mg)	1.46 ± 0.73	1
Riboflavin (mg)	1.70 ± 0.55	1.2
Niacin (mg)	16.9 ± 7.5	13
Vitamin B6 (mg)	1.58 ± 0.69	1.6
Vitamin B12 (mcg)	3.58 ± 2.77	2
Vitamin D (mcg)	3.26 ± 2.24	5
Folic acid (mcg)	265 ± 125	400
Vitamin K (mcg)	96 ± 104	65
Calcium (mg)	943 ± 340	1200
Iron (mg)	13.5 ± 6.0	10
Magnesium (mg)	271 ± 92	320
Phosphate (mg)	1154 ± 388	700
Potassium (mg)	2893 ± 754	2000
Selenium (mg)	57 ± 30	55
Sodium (mg)	2484 ± 921	2400
Zinc (mg)	8.5 ± 3.8	12

[1]Recommended: The recommended amounts of thiamin, riboflavin, niacin, vitamin B12, vitamin D, folic acid, calcium, magnesium, phosphate, and potassium are from the Dietary Reference Intake (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Institute of Medicine, 1999) The recommended amounts of all other nutrients were taken from 10th edition, 1989, RDA 10th edition (107).

No significant amount of oxidation of the oils was found during the study as measured by the peroxide value or p-anisidine tests as shown in **Table 3.7**. The peroxide value and p-anisidine value for FO reported from NIH were 1.0 mg peroxide/ kg oil and 18.8 absorbance units/g oil, respectively.

TABLE 3.7
Measures of lipid peroxidation of three dietary oils

	Peroxide Value (mg peroxide / kg oil)			p-anisidine (Absorbance units / g oil)		
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
TS	1.36	1.18	1.10	1.01	1.01	ND[1]
SO	6.24	10.19	2.51	11.98	11.25	10.16
FO	2.52	6.05	4.69	16.72	17.58	16.30

[1]ND refers to not detectable

The effects of supplementation of dietary oils on plasma fatty acid profile: The plasma fatty acid profile before supplementation of dietary oils was not significantly different among groups except for 14:0, 22:6n-3 and total n-3 as shown in **Table 3.8A**. The amount of 14:0 ranged from 1.0 to 1.2%, that of 22:6n-3 from 1.5 to 1.9 %, and the total n-3 fatty acids from 3.3 to 3.9%. These differences reflect random variation in the subjects and are not the result of dietary intervention.

The plasma fatty acid profile was changed dramatically by the oil supplements as shown in **Table 3.8B**. The amount of total saturated fatty acid was 4% higher in the TS-supplemented group than the SO-supplemented group. The difference was reflected in the amount of the two major saturated fatty acids (14:0, 16:0) but not that of 18:0. After the TS supplementation the relative weight percent of 14:0 was 22% higher than after both the SO and FO supplementation. The relative weight percent of 16:0 was also 6%

and 4% higher in the TS-supplemented group than in the SO- and FO-supplemented groups, respectively. There were also minor significant differences among the other saturated fatty acids.

The amount of total MUFA was 23% and 30% higher in TS-supplemented group than the SO- and FO- supplemented groups, respectively. The amount of oleic acid (18:1n-9c) was significantly different among the groups consuming the dietary oils. After the TS supplementation the relative weight percent was 28% and 41% higher than after the SO and FO supplementation, respectively. The relative weight percent of oleic acid was also 10% higher after the SO supplementation than after the FO supplementation.

The total amount of PUFA was statistically equivalent in the SO- and FO-supplemented groups but 13% and 14% lower in TS-supplemented group than the SO- and FO- supplemented groups, respectively. The relative weight percent of linoleic acid (18:2n-6) was significantly increased in SO-supplemented group compared to the TS (22%) or FO- (28%) supplemented groups.

Interestingly, the FO-supplemented group had significantly lower achidonic acid (20:4n-6) level than the TS- and SO-supplemented groups. After the FO supplementation the relative weight percent was 7% and 13% lower than after the TS and SO supplementation, respectively.

The amount of total n-6 fatty acids was significantly different among different dietary oil supplemented groups. The relative weight percent was 18% and 27% higher in the SO-supplementation group than in the TS- and FO- supplemented groups,

respectively. The relative weight percent of total n-6 fatty acidwas also 7% higher after the TS supplementation than after the FO supplementation.

The amount of total n-3 fatty acid was 288% and 340% higher in FO-supplemented group than the TS- and SO- supplemented groups, respectively. The relative weight percent of EPA (20:5n-3) was significantly increased in FO-supplemented group compared with in TS or SO-supplemented groups. After the FO supplementation the relative weight percent was 1180% and 2030% higher than after the TS and SO supplementation, respectively. The relative weight percent of DHA (22:6n-3) also was significantly increased in FO-supplemented group compared with in TS or SO-supplemented groups. The relative weight percent was 200% higher in the FO-supplemented group than both the TS-and SO-supplementated groups.

TABLE 3.8A

Plasma fatty acid profile of postmenoupausal women before supplementation of the three dietary oils (Relative weight %) [1]

	TS	SO	FO	P-value			
				Overall	TS:SO	TS:FO	SO:FO
14:0	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	0.0108	0.1748	0.0607	0.0031
16:0	19.4 ± 1.0	21.1 ± 1.0	21.0 ± 1.0	0.4301	0.2619	0.2708	0.9602
18:0	6.3 ± 0.3	6.2 ± 0.3	7.0 ± 0.3	0.2030	0.7950	0.1535	0.1038
20:0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.0825	0.1060	0.0334	0.6085
22:0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.6120	0.5625	0.3306	0.7080
23:0	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5211	0.6078	0.5191	0.2605
24:0	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.1945	0.0797	0.2303	0.5345
∑SFA[2]	29.1 ± 1.0	30.2 ± 0.1	31.5 ± 0.1	0.2214	0.4021	0.0858	0.3820
16:1n-7	2.6 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	0.6655	0.6324	0.3728	0.6935
18:1n-9*t*	1.2 ± 0.3	0.8 ± 0.3	1.4 ± 0.3	0.2267	0.2519	0.5522	0.0924
18:1n-7	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	0.7364	0.5966	0.4527	0.8382
18:1n-9*c*	17.6 ± 0.5	16.9 ± 0.5	17 ± 0.4	0.5483	0.3017	0.4258	0.7930
221n-9	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	0.6097	0.3640	0.9178	0.4200
∑MUFA[3]	25.7 ± 0.8	24.2 ± 0.8	25.0 ± 0.7	0.4242	0.1961	0.4777	0.5377
18:2n-6*tt*	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1106	0.1031	0.6856	0.4800
18:2n-6*cc*	27.9 ± 0.8	28 5 ± 0.8	27.4 ± 0.7	0.6040	0.5843	0.6393	0.3214
20:3n-6	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	0.9849	0.9070	0.9565	0.8656
20:4n-6	7.4 ± 0.3	7.4 ± 0.3	7.0 ± 0.3	0.4877	0.9810	0.3020	0.3061
20:5n-3	0.6 ± 0.0	0.6 ± 0.5	0.6 ± 0.0	0.3519	0.7000	0.1641	0.3248
22;4n-6	2.3 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	0.3248	0.1420	0.5957	0.3313
22:6n-3	1.8 ± 0.1	1.9 ± 0.1	1.5 ± 0.1	0.0001	0.9327	0.0003	0.0005
∑PUFA[4]	44.4 ± 1.0	44.8 ± 1.0	42.8 ±1.0	0.3454	0.7553	0.2722	0.1732
∑n-6[5]	40.0 ± 1.0	40.1 ± 1.0	39.1 ± 1.0	0.5473	0.6004	0.5547	0.2782
∑n-3[6]	3.9 ± 0.1	3.8 ± 0.1	3.3 ± 0.1	0.0003	0.6569	0.0002	0.0007

[1] Mean ± SE

[2] ∑SFA=14:0+16:0+18:0+20:0+22:0+23:0+24:0

[3] ∑MUFA=16:1n-7+18:1n-9*t*+18:1n-7+18:1n-9*c*+22:1n-9+24:1

[4] ∑PUFA=18:2n-6*tt*+18:2n-6*cc*+20:3n-6+20:4n-6+20:5n-3+22:4n-6+22:6n-3

[5] ∑n6=18:2n-6*tt*+18:2n-6*cc*+20:3n-6+20:4n-6

[6] ∑n3=20:5n-3+22:6n-3

TABLE 3.8B

Plasma fatty acid profile of postmenoupausal women after supplementation of the three dietary oils (Relative weight %) [1]

	TS	SO	FO	P-value			
				Overall	TS:SO	TS:FO	SO:FO
14:0	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	0.0098	0.0283	0.0035	0.4144
16:0	21.1 ± 0.3	20.0 ± 0.3	20.2 ± 0.3	0.0322	0.0148	0.0401	0.6115
18:0	6.4 ± 0.1	6.5 ± 0.1	6.8 ± 0.1	0.0794	0.4914	0.0289	0.1313
20:0	0.6 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.0009	0.9675	0.0009	0.0001
22:0	0.6 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	0.0001	0.1636	0.0011	0.0001
23:0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4235	0.9907	0.2519	0.2707
24:0	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.4685	0.3073	0.9205	0.2670
∑SFA[2]	30.9 ± 0.4	29.8 ± 0.4	30.0 ± 0.4	0.1090	0.0503	0.1049	0.6788
16:1n-7	2.2 ± 0.1	1.7 ± 0.1	2.4 ± 0.2	0.0094	0.0025	0.1547	0.0637
18:1n-9*t*	1.1 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.0050	0.1676	0.0013	0.0448
18:1n-7	1.7 ± 0.1	1.6 ± 0.1	1.5 ± 0.5	0.0981	0.0865	0.0485	0.8141
18:1n-9*c*	19.0 ± 0.4	14.8 ± 0.4	13.5 ± 0.4	0.0001	0.0001	0.0001	0.0210
221n-9	1.3 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	0.5410	0.7598	0.4340	0.2910
24:1	0.9 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	0.0016	0.0074	0.0006	0.3577
∑MUFA[3]	26.2 ± 0.5	21.3 ± 0.5	20.2 ± 0.4	0.0001	0.0001	0.0001	0.1242
18:2n-6*tt*	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.1	0.0972	0.7091	0.0882	0.0465
18:2n-6*cc*	26.5 ± 0.7	32.4 ± 0.7	25.2 ± 0.6	0.0001	0.0001	0.1813	0.0001
20:3n-6	1.7 ± 0.1	1.7 ± 0.1	0.9 ± 0.1	0.0001	0.7428	0.0001	0.0001
20:4n-6	7.1 ± 0.2	7.6 ± 0.2	6.6 ± 0.2	0.0012	0.0305	0.0525	0.0003
20:5n-3	0.5 ± 0.3	0.3 ± 0.3	6.4 ± 0.3	0.0001	0.6457	0.0001	0.0001
22:4n-6	2.3 ± 0.3	2.8 ± 0.3	2.7 ± 0.3	0.4464	0.2218	0.3907	0.6916
22:6n-3	1.6 ± 0.2	1.6 ± 0.2	4.8 ± 0.2	0.0001	0.8904	0.0001	0.0001
∑PUFA[4]	42.1 ± 0.7	48.3 ± 0.7	49.0 ± 0.7	0.0001	0.0001	0.0001	0.5153
∑n-6[5]	38.0 ± 0.7	45.0 ± 0.7	35.4 ± 0.7	0.0001	0.0001	0.0130	0.0001
∑n-3[6]	3.4 ± 0.4	3.0 ± 0.4	13.2 ± 0.4	0.0001	0.5698	0.0001	0.0001

[1]Mean ± SE

[2]∑SFA=14:0+16:0+18:0+20:0+22:0+23:0+24:0

[3]∑MUFA=16:1n-7+18:1n-9*t*+18:1n-7+18:1n-9*c*+22:1n-9+24:1

[4]∑PUFA=18:2n-6*tt*+18:2n-*6cc*+20:3n-6+20:4n-6+20:5n-3+22:4n-6+22:6n-3

[5]∑n6=18:2n-6*tt*+18:2n-*6cc*+20:3n-6+20:4n-6

[6]∑n3=20:5n-3+22:6n-3

The effects of supplementation of dietary oils on plasma lipids, lipoproteins, apo B concentrations and LDL size: Plasma lipids, lipoproteins, and LDL size before supplementation of dietary oils were not different among groups. However, apo B concentration was significantly lower in the group to be supplemented with SO than the either of the groups to be supplemented with TS or FO as shown in **Table 3.9.** This was a random variation in the population and not the result of dietary intervention.

TABLE 3.9A
Plasma lipid profile, apo B concentration, and LDL size of postmenoupausal women before supplementation with the three dietary oils[1]

	TS	SO	FO	P			
				Overall	TS:SO	TS:FO	SO:FO
TC[2]	204 ± 4	197 ± 4	205 ± 4	0.2367	0.1723	0.8451	0.1215
LDL-C[3]	116 ± 3	112 ± 3	116 ± 3	0.5811	0.3750	0.9857	0.3656
HDL-C[4]	62 ± 1	61 ± 1	63 ± 1	0.4850	0.8427	0.3549	0.2637
TG[5]	130 ± 5	117 ± 5	127 ± 5	0.2287	0.1021	0.6843	0.2107
Apo B[6]	68 ± 1	61 ± 1	66 ± 1	0.0028	0.0010	0.3239	0.0115
LDL size	26.2 ± 0.1	26.2 ± 0.1	26.1 ± 0.1	0.8015	0.9566	0.5510	0.5876

[1]Mean ± SE
[2]TC ; Total cholesterol (mg/dl)
[3]LDL-C; LDL cholesterol (mg/dl)
[4]HDL-C; HDL cholesterol (mg/dl)
[5]TG; Plasma triacylglycerol (mg/dl)
[6]Apo B; Apolipoprotein B (mg/dl)

Plasma lipid changes in response to the different oil supplements are shown in **Table 3.9B**. There were no significant differences among the different oil supplemented groups in plasma total cholesterol or LDL-C concentrations. HDL-C concentrations, however, tended to increase in the FO-supplemented group compared to the TS- and SO-supplemented groups. After the FO supplementation the concentration of plasma HDL-C was 5% and 6% higher than after the TS and SO supplementation, respectively.

The concentration of plasma triacylglycerol significantly differed among three different dietary oil supplemented groups. The concentration was 35% and 20% lower in the FO-supplemented group than in the TS- and SO- supplemented groups, respectively. The concentration of triacylglycerol also 22% lower after the SO supplementation than the TS supplementation.

The ratio of plasma triacylglycerol to HDL-C significantly differed among the three different dietary oil supplemented groups. The ratio was 0.9 units and 0.5 units lower in the FO-supplemented group than in the TS- and SO- supplemented groups, respectively. The ratio was 0.4 units lower after the SO supplementation than the TS supplementation. However, the ratio of LDL-C to HDL-C was not different among three dietary oils-supplemented groups.

Since the initial values of apo B concentration differed, the difference between before and after supplementation was calculated for each supplemented group and used in the statistical evaluation. Supplementation of FO significantly decreased plasma apo B concentration compared with supplementation of TS and SO. The concentration of apo B was decreased 14% in the FO- supplemented group; however, the concentration was decreased only 1% and 3% in the TS- and SO-supplemented groups, respectively.

The LDL particle size was significantly larger in the FO-supplemented group compared to the TS-supplemented group and tended to be larger in the SO-supplemented group compared to the TS-supplemented one. The size of the particle after supplementation of the SO and FO were statistically equivalent.

TABLE 3.9B
Plasma lipid profile, apo B concentration, and LDL size of postmenopausal women after supplementation with the three dietary oils [1]

	TS	SO	FO	P			
				Overall	TS:SO	TS:FO	SO:FO
TC[2]	193 ± 3	187 ± 3	188 ± 3	0.3394	0.1884	0.2250	0.9149
LDL-C[3]	105 ± 3	104 ± 3	105 ± 3	0.9545	0.8629	0.8969	0.7625
HDL-C[4]	63 ± 1	62 ± 1	66 ± 1	0.0968	0.8938	0.0702	0.0535
TG[5]	126 ± 6	103 ± 6	82 ± 6	0.0001	0.0125	0.0001	0.0175
TG/HDL-C	2.3 ± 0.1	1.9 ± 0.3	1.4 ± 0.3	0.0004	0.0377	0.0001	0.0228
LDLC/HDL-C	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	0.6983	0.8272	0.5513	0.4173
Apo B[6]	67 ± 2	59 ± 2	57 ± 2	0.0010	0.0039	0. 0005	0.4146
Δ Apo B[7]	-1.2 ± 1.9	-2.3 ± 1.9	-9.2 ± 1.9	0.0010	0.6764	0.0050	0.0100
LDL size	26.0 ± 0.1	26.3 ± 0.1	26.4 ± 0.2	0.0751	0.0679	0.0363	0.7641

[1]Mean ± SE
[2]TC ; Total cholesterol (mg/dl)
[3]LDL-C; LDL cholesterol (mg/dl)
[4]HDL-C; HDL cholesterol (mg/dl)
[5]TG; Plasma triacylglycerol (mg/dl)
[6]Apo B; apolipoprotein B (mg/dl)
[7]Δ Apo B; Apo B concentration after supplementation of dietary oils - Apo B concentration before supplementation of dietary oils

Discussion

The effect of n-9 (TS, MUFA), n-6 (SO, PUFA), and n-3 (FO, PUFA) fatty acids on plasma lipid, lipoprotein and apo B concentrations, and ratios of triacylglycerol to HDL-C and LDL-C to HDL-C, and LDL particle size was examined in healthy, normolipidemic, postmenopausal women. The amount of oil given to the subjects was physiologically relevant. The fifteen gram supplement of TS contained 12.3 g of oleic acid while that of SO contained 10.5 gram of linoleic acid. These amounts could be obtained from approximately 3 teaspoons of canola oil and corn oil, respectively. The quantity of fish oil given to the participants was equivalent to two servings (180g) of fish

a day. Although this amount is higher than usually found in the average American diet, it is an amount that can be consumed.

Correlations between LDL particle size and CVD-risk indices including, BMI, blood pressure, abdominal circumference, sagittal diameter, waist circumference, total cholesterol, LDL-C and apo B before the subjects were supplemented with any of the oils were not significant. Previous studies (85-87) have shown correlation among these variables. However, the present study may not have had enough statistical power due to the small number of subjects. In addition, the subjects in this study were healthy individuals in terms of blood lipid and LDL particle size. Their average blood lipid values were within desirable levels and fourteen subjects had LDL particle size larger than 25.5 nm, above the range of increased CVD risk. The limited range of the subjects' LDL particle size may be a reason for failing to observe any correlation between LDL particle size and some indices of risk of CVD. However, the LDL particle size was strongly inversely related to plasma triacylglycerol concentration. HDL-C concentration tended to be directly related to LDL particle size. These interrelationships have also been observed by others. Stampfer et al. (88) and Austin et al. (19) found that the LDL diameter had a high inverse correlation with triacylglycerol concentration and a high direct correlation with HDL-C concentration.

After supplementation with the oils, plasma total cholesterol and LDL-C concentrations were not significantly different among the TS, SO, and FO groups. Previous studies have reported a decrease in plasma total cholesterol and LDL-C with MUFA and PUFA enriched diet (27,28,31,89-91). Differences in the design and dietary composition of this study make it difficult to compare the results with those of other

studies. Other investigators (90-93) have often used diets with an extreme fatty acid composition, large amounts of MUFA and PUFA and a diminished amount of saturated fatty acids. In these studies the ratio of MUFA and/or PUFA to saturated fatty acid was raised by replacing saturated fatty acids with MUFA and /or PUFA. Saturated fatty acids have clearly been shown to reduce total cholesterol and LDL-C by suppressing LDL receptor activity and decreasing apo B synthesis (94). Dietschy et al. (94) studied the activity of the hepatic LDL receptor in hamsters fed single, specific fatty acids. The activity was significantly decreased when saturated fatty acids including 12:0, 14:0, and 16:0 were fed to the hamsters.

In the present study the total cholesterol and LDL-C concentrations were not significantly different in the TS-, SO-, and FO- supplemented groups. Supplementation with TS, SO, and FO did not influence the average level of saturated fatty acid intake. Subjects consumed about 9 % of their total energy as saturated fatty acids and this amount remained constant during the entire study. The same level of saturated fatty acid intake among the different oil-supplemented groups may explain the fact that no difference in total cholesterol and LDL-C concentrations among three different fatty acid-supplemented groups was found. This finding corresponds to the results of Nydahl et al. (31). They found no difference on total cholesterol and LDL-C concentrations between MUFA- and PUFA- supplemented groups with the same level of saturated fatty acids intake. However, HDL-C concentration tended to increase in the FO-supplemented group compared to the TS-supplemented group in the present study and was significantly higher in the FO-supplemented group compared to the SO supplemented one. The

consumption of fish or supplementation with fish oil has previously been found to independently increase HDL-C concentrations by 5-10% (57).

Since plasma total cholesterol, LDL-C and HDL-C concentrations were established as risk factors for CVD, a number of additional markers have been identified to better characterize the potential for CVD. Recent data suggest that plasma triacylglycerol concentrations may play an important role. Almost without exception n-3 fatty acid feeding decreases plasma triacylglycerol concentration. Harris et al. (95) calculated from previous studies that approximately 3 g of n-3 fatty acids can reduce plasma triacylglycerol concentration by 30%. This may be explained by reduced hepatic triacylglycerol output and increased chylomicron remnant and VLDL triacylglycerol clearance. In addition, Harris et al. (96) found that n-3 fatty acid supplementation increased the activity of lipoprotein lipase in the plasma of both healthy subjects and hypertriglyceridemic patients. A significant correlation between plasma triacylglycerol concentration and lipoprotein lipase activity has also been reported (96). Therefore, n-3 fatty acid supplementation increases lipoprotein lipase activity and this may lead to a decrease in the concentration of plasma triacylglycerol. A significant decrease in plasma triacylglycerol concentration was observed in the FO group when compared with the TS and SO groups in the present study. This favorable plasma lipid change after intake of fish oil was also observed in other studies (97-99).

The ratio of plasma triacylglycerol to HDL-C has been suggested to be a better predictor of myocardial infarction than ratios previously used (total cholesterol to HDL-C and LDL-C/HDL-C) (15). In the present study fish oil supplementation lowered the ratio

of plasma triacylglycerol to HDL-C significantly compared to the TS- and SO-supplemented groups.

LDL particle size was also significantly increased after the FO supplementation compared to the TS supplementation. Since plasma triacylglycerol concentration is inversely related to LDL particle size, the reduction in plasma triacylglycerol concentration, as a result of n-3 fatty acid intake, would be expected to cause an increase in LDL particle size. The results of this study support this interrelationship after supplementation with FO.

After the FO supplementation, plasma apo B concentrations were significantly decreased compared with baseline but not after the TS and SO supplementation. Each LDL particle contains a single apo B molecule. Therefore, for the same concentration of LDL cholesterol, an increase of LDL particle size lowers apo B concentration, or number of LDL particles. This relationship was supported by the results of this study. A reduction of apo B and triacylglycerol concentration, a decrease in the ratio of triacylglycerol to HDL-C, and an increased LDL particle size as a result of FO supplementation clearly suggested a favorable effect of FO in reducing the risks of CVD.

Although epidemiological and interventional studies provide evidence that dietary fatty acids influence plasma lipids and lipoproteins, there are limited trials of the effects of the dietary fatty acids on LDL particle size, and even existing data are unequivocal. Several groups have studied the effects of n-3 fatty acids on LDL particle size in humans and monkeys and reported conflicting data. Supplementation of n-3 fatty acids increased LDL size in humans (99,100) while it decreased LDL size in monkeys (101,102). However, it can not be ascertained that these results were caused by a physiological

difference between species since different methodologies were used to measure LDL size in humans and monkeys. The PGGE method was used in humans, while an agarose gel chromatography method was used in monkeys. Moreover, there is no evidence that LDL size measured from PGGE corresponds to that measured with agarose gel chromatography. In the PGGE method the diameter of the LDL particle is measured by migration distance in non denaturing gradient gel, while the molecular weight of the LDL particle is measured by comparing elution volume of standard LDL in agarose gel chromatography. Developing a standardized method for measuring LDL particle size is necessary to compare results from the different groups. Furthermore, although LDL size was decreased after n-3 fatty acid feeding as compared with saturated fatty acid feeding in the studies of Parks et al.(101) and Ligna et al. (102), neither reported baseline LDL size. Supplementation with saturated fatty acid has frequently been shown to increase LDL size compared to that of baseline (101,102,105,106). Decreasing LDL size after n-3 fatty acid feeding as compared with saturated fatty acid feeding does not mean decreasing LDL size with n-3 fatty acid feeding as compared with baseline. LDL size should be compared with baseline to establish the effect of n-3 fatty acids.

Sorensen et al. (103) and Nenseter et al. (104) reported that dietary n-3 fatty acids had no effect on LDL size in humans. Nenseter et al. (104) used electron microscopy to measure LDL particle size. In the study of Sorensen et al. (103) LDL particle size did not differ in n-3 fatty acid-rich oil supplemented group compared to linoleic acid-rich oil supplemented group. The present study also showed that LDL particle size was not statistically different between n-3 fatty acid-rich oil supplement group and linoleic acid-rich oil supplemented group.

In conclusion, the results of this study showed that the FO supplementation increased the LDL particle size, reduced the concentration of plasma triacylglycerol and apo B and the ratio of triacylglycerol to HDL-C compared to TS and SO supplementation. These findings demonstrate a greater potential antiatherogenic property of dietary fish oil than either oleic acid-rich or linoleic acid-rich oil in postmenopausal women using hormone replacement therapy.

References

1. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N.Engl.J.Med. 1991;325:373-381.

2. NIH Consensus conference. Triglyceride, high-density lipoprotein, and coronary heart disease. NIH Consensus Development Panel on Triglyceride, High-Density Lipoprotein, and Coronary Heart Disease. JAMA 1993;269:505-510.

3. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. Ann.Intern.Med. 1971;74:1-12.

4. Miller GJ, Miller NE. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. Lancet 1975;1:16-19.

5. Pearson TA, Bulkley BH, Achuff SC, Kwiterovich PO, Gordis L. The association of low levels of HDL cholesterol and arteriographically defined coronary artery disease. Am.J.Epidemiol. 1979;109:285-295.

6. Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 1986;256:2835-2838.

7. Kameda K, Matsuzawa Y, Kubo M, et al. Increased frequency of lipoprotein disorders similar to type III hyperlipoproteinemia in survivors of myocardial infarction in Japan. Atherosclerosis 1984;51:241-249.

8. Thind IS, Sandhu RS. Signification of high density and total cholesterol and triglycerides in acute myocardial infarction -- a case-control study. Clin.Biochem. 1981;14:57-60.

9. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J.Clin.Invest. 1973;52:1544-1568.

10. Fager G, Wiklund O, Olofsson SO, Wilhelmsen L, Bondjers G. Multivariate analyses of serum apolipoproteins and risk factors in relation to acute myocardial infarction. Arteriosclerosis 1981;1:273-279.

11. Hamsten A, Walldius G, Dahlen G, Johansson B, De Faire U. Serum lipoproteins and apolipoproteins in young male survivors of myocardial infarction. Atherosclerosis 1986;59:223-235.

12. Austin MA. Plasma triglyceride and coronary heart disease. Arterioscler.Thromb. 1991;11:2-14.

13. Havel R. McCollum Award Lecture, 1993: triglyceride-rich lipoproteins and atherosclerosis--new perspectives. Am.J.Clin.Nutr. 1994;59:795-796.

14. Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. Am.J.Cardiol. 1998;81:7B-12B.

15. Gaziano JM, Hennekens CH, O'Donnell CJ, Breslow JL, Buring JE. Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. Circulation 1997;96:2520-2525.

16. Genest JJJ, Bard JM, Fruchart JC, Ordovas JM, Wilson PF, Schaefer EJ. Plasma apolipoprotein A-I, A-II, B, E and C-III containing particles in men with premature coronary artery disease. Atherosclerosis 1991;90:149-157.

17. Brunzell JD, Sniderman AD, Albers JJ, Kwiterovich POJ. Apoproteins B and A-I and coronary artery disease in humans. Arteriosclerosis 1984;4:79-83.

18. Austin MA, Krauss RM. Genetic control of low-density-lipoprotein subclasses. Lancet 1986;2:592-595.

19. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988;260:1917-1921.

20. Rudel LL, Pitts LL, Nelson CA. Characterization of plasma low density lipoproteins on nonhuman primates fed dietary cholesterol. J.Lipid Res. 1977;18:211-222.

21. Kalkhoff RK, Hartz AH, Rupley D, Kissebah AH, Kelber S. Relationship of body fat distribution to blood pressure, carbohydrate tolerance, and plasma lipids in healthy obese women. J.Lab.Clin.Med. 1983;102:621-627.

22. Despres JP, Nadeau A, Tremblay A, et al. Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. Diabetes 1989;38:304-309.

23. Keys A. Coronary heart diseas in seven countries. Circulation 1970;41:1-211.

24. Grundy SM, Florentin L, Nix D, Whelan MF. Comparison of monounsaturated fatty acids and carbohydrates for reducing raised levels of plasma cholesterol in man. Am.J.Clin.Nutr. 1988;47:965-969.

25. Colquhoun DM, Moores D, Somerset SM, Humphries JA. Comparison of the effects on lipoproteins and apolipoproteins of a diet high in monounsaturated fatty acids, enriched with avocado, and a high- carbohydrate diet. Am.J.Clin.Nutr. 1992;56:671-677.

26. Mensink RP, de Groot MJ, van den Broeke LT, Severijnen-Nobels AP, Demacker PN, Katan MB. Effects of monounsaturated fatty acids v complex carbohydrates on serum lipoproteins and apoproteins in healthy men and women. Metabolism 1989;38:172-178.

27. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. Lancet 1987;1:122-125.

28. Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N.Engl.J.Med. 1986;314:745-748.

29. Berry EM, Eisenberg S, Friedlander Y, et al. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins--the Jerusalem Nutrition Study. II. Monounsaturated fatty acids vs carbohydrates. Am.J.Clin.Nutr. 1992;56:394-403.

30. Baggio G, Pagnan A, Muraca M, et al. Olive-oil-enriched diet: effect on serum lipoprotein levels and biliary cholesterol saturation. Am.J.Clin.Nutr. 1988;47:960-964.

31. Nydahl MC, Gustafsson IB, Vessby B. Lipid-lowering diets enriched with monounsaturated or polyunsaturated fatty acids but low in saturated fatty acids have similar effects on serum lipid concentrations in hyperlipidemic patients. Am.J.Clin.Nutr. 1994;59:115-122.

32. Hodgson JM, Wahlqvist ML, Boxall JA, Balazs ND. Can linoleic acid contribute to coronary artery disease? Am.J.Clin.Nutr. 1993;58:228-234.

33. Gordon DJ, Rifkind BM. High-density lipoprotein--the clinical implications of recent studies. N.Engl.J.Med. 1989;321:1311-1316.

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

35. Reaven PD. Mechanisms of atherosclerosis: role of LDL oxidation. Adv.Exp.Med.Biol. 1994;366:113-128.

36. Lardinois CK, Starich GH, Mazzaferri EL, DeLett A. Polyunsaturated fatty acids augment insulin secretion. J.Am.Coll.Nutr. 1987;6:507-515.

37. Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high-fat feeding in rats. Science 1987;237:885-888.

38. Reaven GM, Chen YD. Role of insulin in regulation of lipoprotein metabolism in diabetes. Diabetes Metab.Rev. 1988;4:639-652.

39. DeFronzo RA. Insulin resistance, hyperinsulinemia, and coronary artery disease: a complex metabolic web. J.Cardiovasc.Pharmacol. 1992;20 Suppl 11:S1-16.

40. Bang HO, Dyerberg J, Sinclair HM. The composition of the Eskimo food in north western Greenland. Am.J.Clin.Nutr. 1980;33:2657-2661.

41. Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? Lancet 1978;2:117-119.

42. Kromhout D, Feskens EJ, Bowles CH. The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. Int.J.Epidemiol. 1995;24:340-345.

43. Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and risk of sudden cardiac death. JAMA 1998;279:23-28.

44. Norell SE, Ahlbom A, Feychting M, Pedersen NL. Fish consumption and mortality from coronary heart disease. Br.Med.J.(Clin.Res.Ed.) 1986;293:426

45. Kromhout D, Bosschieter EB, de Lezenne C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N.Engl.J.Med. 1985;312:1205-1209.

46. Daviglus ML, Stamler J, Orencia AJ, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. N.Engl.J.Med. 1997;336:1046-1053.

47. Zhang J, Sasaki S, Amano K, Kesteloot H. Fish consumption and mortality from all causes, ischemic heart disease, and stroke: an ecological study. Prev.Med. 1999;28:520-529.

48. Burchfiel CM, Reed DM, Strong JP, Sharp DS, Chyou PH, Rodriguez BL. Predictors of myocardial lesions in men with minimal coronary atherosclerosis at autopsy. The Honolulu heart program. Ann.Epidemiol. 1996;6:137-146.

49. Bjerregaard P, Dyerberg J. Mortality from ischaemic heart disease and cerebrovascular disease in Greenland. Int.J.Epidemiol. 1988;17:514-519.

50. Davidson M, Bulkow LR, Gellin BG. Cardiac mortality in Alaska's indigenous and non-Native residents. Int.J.Epidemiol. 1993;22:62-71.

51. Iso H, Folsom AR, Wu KK, et al. Hemostatic variables in Japanese and Caucasian men. Plasma fibrinogen, factor VIIc, factor VIIIc, and von Willebrand factor and their relations to cardiovascular disease risk factors. Am.J.Epidemiol. 1989;130:925-934.

52. Morris MC, Manson JE, Rosner B, Buring JE, Willett WC, Hennekens CH. Fish consumption and cardiovascular disease in the physicians' health study: a prospective study. Am.J.Epidemiol. 1995;142:166-175.

53. Curb JD, Reed DM. Fish consumption and mortality from coronary heart disease. N.Engl.J.Med. 1985;313:821-822.

54. Ascherio A, Rimm EB, Stampfer MJ, Giovannucci EL, Willett WC. Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men. N.Engl.J.Med. 1995;332:977-982.

55. Fraser GE, Sabate J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary heart disease. The Adventist Health Study. Arch.Intern.Med. 1992;152:1416-1424.

56. Wander RC, Du SH, Ketchum SO, Rowe KE. Effects of interaction of RRR-alpha-tocopheryl acetate and fish oil on low-density-lipoprotein oxidation in postmenopausal women with and without hormone-replacement therapy. Am.J.Clin.Nutr. 1996;63:184-193.

57. Harris WS. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J.Lipid Res. 1989;30:785-807.

58. Illingworth DR, Harris WS, Connor WE. Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans. Arteriosclerosis 1984;4:270-275.

59. Leaf A, Weber PC. Cardiovascular effects of n-3 fatty acids. N.Engl.J.Med. 1988;318:549-557.

60. Bang HO, Dyerberg J. Plasma lipids and lipoproteins in Greenlandic west coast Eskimos. Acta Med.Scand. 1972;192:85-94.

61. Nestel PJ, Connor WE, Reardon MF, Connor S, Wong S, Boston R. Suppression by diets rich in fish oil of very low density lipoprotein production in man. J.Clin.Invest. 1984;74:82-89.

62. Sullivan DR, Sanders TA, Trayner IM, Thompson GR. Paradoxical elevation of LDL apoprotein B levels in hypertriglyceridaemic patients and normal subjects ingesting fish oil. Atherosclerosis 1986;61:129-134.

63. Frisancho AR. Anthropometric standards for the assessment of growth and nutritional status. University of Michigan Press, 1990.

64. Reeves RA. Does this patient have hypertension? How to measure blood pressure. JAMA 1995;273:1211-1218.

65. National Cholesterol Education Program. Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). JAMA 1993;269:3015-3023.

66. Mayer EJ, Alderman BW, Regensteiner JG, et al. Physical-activity-assessment measures compared in a biethnic rural population: the San Luis Valley Diabetes Study. Am.J.Clin.Nutr. 1991;53:812-820.

67. Davidson SB, Wander RC, Du SH, Riddle M. Effect of dietary MUFAs in lipid, lipoproteins, and lipid peroxidation in NIDDM. American Oil Chemists' Society (AOCS) 1997;(abst)

68. Song J, Wander RC. Effects of dietary selenium and fish oil (MaxEPA) on arachidonic acid metabolism and hemostatic function in rats. J.Nutr. 1991;121:284-292.

69. Astill BD, Terhaar CJ, Krasavage WJ, Wlof GL, Roudabush RL, Fassett DW. Safety evaluation and biochemical behavior of monotertiarybutylhydroquinone. J.Am.Oil Chem.Soc. 1975;52:53-58.

70. Wander RC, Du SH, Ketchum SO, Rowe KE. alpha-Tocopherol influences in vivo indices of lipid peroxidation in postmenopausal women given fish oil. J.Nutr. 1996;126:643-652.

71. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin.Chem. 1974;20:470-475.

72. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin.Chem. 1983;29:538-542.

73. Marz W, Gross W. Analysis of plasma lipoproteins by ultracentrifugation in a new fixed angle rotor: evaluation of a phosphotungstic acid/MgCl2 precipitation

and a quantitative lipoprotein electrophoresis assay. Clin.Chim.Acta 1986;160:1-18.

74. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin.Chem. 1972;18:499-502.

75. Gerhard GT, Patton BD, Lindquist SA, Wander RC. Comparison of three species of dietary fish: effects on serum concentrations of low-density-lipoprotein cholesterol and apolipoprotein in normotriglyceridemic subjects. Am.J.Clin.Nutr. 1991;54:334-339.

76. McNamara JR, Campos H, Ordovas JM, Peterson J, Wilson PW, Schaefer EJ. Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution. Results from the Framingham Offspring Study. Arteriosclerosis 1987;7:483-490.

77. Nichols AV, Krauss RM, Musliner TA. Nondenaturing polyacrylamide gradient gel electrophoresis. Methods Enzymol. 1986;128:417-31:417-431.

78. Kuehl RO. Crossover Design. In: AnonymousStatistical Principles of Research Design and Analysis. Belmont, CA: Duxbury Press, 1994:529-559.

79. Rowland ML. Reporting bias in height and weight data. Stat.Bull.Metrop.Insur.Co. 1989;70:2-11.

80. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual, human kinetics books. 1991.

81. Pouliot MC, Despres JP, Lemieux S, et al. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. Am.J.Cardiol. 1994;73:460-468.

82. Lemieux S, Prud'Homme D, Bouchard C, Tremblay A, Despres JP. A single threshold value of waist girth identifies normal-weight and overweight subjects with excess visceral adipose tissue. Am.J.Clin.Nutr. 1996;64:685-693.

83. U.S.Department of Agriculture and U.S.Department of Health and Human Service. Nutrition and your health, Dietary guidelines for Americans, 4th ed. 1995.

84. National Cholesterol Education Program. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. The Expert Panel. Arch.Intern.Med. 1988;148:36-69.

85. Krauss RM. Dense low density lipoproteins and coronary artery disease. Am.J.Cardiol. 1995;75:53B-57B.

86. Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J.Clin.Invest. 1993;92:141-146.

87. Selby JV, Austin MA, Newman B, et al. LDL subclass phenotypes and the insulin resistance syndrome in women. Circulation 1993;88:381-387.

88. Stampfer MJ, Krauss RM, Ma J, et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996;276:882-888.

89. Gumbiner B, Low CC, Reaven PD. Effects of a monounsaturated fatty acid-enriched hypocaloric diet on cardiovascular risk factors in obese patients with type 2 diabetes. Diabetes Care 1998;21:9-15.

90. Keys A, Parlin RW. Serum cholesterol response to changes in dietary lipids. Am.J.Clin.Nutr. 1966;19:175-181.

91. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am.J.Clin.Nutr. 1965;17:281-295.

92. Shepherd J, Packard CJ, Grundy SM, Yeshurun D, Gotto AMJ, Taunton OD. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. J.Lipid Res. 1980;21:91-99.

93. Schaefer EJ, Levy RI, Ernst ND, Van Sant FD, Brewer HBJ. The effects of low cholesterol, high polyunsaturated fat, and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholesterolemic subjects. Am.J.Clin.Nutr. 1981;34:1758-1763.

94. Dietschy JM. Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. J.Nutr. 1998;128:444S-448S.

95. Harris WS. n-3 fatty acids and serum lipoproteins: human studies. Am.J.Clin.Nutr. 1997;65:1645S-1654S.

96. Harris WS, Lu G, Rambjor GS, et al. Influence of n-3 fatty acid supplementation on the endogenous activities of plasma lipases. Am.J.Clin.Nutr. 1997;66:254-260.

97. Green P, Fuchs J, Schoenfeld N, et al. Effects of fish-oil ingestion on cardiovascular risk factors in hyperlipidemic subjects in Israel: a randomized, double-blind crossover study. Am.J.Clin.Nutr. 1990;52:1118-1124.

98. Harats D, Dabach Y, Hollander G, et al. Fish oil ingestion in smokers and nonsmokers enhances peroxidation of plasma lipoproteins [published erratum appears in Atherosclerosis 1991 Dec;91(3):279]. Atherosclerosis 1991;90:127-139.

99. Suzukawa M, Abbey M, Howe PR, Nestel PJ. Effects of fish oil fatty acids on low density lipoprotein size, oxidizability, and uptake by macrophages. J.Lipid Res. 1995;36:473-484.

100. Contacos C, Barter PJ, Sullivan DR. Effect of pravastatin and omega-3 fatty acids on plasma lipids and lipoproteins in patients with combined hyperlipidemia. Arterioscler.Thromb. 1993;13:1755-1762.

101. Parks JS, Bullock BC. Effect of fish oil versus lard diets on the chemical and physical properties of low density lipoproteins of nonhuman primates. J.Lipid Res. 1987;28:173-182.

102. Linga V, Leight MA, St.Clair RW, Parks JS. Dietary fish oil modification of cynomolgus monkey low density lipoproteins results in decreased binding and cholesteryl ester accumulation by cultured fibroblasts. J.Lipid Res. 1993;34:769-778.

103. Sorensen NS, Marckmann P, Hoy CE, van Duyvenvoorde W, Princen HM. Effect of fish-oil-enriched margarine on plasma lipids, low-density- lipoprotein particle composition, size, and susceptibility to oxidation. Am.J.Clin.Nutr. 1998;68:235-241.

104. Nenseter MS, Rustan AC, Lund-Katz S, et al. Effect of dietary supplementation with n-3 polyunsaturated fatty acids on physical properties and metabolism of low density lipoprotein in humans. Arterioscler.Thromb. 1992;12:369-379.

105. Dreon DM, Fernstrom HA, Campos H, Blanche P, Williams PT, Krauss RM. Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men. Am.J.Clin.Nutr. 1998;67:828-836.

106. Rudel LL, Parks JS, Sawyer JK. Compared with dietary monounsaturated and saturated fat, polyunsaturated fat protects African green monkeys from coronary artery atherosclerosis. Arterioscler.Thromb.Vasc.Biol. 1995;15:2101-2110.

107. Subcommittee on the Tenth Edition of the RDAs. Food and Nutrition Board,Commission on Life Sciences,National Research Council. Recommended Dietary Allowances. 10 ed. Washington, D.C.: National Academy Press, 1989.

CHAPTER 4

THE EFFECT OF ENRICHMENT WITH N-9, N-6, AND N-3 FATTY ACIDS ON OXIDATIVE SUSCEPTIBILITY OF LDL SUBFRACTIONS IN POSTMENOPAUSAL WOMEN

Ye-Sun Lee
Shi-Hua Du
Jane Higdon
Tianyiang Wu
Rosemary C. Wander

Oregon State University
Department of Nutrition of Food Management
Corvallis, Oregon

Abstract

Individuals with high proportions of small low density lipoprotein (LDL) are at a greater risk for atherosclerosis than those with high proportions of large LDL. The reasons for this are unknown but may relate to changes produced in oxidizability or lipid classes in large and small LDL by different dietary fatty acids. The influence of these parameters on oxidative susceptibility and lipid composition of LDL subfractions has not been clearly defined, especially in postmenopausal women who are at risk for cardiovascular disease. Consequently, the purpose of this study was to investigate the effect of supplements of different fatty acids on oxidizability and composition of large and small subfractions of LDL. The experimental approach was a three-period, three-treatment, single-blind crossover trial. Sixteen healthy, postmenopausal women whose mean consumption of fat was 30% of their total energy were assigned to one of three treatment groups. They were given 15 g/d supplements of high oleic acid sunflower oil (TS, n-9), high linoleic acid safflower oil (SO, n-6), or fish oil (FO, n-3). Each period lasted 5 weeks followed by a 7-week washout interval. The fatty acid profile was significantly changed by oil supplementation in both large and small LDL. Although the concentration of total cholesterol, free cholesterol, cholesterol ester, phospholipid, triacylglycerol, α-tocopherol, and γ-tocopherol was not changed by any of the oil supplements, it was significantly higher in large LDL than small. The oxidation of the unsaturated fatty acids after the supplementation of the three oils in the two LDL subfractions was monitored by following the formation of conjugated dienes. There was a significant interaction between the oil supplement and subfraction on the lag time. This

occurred because the changes produced in lag time by the oil supplementation were greater in the large subfraction than small. The lag time was 14 min and 11 min shorter in the FO-supplemented group compared to the TS- (P= 0.0001) and SO-supplemented group (P = 0.0001), respectively, in large LDL. However, in small LDL the lag time was only 8 min shorter in the FO-supplemented group compared to both the TS- (P = 0.001) and SO- (P = 0.006) supplemented groups. The maximum rate of formation of conjugated dienes was 15% slower in the FO-supplemented group compared to the TS-supplemented group (P = 0.02) and 37% slower than in the SO-supplemented group (P= 0.001). The rate of formation of conjugated dienes was 19% slower in the small LDL subfraction than the large (P = 0.002). The effect of the oil supplements on the maximum amount of conjugated dienes formed was modest with the value being 8% lower (P= 0.04) in the TS-supplemented group compared to the SO-supplemented group and 9% lower compared to the FO-supplemented group (0.05). It was 25% lower in the small compared to the large subfraction (P= 0.001). The oxidative modification to apolipoproten B (apo B) was monitored by following its increase in negative charge. Few changes were produced by either the oil treatment or the subfraction over the 23 hr this was measured except after 1 hr of oxidation. At this time, the increase in negative charge was 28% higher in the FO-supplemented group than either the TS- or SO-supplemented groups (P= 0.0001 in both cases). In addition, the increase in negative charge was 12% larger in the small LDL compared to the large subfraction. In conclusion, the oxidative susceptibility of LDL was reduced in n-9 fatty acid-enriched LDL compared to the n-6 fatty acid-enriched LDL in postmenopausal women. However, the influence of n-3 fatty acid supplementation on

oxidative susceptibility of LDL was less clear. The data suggest that manipulation of the type of dietary fat may influence on small and large LDL similarly.

Introduction

The underlying primary cause of most cardiovascular disease is believed to be atherosclerosis, a progressive multifactorial disease of the artery wall (1). It is currently thought that oxidation of low-density lipoprotein (LDL) by cells in the artery wall leads to a proatherogenic particle that may help initiate early lesion formation (2,3). The factors that contribute to the susceptibility of LDL to oxidize have not been clearly defined.

Oxidation begins when a reactive radical abstracts a hydrogen atom from an unsaturated fatty acid on the LDL particle. Although this process presumably begins in the surface phospholipids, it spreads to involve the cholesterol esters and triacylglycerol in the core of the LDL particles.

Among the factors that may contribute to the oxidative susceptibility of the LDL particle is its concentration of the different fatty acid, such as oleic acid (18:1n-9), linoleic acid (18:2n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). The fatty acid content of LDL particles has been shown to be easily modified by dietary fatty acids (3,4). Diets enriched in linoleic acid increased the content of linoleic acid in LDL. In contrast, diets enriched in oleic acid produced LDL particles rich in oleic acid (5,6). Since dietary fatty acids influence the fatty acid composition of LDL, the amount and type of fat in the diet may affect susceptibility of LDL to oxidative damage. Reaven et al. (7) reported that oxidative susceptibility of the LDL obtained from

subjects fed diets enriched with oleic acid was significantly lower than that of LDL obtained from subjects fed linoleic acid-enriched diets (p=0.0001). Similar results have been found in other studies (6,8,9).

Oxidative susceptibility of LDL enriched in the long chain n-3 fatty acids (EPA and DHA) is less clear. Wander et al. (4) found that supplementation with fish oil shortened the lag time approximately 18 min (P<0.001), suggesting increased susceptibility, but slowed the oxidation rate 25% (P<0.001), suggesting decreased susceptibility. In support of this study Whitman et al. (10) investigated LDL oxidation in female Yucatan miniature swine fed with either fish oil or a control oil (a mixture of corn oil, safflower oil, and beef tallow). They found that fish oil LDL had a 30% shorter lag time and a 30% slower oxidation rate but no difference in atherosclerotic lesion development of all major blood vessels. On the other hand Nenster et al. (1992) reported that LDL from the fish oil and corn oil fed groups showed similar susceptibility to copper catalyzed lipid peroxidation, as measured by the amount of lipid peroxides formed and the degradation of oxidatively modified LDL in cultured macrophages.

In addition to its fatty acid composition, the size of the LDL particle may also contribute to its oxidative susceptibility. However, studies on the oxidative susceptibility of LDL subfractions have been incomplete. Although previous studies have suggested that small LDL was more susceptible to oxidation than the larger particles (13-19), it has not been clearly demonstrated. For example, Chait et al. (18) compared oxidative modification in six subfractions of LDL isolated from phenotype A and phenotype B subjects. Lag time was significantly shorter in LDL isolated from phenotype B than phenotype A ($P < 0.01$). Although lag time was inversely related ($P < 0.001$) to LDL

density in both groups of subjects, the rate of oxidation of LDL was not correlate with LDL subfractions.

Dejager et al. (19) studied the oxidative susceptibility of LDL particle subfractions in 9 hyperlipidemia subjects. They found a reduction ($P < 0.01$) in lag time in the smallest LDL subfraction; however, they did not report the rate of oxidation.

On the other hand, de Graaf et al. (17) measured oxidative susceptibility in three LDL subfractions in 11 healthy people (LDL1, d =1.030-1.033; LDL2, d = 1.033-1.044; LDL3, d = 1.040-1.045 g/ml). A significant inverse linear relation was found between LDL size and lag time ($P < 0.001$). The LDL size showed a negative linear relation with the rate of oxidation ($P < 0.001$) and the amount of conjugated dienes formed in the LDL subfractions. However, the range of LDL used in this study was only 1.030-1.045 g/ml which corresponds to the large, buoyant part of LDL.

The composition of LDL subfractions may also be an important factor for its oxidative susceptibility although the information gathered to date is contradictory. Tribble et al. (14) reported that small, dense LDL particles had more cholesteryl ester and less free cholesterol than large, buoyant ones. They suggested that free cholesterol, which was found predominantly on the surface of the LDL, could be responsible for protection from lipid oxidation. In support of this theory, Rudel et al. (20) in their study with monkeys suggested that the increased atherogenecity of LDL particles was associated with the enrichment of these particles with cholesteryl esters. However, in humans de Graaf et al. (17) reported that large buoyant LDL particles had more cholesteryl ester than smaller ones whereas Dejager el al. (19) reported that the concentration of cholesteryl ester and free cholesterol was not significantly different between large LDL and small LDL. The

shortened lag time in small LDL is explained in part by a lower content of antioxidants, including ubiquinol-10 and α-tocopherol. Previous studies have shown that small LDL contain significantly less α-tocopherol and ubiquinol-10 than large (15-17). However, Dejager et al. (19) found that the oxidative susceptibility of LDL in large and small was independent of vitamin E content.

As with the entire spectrum of sizes of LDL, the fatty acid contents of its subfractions may influence its oxidative susceptibility although few studies have investigated this. de Graaf et al. (17) suggested that small LDL was less resistant to oxidation than large because they have significantly higher concentrations of polyunsaturated fatty acids including 18:2n-6 and 20:4n-6. In this study the fatty acid content of LDL was expressed relative to the mg of vitamin E.

Postmenopausal women have enhanced CVD-risk profile compared to premenopausal women. Although the CVD-risk increases in this population, minimal experimental data have been accumulated to understand why.

We know of no work that compared the oxidative susceptibility of different LDL subfractions enriched with fatty acids that differ in their degree of unsaturation obtained from postmenopausal women. Consequently, the purpose of this study was to investigate the oxidative susceptibility of different LDL subfractions obtained from postmenopausal women enriched with n-9, n-6, and n-3 fatty acids.

Subjects and Methods

Experimental design: A single-blind, three-treatment, three-period, randomized crossover trial was used. The treatments were 15 g daily supplements of oleic acid-rich sunflower oil, a good source of n-9 fatty acids (TS), linoleic acid-rich safflower oil, a good source of n-6 fatty acids (SO), or EPA/DHA-rich fish oil, a good source of n-3 fatty acids (FO). Each subject received all three treatments. Each of the treatment periods lasted five weeks followed by a 7-week washout interval.

Sixteen subjects were recruited from the Oregon State University campus and surrounding community. They were healthy, nonsmoking, normolipidemic postmenopausal females, aged 53-72 years who were on hormone replacement therapy (HRT). Postmenopausal was defined as menstrual cycles ceased for at least six months or use of HRT for a comparable period of time. Body weights were between 50 and 85 percentiles of their mean weight range for the age (21). Individuals who were on chronic medication, such as corticosteroids, thyroid hormones, lipid lowering drugs, or high blood pressure drugs, were not included in the study. Nutritional supplements were not permitted except calcium (1500 mg/day including diet and supplement).

Approval to work with human subjects was obtained from the Oregon State University's Human Subjects Committee. During the study the participants visited the Metabolic Feeding Unit in the Department of Nutrition and Food Management at Oregon State University on Monday and Wednesday on the first and sixth weeks of each period. Blood samples were drawn, empty supplement bottles were taken, any leftover capsules were counted, new supplements were given if appropriate, and breakfast was provided.

In addition, weight and blood pressure were measured during each visit. A profile of the current physical activity was collected and analyzed at each period by the method of Mayer et al. (22). Subjects were asked to keep 3-day dietary records once during each period. These were analyzed using Food Processor Plus 7.0 (ESHA Research, Salem, OR). These records were used to establish that the subjects did not modify their diets significantly over the course of study. Subjects were also asked to visit the Metabolic Feeding Unit on Monday of the third week of each period. During this visit empty supplement bottles were taken, new supplements were given and breakfast was provided. This visit was made to encourage subjects' involvement in the study thus improving compliance. The fatty acid profile of the two LDL subfractions was measured as well as the disappearance of the capsules monitored to determine compliance of the subjects. The fatty acid profile of the oils was measured by gas chromatography as previously described by Song and Wander (23) and is given in **Table 4.1**. Heptadecanoic acid (Nu-Chek Prep, Elysian, MN) was added as an internal standard.

The TS, obtained in bulk from Humpco, Memphis, TN, and the SO, obtained in bulk from Arista Industries, Darien,CO, were encapsulated by the Professional Compounding Pharmacy, Corvallis, OR. Fifteen grams of oil were equally distributed into 20 capsules for each day. The FO was obtained in 1g soft gel capsules from the National Institute of Health's (NIH) Fish Oil Test Material Program.

TABLE 4.1
Intake of selected fatty acids obtained from the three oils given to the postmenopausal women as dietary supplements

	TS	SO	FO[1]
		g/day	
16:0	0.49	1.04	2.16
18:0	0.53	0.36	0.39
[2]∑SFA	1.26	1.53	3.90
16:1n-7	0.02	0.02	1.34
18:1n-9t	ND	ND	ND
18:1n-9c	12.3	1.85	0.76
20:1n-9	0.04	0.03	0.12
24:1	ND	0.02	0.04
[3]∑MUFA	12.3	2.04	2.63
18:2n-6cc	0.58	10.5	0.21
18:3n-3	0.02	0.02	0.15
20:4n-6	ND	ND	0.12
20:5n-3	ND	0.02	1.97
22:5n-3	ND	ND	0.35
22:6n-3	ND	ND	1.44
[4]∑PUFA	0.59	10.5	4.56

[1]Analysis was performed by National Institute of Health, Fish Oil Test Material Program.
[1]ND is not detectable.
[2]∑SFA is 14:0+16:0+18:0+20:0+22:0+24:0
[3]∑MUFA is 16:1n-7+18:1n-9*t* +18:1n-9*c* +20:1n-9+24:1
[4]∑PUFA is 18:2n-6*cc*+18:3n-3+20:4n-6+20:5n3+22:5n-3+22:6n-3

The participants consumed 15g of oil /day. Each fish oil capsule contained 1 mg/g α-tocopherol, 1 mg/g γ-tocopherol, and 0.02% tertiary butylhydroquinone (TBHQ). The α-tocopherol, γ-tocopherol and TBHQ content of the TS and SO were matched to that of the NIH fish oil before they were encapsulated. Peroxide value, a measure of lipid

peroxidation, and p-anisidine value, an indication of the aldehyde content of fats, were measured before each period to track oxidation of the oils by methods reported previously (4,24).

Blood was collected after an overnight fast of $\geq$ 10 hours into tubes containing Na_2EDTA (1.5 mg/ml plasma). Plasma was separated by low-speed centrifugation at 600 x g for 15 min at 4°C (TJ-6 Centrifuge, Beckman, Palo Alto, CA). Aliquots were made for each essay and stored at -80°C until processed.

The two fractions of LDL were prepared by density gradient ultracentrifugation using a near vertical rotor (Beckman NVT 65, Palo Alto, CA). Briefly, 1.006 g/ml NaCl was gently added to Quick-seal centrifuge tubes (Cat. No. 34413, Beckman, Palo Alto, CA) over 1.065 g/ml KBr solution. The density of plasma was adjusted to 1.080 g/ml by addition of solid KBr, then under-layered to the KBr solution. The tubes were centrifuged at 60,000 rpm for 105 min (L5-75 Ultracentriguge Beckman, Palo Alto, CA). To determine the density of LDL, a pure KBr solution was adjusted to the same density and centrifuged under the same conditions as the plasma. The densities of multiple contiguous bands of the KBr solution were measured using a refractometer (7187 VD, Bausch & Lomb, Rochester, NY). These known densities were used to prepare the constant density range for buoyant and dense LDL fractions. The LDL subfractions were separated with a syringe. The density range of 1.032-1.045 g/ml was prepared for large, buoyant LDL and 1.045-1.060 g/ml for small, dense LDL. By this procedure the LDL was separated from other lipoproteins in about 3 hours. The density of LDL was confirmed by non-denaturing gradient gel electrophoresis using 2-16% polyacrylamide gradient gels (Isolab, Arkon, OH).

Oxidation of LDL: The LDL subfractions were immediately dialyzed against 0.01M phosphate-buffer-saline (PBS) in the dark at 4°C for 20 h with four changes of buffer to remove EDTA (LDL:PBS = 1:100). The buffer was made oxygen free by vacuum degassing for 10 min followed by purging with nitrogen for 1 min. The dialyzed LDL was used immediately.

From one aliquot of the two dialyzed LDL subfractions, oxidation was measured using the continuous production of conjugated dienes as developed by Esterbauer et al.(25) and currently in use in our laboratory (4). In this method LDL was oxidized with 1.66 μM copper and the production of conjugated dienes was measured at 234 nm. The lag time, maximum rate of oxidation, and maximum amount of conjugated dienes formed were measured. To evaluate change in apo B produced by the oxidation its change in electronegativity was measured on another aliquot of the two dialyzed LDL subfractions. This fraction was oxidized at 37°C in oxygen-saturated PBS with 5 μM $CuCl_2$. Aliquots of these oxidized subfractions were withdrawn at 0, 0.5, 1, 3, 5, 7, and 23 hours. Increased negative charge of apo B was measured by agarose-gel electrophoresis (26).

Composition of the two LDL subfractions: The fatty acid profile of the two LDL subfractions was measured by gas chromatography as previously described by Song and Wander (23). LDL cholesterol concentration was measured enzymatically by a modification of the method of Allain et al. (27) (Sigma Kit, Cat.No. 352-100, St. Louis, MO). Triacylglycerol concentration was determined by a modification of the method of McGowan et al. (28) (Sigma Kit, Cat.No. 336-10). Free cholesterol (FC) concentration was determined enzymatically using a product from Wako Pure Chemical Industry (Cat.

No.274-47109, Richmond, VA). Cholesteryl ester concentration was calculated as (Total cholesterol-FC) x 1.68 (29). Phospholipids was determined enzymatically as phosphotidyl choline (Wako Pure Chemical Industry, Cat. No., 996-54001). The concentration of total phospholipids was calculated by multiplying the concentration of phosphotidyl choline by 1.1. Protein was determined by the method of Lowry (30). α-Tocopherol and γ- tocopherol concentration was measured by isocratic HPLC (4,31).

Statistical analysis: Summary statistics were calculated (mean and standard error). Normal distribution and homogeneity of variance were checked before data analysis and all data obeyed these two assumptions for analysis of variance. The balanced crossover design of this study allowed examination for carryover effects. No carryover was demonstrated for any variable. The effects of each supplement on the variables of interest in the two LDL subfractions were compared using a between-within subject ANOVA procedure with repeated measure on the two LDL subfractions. Between subject sources of variation included sequence of treatment and individual subjects nested within sequence and within subject sources of variation included period and treatment (32). If there was a significant interaction between treatment and LDL subfractions, the effect of treatment was separately compared in the two LDL subfractions using pair-wise t-tests. Pair-wise comparisons were made using Fisher's least significant difference test to determine significant differences among treatments (32). The calculations were performed using the general linear model (GLM) of the Statistical Analysis System (SAS) software (version 6.12; SAS Institute, Cary NC). P-values ≤ 0.05 were reported as significant.

Results

Subjects: Fifteen subjects completed this study. Overall, compliance to the study was excellent as indicated by the change in the fatty acid profile of the two LDL subfractions and the disappearance of the oil supplementation capsules. No side effects were reported from the supplementation. All subjects were in good physical condition at the time of the study as indicated from their medical records.

The initial characteristics of the subjects are given in **Table 4.2**. According to average BMI, the subjects were marginally overweight since the upper limit of a healthy weight BMI is 24.9 kg/m^2 (33). The average value of plasma total cholesterol of the subjects was 1 % higher than the desirable level according to the guideline issued by the U.S. National Cholesterol Education Program Adult Treatment Panel II (34) (<200 mg/dl, 5.2 mmol/L). However, the average LDL and HDL cholesterol of the subjects met or surpassed the desirable level (<130 mg/dl, 3.4 mmol/L, ≥ 60 mg/dl, 1.6 mmol/L, respectively). The average plasma triacylglycerol concentration also met the desirable level (<200 mg/dl, 2.3 mmol/L). Blood pressure was within normal ranges. The alcohol consumption ranged from 0 to 16 drinks/wk and the mean was 2.3 drinks/wk. Eleven subjects used 0.625, two subjects used 0.3, and one subject used 1 mg of conjugated equine estrogen daily. Only one subject used 1 mg of estradiol for her hormone replacement therapy. Not all subjects used combined HRT. Of those who did six subjects used 2.5 and one used 5 mg progesterone. Their average leisure physical activity level was 2.5 hours/wk and did not change during the study.

TABLE 4.2
Characteristics of subjects before supplementation with dietary oils

	Mean ± SD (n = 15)
Age (y)	57.9 ± 5.8
BMI (kg/m^2)	25.9 ± 3.5
Plasma cholesterol (mg/dl)	202 ± 21
HDL cholesterol (mg/dl)	60 ± 15
LDL cholesterol (mg/dl)	115 ± 24
Plasma triacylglycerol (mg/dl)	132 ± 41
Systolic blood pressure (mmHg)	129 ± 10
Diastolic blood pressure (mmHg)	76 ± 8

The average value of the 3-day diet records calculated at each of the three periods was reported in detail previously (35). Values for selected nutrients are reported in **Table 4.3**. The intake of carbohydrate, protein, total fat, saturated fat, and cholesterol was within the recommendations of the Dietary Guidelines (36). All other nutrients met or exceeded the recommended amounts. The fifteen gram oil supplement provided an additional 565 kJ (135 Kcal) and 15 mg α-tocopherol each day. The subjects gained an average of 0.6 kg over the 29 weeks of the study. The amounts of oleic acid provided by Trisun, linoleic acid provided by safflower oil, and EPA/DHA provided by fish oil were physiologically relevant. The 12.3 g of oleic acid provided by the Trisun and the 10.5 g linoleic acid provided by the safflower oil could be obtained from approximately 3 teaspoons of Canola oil and corn oil, respectively. The quantity of fish oil given to the participants was equivalent to two servings (180g) of fish a day. No significant amount of oxidation of the oils was found during the study as measured by the peroxide value or p-anisidine.

TABLE 4.3
Composition of selected dietary components of subjects averaged from 3-day dietary records obtained at each of the three periods

	Intake Mean ± SE
KJ	1790 ± 460
% Cabohydrates	55 ± 8
% Protein	16 ± 3
% Fat	29 ± 6
Saturated fat (g)	19 ± 8
MUFA (g)	18 ± 8
PUFA (g)	9 ± 5
EPA, DHA (mg)	0, 0.2
Cholesterol (mg)	178 ± 89
Fiber (g)	20 ± 8
Vitamin E (α-TE, mg)	6.4 ± 3.1
Vitamin C (mg)	140 ± 83
Calcium (mg)	943 ± 340
Iron (mg)	13.5 ± 6.0
Selenium (mg)	57 ± 30

Fatty acid profile in the LDL subfractions: The fatty acid composition of the two LDL subfractions after oil supplementation is shown **Table 4.4**. The concentration of the major saturated fatty acids as well as the total amount of saturated fatty acid were not changed by the oil supplements. These concentrations did, however, differ in the small and large LDL. In all cases they were greater in the large LDL.

TABLE 4.4
Concentration of selected fatty acid in LDL subfractions of subjects after supplementation with the three dietary oils[1]

				Main effect of dietary treatment			Main effect of size	Interaction
nmol/mg of LDL protein	TS[2]	SO	FO	TS:SO[3]	TS:FO	SO:FO	L:S[4]	TRT*SIZE[5]
L[6]14:0	54±6	51±6	49±6	0.5271	0.7382	0.5656	0.0374	0.4962
S[7]14:0	37±6	47±6	38±6					
L 16:0	70±2	71±2	68±2	0.6607	0.7845	0.4775	0.0001	0.9342
S 16:0	52±4	53±4	53±4					
L 18:0	429±92	263±92	263±92	0.2829	0.2624	0.9612	0.0236	0.2906
S 18:0	170±13	193±13	186±13					
L SFA[8]	1215±98	1087±98	1057±98	0.2733	0.1940	0.8322	0.0010	0.3289
S SFA	775±58	833±58	779±58	0.8538	0.6783	0.8172		
L 18:1n-9	656±26	495±26	455±26	0.0001	0.0001	0.2802	0.0001	0.0406
S 18:1n-9	444±27	388±27	332±27	0.1457	0.0061	0.1501		
L MUFA[9]	903±37	718±37	680±37	0.0067	0.0003	0.2474	0.0001	0.1223
S MUFA	617±38	563±38	506±38					
L 18:2n-6	1172±42	1380±42	998±42	0.0078	0.0127	0.0001	0.0001	0.3344
S 18:2n-6	904±57	1023±57	783±57					
L 18:3n-3	27±2	21±2	26±2	0.0797	0.8003	0.0476	0.0001	0.5404
S 18:3n-3	18±2	16±2	20±2					
L 20:4n-6	259±12	262±12	218±12	0.5818	0.0241	0.0066	0.0004	0.9320
S 20:4n-6	203±18	217±12	169±12					
L 20:5n-3	23±8	21±8	215±8	0.9474	0.0001	0.0001	0.1623	0.0115
S 20:5n-3	17±18	43±18	150±18	0.3132	0.0001	0.0002		
L 22:6n-3	45±5	47±5	119±5	0.7694	0.0001	0.0001	0.0001	0.0099
S 22:6n-3	38±4	37±4	91±4	0.7860	0.0001	0.0001		

TABLE 4.4 (Continued)

				Main effect of dietary treatment	Main effect of size	Interaction		
Numol/mg of LDLprotein	TS[2]	SO	FO	TS:SO[3]	TS:FO	SO:FO	L:S[4]	TRT*SIZE[5]
L PUFA[10]	1868±73	2071±73	1927±73	0.0476	0.5912	0.1368	0.0001	0.9876
S PUFA	1367±89	1578±89	1417±89					
L n-6[11]	1513±50	1718±50	1282±50	0.0217	0.0065	0.0001	0.0001	0.5591
S n-6	1170±76	1304±76	993±76					
L n-3[12]	121±14	116±14	383±14	0.7925	0.0001	0.0001	0.0006	0.0237
S n-3	85±19	104±19	289±19	0.4929	0.0001	0.0001		
L UI[13]	4822±200	5233±200	5710±200	0.1409	0.0003	0.0140	0.0001	0.5383
S UI	3535±247	4063±247	4211±247					

[1] Mean ± SE, mmol fatty acid per mg of LDL protein

[2] TS = Oleic acid enriched-oil (n-9), SO = Linoleic acid enriched-oil (n-6), FO Fish oil

[3] TS:SO = Paired t-test between TS and SO; TS:FO = Paired t-test between TS and FO; SO:FO = Paired t-test between SO and FO

[4] L:S = Paired t-test between large and small LDL

[5] TRT*SIZE = Treatment-LDL subfraction interaction

[6] L is large LDL

[7] S is small LDL

[8] ∑SFA=14:0+16:0+18:0+20:0+22:0

[9] ∑MUFA=16:4n-1+18:1n-9

[10] ∑PUFA= 18:2n-6+18:3n-3+20:4n-6+20:5n-3+22:4n-6+22:6n-3

[11] ∑n6=18:2n-6+20:4n-6

[12] ∑n3=18:3n-3+20:5n-3+22:6n-3

[13] UI= (∑MUFA) + (∑PUFA with 2 double bonds * 2) + (∑PUFA with 3 double bonds * 3)...+ (∑PUFA with 6 double bonds * 6)

Although the change in the concentration of total MUFA occurred primarily because of changes in the concentration of oleic acid, there was a significant treatment-LDL subfraction interaction for this fatty acid. This occurred because in the large fraction its concentration in the SO and FO groups were statistically equivalent but lower (26% and 30%, respectively) than the TS groups. In contrast, in the small subfraction although the concentration in the FO group remained significantly lower than in the TS group (25%) that in the SO group did not.

The concentration of total n-6 fatty acids was significantly higher in large LDL than that in small (1.51 ± 0.05 vs 1.18 ± 0.08 umol/mg LDL protein, respectively, (P = 0.0001). After the oil supplementation the concentration was also significantly changed. The concentration was 13% and 32% higher in SO-supplemented group than the TS- and FO- supplemented groups, respectively. The concentration was also 18% higher in the TS-supplemented group than the FO-supplemented group. These changes were produced primarily by changes in the concentration of linoleic acid but additionally by changes in the concentration of arachidonic acid.

Large LDL had significantly higher arachidonic acid than small (0.25 ± 0.01 vs 0.20 ±0.02 umol/mg LDL protein, respectively, P = 0.0004). Despite the fact that the fish oil supplement was the only one that provided arachidonic acid, the concentration was significantly decreased in the FO-supplemented group compared to the TS and SO-supplemented groups. The concentration was 17% lower after the FO supplementation than after both the TS and SO supplementation. The fact that there was no interaction between treatment and LDL subfractions in these variables indicated that the change

produced by the oil treatment in the small subfractions paralled those seen in the large subfractions.

There was a significant treatment-LDL subfraction interaction in the concentration of n-3 fatty acids. The concentration of EPA was higher after the FO supplementation than after the TS and SO supplementation in both large and small LDL. However, in large LDL the increase in EPA concentration was greater than that in small one. The concentration was 10-fold higher after FO supplementation than after both the TS and SO supplementation in large LDL. The concentration was 650% and 275% higher in the FO-supplemented group than after the TS and SO supplementation, respectively, in small LDL.

The concentration of DHA was significantly higher in the FO-supplemented group than in the TS- and SO-supplemented group in both large and small LDL. Again the increase was greater in the large LDL. It was 200% and 140% higher in the FO-supplemented group than the TS- and SO-supplemented groups, respectively, in large LDL. The concentration was 125% higher in the FO-supplemented group than both the TS- and SO- supplemented groups in small LDL.

The changes in the concentration of total n-3 fatty acids mirrored those that occurred in the EPA and DHA. As expected, there was an interaction between LDL subfractions and dietary oil treatment. This occurred because the increase in total n-3 fatty acids was greater in the large LDL. It was significantly increased in the FO-supplemented group compared to in the TS- and SO-supplemented groups in both large and small LDL.

The unsaturation index was 29% and 12% higher in the FO-supplemented group compared to the TS- and SO supplemented groups, respectively. The unsaturation index was significantly higher in large LDL than that in small LDL (5.3 ± 0.2 vs 4.0 ± 0.2, respectively, P = 0.0001).

Composition of LDL subfractions: The composition of the two LDL subfractions is shown in **Table 4.5**. The concentrations of triacylglycerol, total cholesterol, free cholesterol, cholesterol ester, phospholipid, α-tocopherol, and γ-tocopherol were significantly higher in large than small LDL when the values were expressed as mg of each constituent per mg of LDL protein. Triacylglycerol concentration was 71% higher (0.24 ± 0.02 vs 0.14 ± 0.01 mg/mg LDL protein, respectively, P = 0.0001); total cholesterol was 29% higher (1.06 ± 0.03 vs 0.82 ± 0.03 mg/mg LDL protein, respectively, P = 0.0001); free cholesterol was 35% higher (0.27 ± 0.01 vs 0.20 ± 0.01 mg/mg LDL protein, respectively, P = 0.0001); cholesterol ester was 27% higher (1.32 ± 0.06 vs 1.04 ± 0.06 mg/mg LDL protein, respectively, P = 0.0001); phospholipid 23% higher (1.06 ± 0.03 vs 0.86 ± 0.02 mg/mg LDL protein, respectively, P = 0.0001); α-tocopherol was 16% higher (10.7 ± 1.2 vs 9.2 ± 0.9 nmol/mg LDL protein, respectively, P = 0.0005); and γ-tocopherol 37% higher (1.15 ± 0.17 vs 0.84 ± 0.12 nmol/mg LDL protein, respectively, P = 0.0065) in large LDL than in small LDL. Dietary oil supplementation did not change the composition of either subfraction.

TABLE 4.5

Composition of the two LDL subfractions obtained from the subjects after supplementation with the three dietary oils[1]

				Main effect of dietary treatment			Main effect of size	Interaction
	TS[2]	SO	FO	TS:SO[3]	TS:FO	SO:FO	L:S[4]	TRT*SIZE[5]
L[6] Triacylglycerol[7]	0.24±0.02	0.25±0.02	0.23±0.02	0.4987	0.4956	0.1439	0.0001	0.6393
S[8] Triacylglycerol	0.13±0.01	0.15±0.01	0.13±0.01					
L Total cholesterol[7]	1.08±0.03	1.05±0.03	1.00±0.03	0.3908	0.0980	0.4065	0.0001	0.6338
S Total cholesterol	0.83±0.03	0.81±0.03	0.79±0.03					
L Free cholesterol[7]	0.28±0.01	0.28±0.01	0.26±0.01	0.3732	0.0727	0.3437	0.0001	0.4977
S Free cholesterol	0.21±0.01	0.20±0.01	0.20±0.01					
L Cholesteryl ester[7]	1.34±0.06	1.34±0.06	1.26±0.06	0.6841	0.2619	0.4687	0.0001	0.5549
S Cholesteryl ester	1.08±0.06	1.02±0.06	1.01±0.06					
L Phospholipid[7]	1.07±0.03	1.07±0.03	1.05±0.03	0.7645	0.1963	0.3155	0.0001	0.6355
S Phospholipid	0.89±0.02	0.87±0.02	0.83±0.02					
L α-Tocopherol[9]	10.7±1.1	10.7±1.0	11.6±0.8	0.5243	0.0973	0.2456	0.0005	0.2547
S α-Tocopherol	9.24±0.90	8.85±1.11	8.08±032					
L γ-Tocopherol[9]	1.15±0.17	1.30±0.23	1.03±0.23	0.4356	0.5878	0.2454	0.0065	0.4356
S γ-Tocopherol	0.84±0.19	0.94±0.14	0.77±0.21					

[1] Mean ± SE

[2] TS = Oleic acid enriched-oil (n-9), SO = Linoleic acid enriched-oil (n-6), FO = Fish oil (n-3)

[3] TS:SO = Paired t-test between TS and SO; TS:FO = Paired t-test between TS and FO; SO:FO = Paired t-test between SO and FO

[4] L:S = Paired t-test between large and small LDL

[5] TRT*SIZE = Treatment-LDL subfraction interaction

[6] L is large LDL

[7] mg/mg LDL protein

[8] S is small LDL

[9] mmol/mg LDL protein

Oxidative susceptibility of the two LDL subfractions expressed as conjugated diene formation: The oxidative susceptibility of the two LDL subfractions was measured by monitoring the formation of conjugated dienes. The data are shown in **Table 4. 6**. There was a significant treatment-LDL subfraction interaction on lag time. It was shorter after the FO supplementation than the TS and SO supplementation in both large and small LDL. However, the changes were bigger in the large fraction than those in small one. After the FO supplementation lag time was 27% and 21% shorter than after the TS and SO supplementation, respectively in large LDL. It was only 18% and 19% shorter in the FO-supplemented group than in the TS- and SO-supplemented groups, respectively, in small LDL.

The maximum rate of the formation of conjugated dienes was 14% higher in large LDL than that in small LDL (1.90 ± 0.31 vs 1.67 ± 0.10 nmol/mg LDL protein/min, respectively, P = 0.002). It was also higher after SO supplementation than TS and FO supplementation in both large and small LDL. After the SO supplementation the maximum rate was 16% and 40% higher than after the TS and FO supplementation, respectively. The maximum rate was also 21% higher after the TS supplementation than the FO supplementation. The maximum amount of conjugated dienes was higher in large than small LDL (92.4 ± 3.0 vs 73.9 ± 2.6 nmol/mg LDL protein, respectively, P = 0.0001). It was lower after the TS supplementation than the SO and FO supplementation, however; in SO- and FO- supplemented groups it was statistically equivalent. After the TS supplementation the maximum amount of conjugated dienes was 7% and 8% lower than after the SO and FO supplementation, respectively.

TABLE 4.6

Oxidative susceptibility in two LDL subfractions obtained from the subjects after supplementation with the three dietary oils expressed as conjugated dienes formation[1]

				Main effect of dietary treatment			Main effect of size	Interaction
	TS[2]	SO	FO	TS:SO[3]	TS:FO	SO:FO	L:S[4]	TRT*SIZE[5]
L[6] Lag Time	52.5±2.5	49.1±2.5	38.6±2.6	0.1034	0.0001	0.0001	0.0001	0.0125
S[7] Lag Time	43.6±3.0	44.0±2.7	35.8±2.1	0.8747	0.0098	0.0057		
L Max Rate[8]	1.90±0.16	2.28±0.13	1.53±0.10	0.0153	0.0194	0.0001	0.0020	0.0853
S Max Rate	1.66±0.11	1.86±0.08	1.50±0.09					
L Max Conc[9]	85.9±2.8	97.0±3.0	94.3±3.1	0.0401	0.0501	0.8714	0.0001	0.1505
S Max Conc	71.2±3.4	73.3±2.3	77.2±2.2					

[1] Mean ± SE

[2] TS = Oleic acid enriched-oil (n-9), SO = Linoleic acid enriched-oil (n-6), FO = Fish oil (n-3)

[3] TS:SO = Paired t-test between TS and SO; TS:FO = Paired t-test between TS and FO; SO:FO = Paired t-test between SO and FO

[4] L:S = Paired t-test between large and small LDL

[5] TRT*SIZE = Treatment-LDL subfraction interaction

[6] L is large LDL

[7] S is small LDL

[8] Max Rate; Maximum rate, nmol /mg LDL protein /min

[9] Max Conc; Maximum amount of conjugated diene, nmol /mg LDL protein

Since there was no interaction between treatment and subfraction for either of these last two variables, the changes produced by the oil supplements in the large fraction paralled those in the small.

Apo B oxidation in the two LDL subfractions: The changes produced in apo B by oxidation in the two LDL subfractions measured as the increase in their negative charge are shown in **Table 4.7**. These values were statistically equivalent in large and small LDL except at 1 hr and 23 hr. At 1 hr it was 13% lower in large LDL compared to small LDL (1.64 ± 0.03 vs 1.86 ± 0.02, respectively, P = 0.0065). However, it was 5% higher at 23hr in large LDL compared to that of small LDL (6.01 ± 0.05 vs 5.71 ± 0.03, respectively, P = 0.0027).

The production of the negative charge was higher in the FO-supplemented group until 7 hr of oxidation. It was 18% and 16% higher in the FO-supplemented group than in the TS and SO-supplemented groups, respectively, at 0.5 hr, but statistically equivalent in the TS and SO-supplemented groups. At 1 hr it was 39% and 34% higher in the FO-supplemented group compared to the TS and SO-supplemented groups, respectively, and it was statistically equivalent in the TS and SO-supplemented groups. At 3 hr it was 7% and 12% lower in the TS-supplemented group compared to the SO and FO-supplemented groups, respectively, and it was statistically equivalent in the SO and FO-supplemented groups. The differences of the production of the negative charge gradually decreased in three dietary oil treatment groups after 5 hr. Ultimately the differences were statistically equivalent in three dietary oil treatment groups at 23 hr.

TABLE 4.7

Apo B oxidation in the two LDL subfractions obtained from the subjects after supplementation with the three dietary oils

				Main effect of dietary treatment			Main effect of size	Interaction
	TS[2]	SO	FO	TS:SO[3]	TS:FO	SO:FO	L:S	TRT*SIZE[4]
L[5] 0.5hr	1.10±0.06	1.11±0.06	1.34±0.07	0.6304	0.0001	0.0002	0.5599	0.5480
S[6] 0.5hr	1.14±0.03	1.17±0.03	1.30±0.04					
L 1hr	1.39±0.09	1.48±0.09	2.04±0.11	0.5197	0.0001	0.0001	0.0065	0.8656
S 1hr	1.67±0.07	1.70±0.07	2.22±0.08					
L 3hr	3.29±0.15	3.75±0.15	3.89±0.16	0.0284	0.0005	0.1544	0.2819	0.3764
S 3hr	3.60±0.12	3.72±0.12	3.94±0.13					
L 5hr	4.41±0.15	4.82±0.15	4.92±0.16	0.5723	01024	0.0960	0.5764	0.4649
S 5hr	5.02±0.09	4.66±0.10	4.30±0.10					
L 7hr	4.98±0.14	5.38±0.14	5.41±0.15	0.1711	0.1720	0.1240	0.1288	0.4360
S 7hr	5.38±0.10	5.13±0.10	4.78±0.11					
L 23hr	5.86±0.15	6.17±0.14	6.00±0.14	0.1691	0.2027	0.9294	0.0027	0.1704
S 23hr	5.56±0.10	5.68±0.09	6.12±0.09					

[1] Mean ± SE, Relative electrophoretic mobility

[2] TS = Oleic acid enriched-oil (n-9), SO = Linoleic acid enriched-oil (n-6), FO = Fish oil (n-3)

[3] TS:SO = Paired t-test between TS and SO; TS:FO = Paired t-test between TS and FO; SO:FO = Paired t-test between SO and FO

[4] L:S = Paired t-test between large and small LDL

[5] TRT*SIZE = Treatment-LDL subfraction interaction

[6] L is large LDL

[7] S is small LDL

Discussion

With a gradual understanding of the mechanisms contributing to the development of atherosclerosis, it has become apparent that dietary fat composition may also influence its development by mechanisms other than lowering plasma cholesterol concentrations. When LDL becomes oxidized it takes on a variety of atherogenic properties. The oxidative susceptibility of LDL depends on its content of fatty acids (37), the main targets of the oxidative reactions, and also on the presence of antioxidants, such as ascorbic acid, uric acid, α-tocopherol, carotenoids, lycopenes, ubiquinol-10, or phenols (38,39).

In the present study dietary fatty acids supplements significantly changed the concentrations of fatty acids in the two LDL subfractions. Supplementation with an oil rich n-9 fatty acids resulted in LDL with about 30% more oleic acid, supplementation with an oil rich in n-6 fatty acids caused about a 20% increase in the amount of linoleic acid, and supplementation of fish oil caused a many-fold increase in the amount of EPA and DHA. In addition, the total amount of each fatty acid was much greater in the large subfraction. These modulations in the fatty acids composition of LDL influenced its oxidation. Supplementation with the n-9 fatty acid-rich oil resulted in LDL more resistant to oxidation as compared with supplementation with oil rich in n-6 fatty acids as measured by the formation of conjugated dienes and increase in the negative charge on apo B. However, the oxidative susceptibility of LDL after supplementation with n-3 fatty acids was less clear. N-3 fatty acid-enriched LDL demonstrated a shorter lag time than that measured in n-9 and n-6 fatty acid-enriched LDL but its maximum rate of oxidation was the lowest among the three oil-enriched LDL. This behavior was more

pronounced in the large subfraction. In addition, although the maximum amount of conjugated diene formation was higher than that in n-9 fatty acid-enriched LDL, it was statistically equivalent with that in n-6 fatty acid-enriched LDL. The formation of negative charge on apo B after FO supplementation was higher compared to both the TS and SO supplementation at 0 hr and 1 hr and it was higher after the FO supplementation than the TS supplementation at 3 hr. It was statistically equivalent in three oil treatments at 5, 7, and 23 hr.

These differences in the oxidizability of LDL subfractions enriched with different fatty acids may relate to their α-tocopherol content. Although the amount of α-tocopherol in the two LDL subfractions was statistically equivalent in the three treatment groups, the ratio of unsaturated fatty acids to α-tocopherol was 23% and 7% higher in the FO-supplemented group compared to the TS- and SO-supplemented groups, respectively. The shorter lag time and faster formation of negative charge on apo B after FO supplementation compared to that of TS may be explained by its higher number of unsaturated fatty acids molecules per molecule of α-tocopherol in each of the LDL subfractions.

Based on classical autoxidation kinetics, the rate of oxidation of oleic acid is much slower than that of fatty acids such as EPA and DHA which have 5 and 6 double bonds, respectively (61). Thus, it can be argued that highly unsaturated fatty acids such as EPA and DHA would increase oxidative stress, encourage LDL oxidation, increase atherogenesis, and ultimately increase cardiovascular mortality while MUFA intake would produce LDL less susceptible to oxidize and be associated with a decrease in cardiovascular mortality. Based on epidemiologic data generated about MUFA, this

appears to be true. The reduced risk of cardiovascular disease in Mediterranean countries has been well documented (40). The Mediterranean diet is rich in MUFA and reduced in PUFA compared to the American diet. The higher MUFA intake in Mediterranean countries may contribute to the reduced cardiovascular disease that has been found there.

However, the association with epidemiologic data and oxidation of LDL enriched with EPA and DHA is less clear. One cannot say definitively that these LDL are more susceptible to oxidize. On the one hand, lag time is shorter suggesting that they are more prone to oxidize. On the other hand, the rate of formation of conjugated dienes is slower, suggesting that they are not. Numerous studies have shown a strong inverse relationship of CVD mortality and fish consumption (41-50). Although the oxidative susceptibility of EPA/DHA-enriched LDL is not yet clear, there are several other ways in which they change risk of heart disease.

A moderate intake of n-3 fatty acids changes the fatty acid content of lipoprotein and cell membranes, which may affect a variety of physiological mechanisms associated with atherosclerosis. For example, human leukocytes generate platelet-activating factor, a lipid mediator of inflammation (a proatherogenic factor) from membrane alkyl phospholipids through the release of arachidonic acid or other fatty acids at the 2-position and subsequent acetylation. N-3 fatty acid suppress human leukocyte arachidonic acid release and metabolism (51). Thus dietary n-3 fatty acids supplements also significantly increase endothelium-dependent relaxation in arteries (52). Decreasing platelet aggregation by reduction in the production of tromboxane A_2 (53,54) and reductions in fibrinogen have been also induced by fish oil supplementation and may contribute to the

cardiovascular benefits of n-3 fatty acids. In a meta-analysis of controlled trials, Morris et al. (55) reported that fish oil lowered blood pressure, another risk factor for CVD. In addition, the n-3 fatty acids supplementation increased the LDL particle size, reduced plasma triacylglycerol and apo B concentration and the ratio of triacylglycerol to high density lipoprotein cholesterol (35).

In addition to the influence of the dietary oil supplement on LDL oxidative susceptibility, their impact of LDL size was also investigated in this study. The lag time was shorter in small LDL compared to that in large LDL. The maximum rate of oxidation and maximum amount of conjugated dienes were higher in large LDL than in small. At 1hr the formation of negative charge on apo B was higher in small LDL, but it was higher at 23 hr in large LDL. A shortened lag time and a slower oxidation rate are contradictory in explaining oxidative susceptibility in LDL. However, the composition of the two LDL subfractions may explain this association. Numerous studies have shown that higher α-tocopherol concentrations lengthens lag time while larger amounts of PUFA increases the rate of oxidation (38,39). The concentration of α-tocopherol was 26% higher and that of PUFA was 34% higher in large LDL compared to small in the present study. The higher concentrations of α-tocopherol may explain the longer lag time in large LDL. In addition, the higher PUFA concentration may also explain the higher rate of oxidation in large LDL. Although previous studies agreed on a shorter lag time with decreasing size of LDL, results of other measurements on LDL oxidation were not clear (13-19).

In trying to explain the enhanced atherogenity of small LDL other than oxidative susceptibility, its compositional differences have also been investigated by several

groups. Recently, Halle et al. (56) and Galeano et al. (57) reported a decreased free cholesterol and phospholipid concentrations in small LDL, which led to a reduced binding affinity to the LDL receptors of human fibroblasts (58). In support of this theory, Nigon et al. (59) reported that small LDL was cleared less effectively by LDL receptors. Galeano et al. (57) also suggested that the structural conformation of apo B was different in small LDL than that of large LDL and these differences modified the binding region of apo B in small LDL. This resulted in a decreased clearance of LDL and prolonged residence time in the intravascular compartment. This allows LDL more time to infiltrate the intimal lining of the artery wall and more time to be attacked by free radicals (60). As a result oxidatively modified small LDL is taken up by artery wall macrophages to a greater extent than large LDL. These compositional differences in small LDL possibly contribute to its enhanced atherogenity. In support of this the present study showed that the composition of small LDL was significantly different than large LDL.

In conclusion, the oxidative susceptibility of LDL was reduced in n-9 fatty acid-enriched LDL compared to the n-6 fatty acid-enriched LDL in postmenopausal women. However, the influence of n-3 fatty acid supplementation on oxidative susceptibility of LDL was less clear. The data suggest that manipulation of the type of dietary fat may influence on small and large LDL similarly.

References

1. Badimon JJ, Fuster V, Chesebro JH, Badimon L. Coronary atherosclerosis. A multifactorial disease. Circulation 1993;87:II3-16.

2. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N.Engl.J.Med. 1989;320:915-924.

3. Reaven PD, Witztum JL. Oxidized low density lipoproteins in atherogenesis: role of dietary modification. Annu.Rev.Nutr. 1996;16:51-71:51-71.

4. Wander RC, Du SH, Ketchum SO, Rowe KE. Effects of interaction of RRR-alpha-tocopheryl acetate and fish oil on low-density-lipoprotein oxidation in postmenopausal women with and without hormone-replacement therapy. Am.J.Clin.Nutr. 1996;63:184-193.

5. Reaven PD, Grasse BJ, Tribble DL. Effects of linoleate-enriched and oleate-enriched diets in combination with alpha-tocopherol on the susceptibility of LDL and LDL subfractions to oxidative modification in humans. Arterioscler.Thromb. 1994;14:557-566.

6. Davidson SB, Wander RC, Du SH, Riddle M. Effect of dietary MUFAs in lipid, lipoproteins, and lipid peroxidation in NIDDM. American Oil Chemists' Society (AOCS) 1997;(abst)

7. Reaven P, Parthasarathy S, Grasse BJ, et al. Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. Am.J.Clin.Nutr. 1991;54:701-706.

8. Berry EM, Eisenberg S, Friedlander Y, et al. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins--the Jerusalem Nutrition Study. II. Monounsaturated fatty acids vs carbohydrates. Am.J.Clin.Nutr. 1992;56:394-403.

9. Bonanome A, Pagnan A, Biffanti S, et al. Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. Arterioscler.Thromb. 1992;12:529-533.

10. Whitman SC, Fish JR, Rand ML, Rogers KA. n-3 fatty acid incorporation into LDL particles renders them more susceptible to oxidation in vitro but not necessarily more atherogenic in vivo. Arterioscler.Thromb. 1994;14:1170-1176.

11. Thiery J, Seidel D. Fish oil feeding results in an enhancement of cholesterol-induced atherosclerosis in rabbits. Atherosclerosis 1987;63:53-56.

12. Suzukawa M, Abbey M, Howe PR, Nestel PJ. Effects of fish oil fatty acids on low density lipoprotein size, oxidizability, and uptake by macrophages. J.Lipid Res. 1995;36:473-484.

13. Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis 1992;93:189-199.

14. Tribble DL, Krauss RM, Lansberg MG, Thiel PM, van den Berg JJ. Greater oxidative susceptibility of the surface monolayer in small dense LDL may contribute to differences in copper-induced oxidation among LDL density subfractions. J.Lipid Res. 1995a;36:662-671.

15. Tribble DL, Thiel PM, van den Berg JJ, Krauss RM. Differing alpha-tocopherol oxidative lability and ascorbic acid sparing effects in buoyant and dense LDL. Arterioscler.Thromb.Vasc.Biol. 1995b;15:2025-2031.

16. Tribble DL, van den Berg JJ, Motchnik PA, et al. Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and alpha-tocopherol content. Proc.Natl.Acad.Sci.U.S.A. 1994;91:1183-1187.

17. de Graaf J, Hak-Lemmers HL, Hectors MP, Demacker PN, Hendriks JC, Stalenhoef AF. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arterioscler.Thromb. 1991;11:298-306.

18. Chait A, Brazg RL, Tribble DL, Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Am.J.Med. 1993;94:350-356.

19. Dejager S, Bruckert E, Chapman MJ. Dense low density lipoprotein subspecies with diminished oxidative resistance predominate in combined hyperlipidemia. J.Lipid Res. 1993;34:295-308.

20. Rudel LL, Bond MG, Bullock BC. LDL heterogeneity and atherosclerosis in nonhuman primates. Ann.N.Y.Acad.Sci. 1985;454:248-53:248-253.

21. Frisancho AR. Anthropometric standards for the assessment of growth and nutritional status. University of Michigan Press, 1990.

22. Mayer EJ, Alderman BW, Regensteiner JG, et al. Physical-activity-assessment measures compared in a biethnic rural population: the San Luis Valley Diabetes Study. Am.J.Clin.Nutr. 1991;53:812-820.

23. Song J, Wander RC. Effects of dietary selenium and fish oil (MaxEPA) on arachidonic acid metabolism and hemostatic function in rats. J.Nutr. 1991;121:284-292.

24. Wander RC, Du SH, Ketchum SO, Rowe KE. alpha-Tocopherol influences in vivo indices of lipid peroxidation in postmenopausal women given fish oil. J.Nutr. 1996;126:643-652.

25. Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic.Res.Commun. 1989;6:67-75.

26. Noguchi N, Niki E. Apolipoprotein B protein oxidation in low-density lipoproteins. Methods Enzymol. 1994;233:490-4:490-494.

27. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin.Chem. 1974;20:470-475.

28. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin.Chem. 1983;29:538-542.

29. Sirtori CR, Gatti E, Tremoli E, et al. Olive oil, corn oil, and n-3 fatty acids differently affect lipids, lipoproteins, platelets, and superoxide formation in type II hypercholesterolemia. Am.J.Clin.Nutr. 1992;56:113-122.

30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measured with folin phenol reagent. J.Biol.Chem. 1951;193:265-275.

31. Arnaud J, Fortis I, Blachier S, Kia D, Favier A. Simultaneous determination of retinol, alpha-tocopherol and beta- carotene in serum by isocratic high-performance liquid chromatography. J.Chromatogr. 1991;572:103-116.

32. Kuehl RO. Crossover Design. In: AnonymousStatistical Principles of Research Design and Analysis. Belmont, CA: Duxbury Press, 1994:529-559.

33. Rowland ML. Reporting bias in height and weight data. Stat.Bull.Metrop.Insur.Co. 1989;70:2-11.

34. National Cholesterol Education Program. Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). JAMA 1993;269:3015-3023.

35. Lee YS, Du SH, Higdon J, Wu T, Wander RC. The effect of supplementation with n-9, n-6, and n-3 fatty acids on plasma lipid, lipoprotein, apolipoprotein B concentrations, and LDL particle size in postmenopausal women. Submitted 1999

36. U.S.Department of Agriculture and U.S.Department of Health and Human Service. Nutrition and your health, Dietary guidelines for Americans, 4th ed. 1995.

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

38. Niki E, Noguchi N, Gotoh N. Inhibition of oxidative modification of low density lipoprotein by antioxidants. J.Nutr.Sci.Vitaminol.(Tokyo.) 1993;39 Suppl:S1-8:S1-S8

39. Thomas MJ, Thornburg T, Manning J, Hooper K, Rudel LL. Fatty acid composition of low-density lipoprotein influences its susceptibility to autoxidation. Biochemistry 1994;33:1828-1834.

40. Keys A. Seven Countries; A multivariate analysis of death and coronary heart disease. Cambridge, Mass: 1980.

41. Kromhout D, Feskens EJ, Bowles CH. The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. Int.J.Epidemiol. 1995;24:340-345.

42. Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and risk of sudden cardiac death. JAMA 1998;279:23-28.

43. Norell SE, Ahlbom A, Feychting M, Pedersen NL. Fish consumption and mortality from coronary heart disease. Br.Med.J.(Clin.Res.Ed.) 1986;293:426

44. Kromhout D, Bosschieter EB, de Lezenne C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N.Engl.J.Med. 1985;312:1205-1209.

45. Daviglus ML, Stamler J, Orencia AJ, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. N.Engl.J.Med. 1997;336:1046-1053.

46. Zhang J, Sasaki S, Amano K, Kesteloot H. Fish consumption and mortality from all causes, ischemic heart disease, and stroke: an ecological study. Prev.Med. 1999;28:520-529.

47. Burchfiel CM, Reed DM, Strong JP, Sharp DS, Chyou PH, Rodriguez BL. Predictors of myocardial lesions in men with minimal coronary atherosclerosis at autopsy. The Honolulu heart program. Ann.Epidemiol. 1996;6:137-146.

48. Bjerregaard P, Dyerberg J. Mortality from ischaemic heart disease and cerebrovascular disease in Greenland. Int.J.Epidemiol. 1988;17:514-519.

49. Davidson M, Bulkow LR, Gellin BG. Cardiac mortality in Alaska's indigenous and non-Native residents. Int.J.Epidemiol. 1993;22:62-71.

50. Iso H, Folsom AR, Wu KK, et al. Hemostatic variables in Japanese and Caucasian

men. Plasma fibrinogen, factor VIIc, factor VIIIc, and von Willebrand factor and their relations to cardiovascular disease risk factors. Am.J.Epidemiol. 1989;130:925-934.

51. Sperling RI, Robin JL, Kylander KA, Lee TH, Lewis RA, Austen KF. The effects of N-3 polyunsaturated fatty acids on the generation of platelet-activating factor-acether by human monocytes. J.Immunol. 1987;139:4186-4191.

52. Shimokawa H, Vanhoutte PM. Dietary omega 3 fatty acids and endothelium-dependent relaxations in porcine coronary arteries. Am.J.Physiol. 1989;256:H968-H973

53. Burr ML. Fish food, fish oil and cardiovascular disease. Clin.Exp.Hypertens.[A.] 1992;14:181-192.

54. Fox PL, DiCorleto PE. Fish oils inhibit endothelial cell production of platelet-derived growth factor-like protein. Science 1988;241:453-456.

55. Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation 1993;88:523-533.

56. Halle M, Berg A, Baumstark MW, Konig D, Huonker M, Keul J. Influence of mild to moderately elevated triglycerides on low density lipoprotein subfraction concentration and composition in healthy men with low high density lipoprotein cholesterol levels. Atherosclerosis 1999;143:185-192.

57. Galeano NF, Milne R, Marcel YL, et al. Apoprotein B structure and receptor recognition of triglyceride-rich low density lipoprotein (LDL) is modified in small LDL but not in triglyceride-rich LDL of normal size. J.Biol.Chem. 1994;269:511-519.

58. Chen GC, Liu W, Duchateau P, et al. Conformational differences in human apolipoprotein B-100 among subspecies of low density lipoproteins (LDL). Association of altered proteolytic accessibility with decreased receptor binding of LDL subspecies from hypertriglyceridemic subjects. J.Biol.Chem. 1994;269:29121-29128.

59. Nigon F, Lesnik P, Rouis M, Chapman MJ. Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. J.Lipid Res. 1991;32:1741-1753.

60. Griffin BA. Lipoprotein atherogenicity: an overview of current mechanisms. Proc.Nutr.Soc. 1999;58:163-169.

61. Frankel EN. Lipid Oxidation. The oily Press, Dundee, 1998.

CHAPTER 5

CONCLUSIONS

The effect of n-9 (TS), n-6 (SO) and n-3 (FO) fatty acids on plasma lipid, lipoprotein and apolipoprotein B (apo B) concentrations, and ratios of triacylglycerol to high density lipoprotein cholesterol (HDL-C), low density lipoprotein (LDL) particle size and oxidative susceptibility of the two LDL subfractions was examined in healthy, normolipidemic, postmenopausal women.

HDL-C concentration tended to increase in the FO-supplemented group compared to the TS-supplemented group in the present study and it was significantly higher in the FO-supplemented group compared to the SO supplemented one. The consumption of fish or supplementation with fish oil has previously been found to increase independently HDL-C concentrations (5-10 %) (Harris 1989; Green et al. 1990; Harats et al. 1991; Suzukawa et al. 1995).

The ratio of plasma triacylglycerol to HDL-C has been suggested as a better predictor of myocardial infarction than the ratios previously used (total cholesterol to HDL-C and LDL-C/HDL-C) (Gaziano et al. 1997). In the present study fish oil supplementation lowered the ratio of plasma triacylglycerol to HDL-C significantly compared to the TS- and SO-supplemented groups.

LDL particle size was also significantly increased after the FO supplementation compared to the TS supplementation. Since plasma triacylglycerol concentration is inversely related to LDL particle size (Austin et al. 1988; Stampfer et al. 1996), the reduction in plasma triacylglycerol concentration, as a result of n-3 fatty acid intake,

would be expected to result in an increase in LDL particle size. The results of this study support this interrelationship after supplementation of fish oil.

After the FO supplementation, plasma apo B concentrations were significantly decreased compared with baseline but not after the TS and SO supplementations. Each LDL particle carries a single apo B molecule. Therefore, for the same level of LDL cholesterol, an increase of LDL particle size lower apo B concentration. This relationship was supported by the results of this study. A reduction of apo B and triacylglycerol concentration, a decrease in the ratio of triacylglycerol to HDL-C, and an increase LDL particle size as a result of FO supplementation suggest a favorable effect of FO in reducing the risk factors for CVD.

Dietary fatty acids supplements significantly changed the concentrations of fatty acids in the two LDL subfractions. Supplementation with an oil rich in n-9 fatty acids resulted in LDL with about 30% more oleic acid, supplementation with an oil rich in n-6 fatty acids caused about a 20% increase in the amount of linoleic acid, and supplementation of fish oil caused a many-fold increase in the amount of EPA and DHA. The fatty acid content also differed between the two subfractions. In addition, the total amount of each fatty acid was much greater in the large subfraction. These modulations in the fatty acids composition of LDL influenced its oxidation. Supplementation with the n-9 fatty acid-rich oil resulted in LDL more resistant to oxidation as compared with supplementation with oil rich in n-6 fatty acids as measured by the formation of conjugated dienes and increase in the negative charge on apo B. However, the oxidative susceptibility of LDL after supplementation with n-3 fatty acids was less clear. N-3 fatty acid-enriched LDL demonstrated a shorter lag time than that measured in n-9 and n-6

fatty acid-enriched LDL but its maximum rate of oxidation was the lowest among the three oil-enriched LDL. This behavior was more pronounced in the large subfraction. In addition, although the maximum amount of conjugated diene formation was higher in n-3 fatty acid enriched LDL than that in n-9 fatty acid-enriched LDL, it was statistically equivalent to that in n-6 fatty acid-enriched LDL. The formation of negative charge on apo B after FO supplementation was higher compared to both the TS and SO supplementation at 0 hr and 1 hr and it was higher after the FO supplementation than the TS supplementation at 3 hr. It was statistically equivalent in three oil treatments at 5, 7, and 23 hr.

These differences in the oxidizability of LDL subfractions enriched with different fatty acids may relate to their α-tocopherol content. Although the amount of α-tocopherol in the two LDL subfractions was statistically equivalent in the three treatment groups, the ratio of unsaturated fatty acids to α-tocopherol was 23% and 7% higher in the FO-supplemented group compared to the TS- and SO-supplemented groups, respectively. The shorter lag time and faster formation of negative charge on apo B after FO supplementation compared to that of TS may be explained by its higher number of unsaturated fatty acids molecules per molecule of α-tocopherol in each of the LDL subfractions. .

In addition to the influence of the dietary oil supplement on LDL oxidative susceptibility, their impact of LDL size was also investigated in this study. The lag time was shorter in small LDL compared to that in large LDL. The maximum rate of oxidation and maximum amount of conjugated dienes were higher in large LDL than in small. At 1hr the formation of negative charge on apo B was higher in small LDL, but it

was higher at 23 hr in large LDL. A shortened lag time and a slower oxidation rate are contradictory in explaining oxidative susceptibility in LDL. However, the composition of the two LDL subfractions may explain this association. Numerous studies have shown that higher concentration of α-tocopherol lengthen lag time while larger amounts of PUFA increased the rate of oxidation (Niki et al. 1993; Thomas et al. 1994). The concentration of α-tocopherol was 26% higher and that of PUFA was 34% higher in large LDL compared to small in the present study. The higher concentrations of α-tocopherol may explain the longer lag time in large LDL. In addition, the higher PUFA concentration may also explain the higher rate of oxidation in large LDL. Although previous studies agreed that lag time shortens with decreasing size of LDL, results of other measurements on LDL oxidation were not clear (de Graaf et al. 1991; Tribble et al. 1992; Chait et al. 1993; Dejager et al. 1993; Tribble et al. 1994; Tribble et al. 1995a; Tribble et al. 1995b).

In trying to explain the enhanced atherogenity of small LDL other than its oxidative susceptibility, its compositional differences have also been investigated by several groups. Recently, Halle et al. (1999) and Galeano et al. (1994) reported a decreased free cholesterol and phospholipid concentrations in small LDL, which led to a reduced binding affinity of human fibroblasts to the LDL receptors (Chen et al. 1994). In support of this theory, Nigon et al. (1991) reported that small LDL was cleared less effectively by LDL receptors. Galeano et al. (1994) also suggested that the structural conformation of apo B was different in small LDL than that of large LDL and these differences modified the binding region of apo B in small LDL. This resulted in decreased clearance of LDL and prolonged residence time in the intravascular

compartment, allowing LDL more time to infiltrate the intimal lining of the artery wall and more time to be attacked by free radicals (Griffin 1999). As a result oxidatively modified small LDL is taken up by artery wall macrophages to a greater extent than large LDL. These compositional differences in small LDL possibly contribute to its enhanced atherogenity. The findings of the present study are consistent with this scenario, in that composition of small LDL was significantly different than large LDL.

In conclusion, the results of this study showed that the FO supplementation increased the LDL particle size, reduced the concentration of plasma triacylglycerol and apo B, and the ratio of triacylglycerol to HDL-C compared with TS and SO feedings. These findings demonstrated a greater potential antiatherogenic property of dietary fish oil than either oleic acid-rich or linoleic acid-rich oil in postmenopausal women using hormone. The oxidative susceptibility of LDL was reduced in n-9 fatty acid-enriched LDL compared to the n-6 fatty acid-enriched LDL in postmenopausal women with hormone replacement therapy. However, the influence of n-3 fatty acid supplementation on oxidative susceptibility of LDL was less clear. The data suggest that manipulation of the type of dietary fat may influence small and large LDL similarly, although the total amount of potential oxidative substrates is greater in large LDL.

BIBLIOGRAPHY

Abbey, M., Clifton, P.M., McMurchie, E.J., McIntosh, G.H. & Nestel, P.J. (1990) Effect of a high fat/cholesterol diet with or without eicosapentaenoic acid on plasma lipids, lipoproteins and lipid transfer protein activity in the marmoset. *Atherosclerosis* 81: 163-174.

Albert, C.M., Hennekens, C.H., O'Donnell, C.J., Ajani, U.A., Carey, V.J., Willett, W.C., Ruskin, J.N. & Manson, J.E. (1998) Fish consumption and risk of sudden cardiac death. *JAMA* 279: 23-28.

Albrink, M.J., Krauss, R.M., Lindgrem, F.T., von der, G., Pan, S. & Wood, P.D. (1980) Intercorrelations among plasma high density lipoprotein, obesity and triglycerides in a normal population. *Lipids* 15: 668-676.

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W. & Fu, P.C. (1974) Enzymatic determination of total serum cholesterol. *Clin.Chem.* 20: 470-475.

American Heart Association. (1998) 1999 Heart and Stroke Statistical Update., Dallas, Tex.

Arnaud, J., Fortis, I., Blachier, S., Kia, D. & Favier, A. (1991) Simultaneous determination of retinol, alpha-tocopherol and beta- carotene in serum by isocratic high-performance liquid chromatography. *J.Chromatogr.* 572: 103-116.

Ascherio, A., Rimm, E.B., Stampfer, M.J., Giovannucci, E.L. & Willett, W.C. (1995) Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men. *N.Engl.J.Med.* 332: 977-982.

Astill, B.D., Terhaar, C.J., Krasavage, W.J., Wlof, G.L., Roudabush, R.L. & Fassett, D.W. (1975) Safety evaluation and biochemical behavior of monotertiarybutylhydroquinone. *J.Am.Oil Chem.Soc.* 52: 53-58.

Austin, M.A. (1989) Plasma triglyceride as a risk factor for coronary heart disease. The epidemiologic evidence and beyond. *Am.J.Epidemiol.* 129: 249-259.

Austin, M.A. (1991) Plasma triglyceride and coronary heart disease. *Arterioscler.Thromb.* 11: 2-14.

Austin, M.A., Breslow, J.L., Hennekens, C.H., Buring, J.E., Willett, W.C. & Krauss, R.M. (1988) Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA* 260: 1917-1921.

Austin, M.A., Hokanson, J.E. & Edwards, K.L. (1998) Hypertriglyceridemia as a cardiovascular risk factor. *Am.J.Cardiol.* 81: 7B-12B.

Austin, M.A., King, M.C., Vranizan, K.M. & Krauss, R.M. (1990) Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 82: 495-506.

Austin, M.A. & Krauss, R.M. (1986) Genetic control of low-density-lipoprotein subclasses. *Lancet* 2: 592-595.

Badimon, J.J., Fuster, V., Chesebro, J.H. & Badimon, L. (1993) Coronary atherosclerosis. A multifactorial disease. *Circulation* 87: II3-16.

Baggio, G., Pagnan, A., Muraca, M., Martini, S., Opportuno, A., Bonanome, A., Ambrosio, G.B., Ferrari, S., Guarini, P. & Piccolo, D. (1988) Olive-oil-enriched diet: effect on serum lipoprotein levels and biliary cholesterol saturation. *Am.J.Clin.Nutr.* 47: 960-964.

Bang, H.O. & Dyerberg, J. (1972) Plasma lipids and lipoproteins in Greenlandic west coast Eskimos. *Acta Med.Scand.* 192: 85-94.

Bang, H.O., Dyerberg, J. & Sinclair, H.M. (1980) The composition of the Eskimo food in north western Greenland. *Am.J.Clin.Nutr.* 33: 2657-2661.

Barakat, H.A., Carpenter, J.W., McLendon, V.D., Khazanie, P., Leggett, N., Heath, J. & Marks, R. (1990) Influence of obesity, impaired glucose tolerance, and NIDDM on LDL structure and composition. Possible link between hyperinsulinemia and atherosclerosis. *Diabetes* 39: 1527-1533.

Berliner, J.A. & Heinecke, J.W. (1996) The role of oxidized lipoproteins in atherogenesis. *Free Radic.Biol.Med.* 20: 707-727.

Berliner, J.A., Navab, M., Fogelman, A.M., Frank, J.S., Demer, L.L., Edwards, P.A., Watson, A.D. & Lusis, A.J. (1995) Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. *Circulation* 91: 2488-2496.

Berry, E.M., Eisenberg, S., Friedlander, Y., Harats, D., Kaufmann, N.A., Norman, Y. & Stein, Y. (1992) Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins--the Jerusalem Nutrition Study. II. Monounsaturated fatty acids vs carbohydrates. *Am.J.Clin.Nutr.* 56: 394-403.

Bjerregaard, P. & Dyerberg, J. (1988) Mortality from ischaemic heart disease and cerebrovascular disease in Greenland. *Int.J.Epidemiol.* 17: 514-519.

Bonanome, A., Pagnan, A., Biffanti, S., Opportuno, A., Sorgato, F., Dorella, M., Maiorino, M. & Ursini, F. (1992) Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. *Arterioscler.Thromb.* 12: 529-533.

Brown, M.S. & Goldstein, J.L. (1990) Atherosclerosis. Scavenging for receptors [news]. *Nature* 343: 508-509.

Brunzell, J.D., Sniderman, A.D., Albers, J.J. & Kwiterovich, P.O.J. (1984) Apoproteins B and A-I and coronary artery disease in humans. *Arteriosclerosis* 4: 79-83.

Burchfiel, C.M., Reed, D.M., Strong, J.P., Sharp, D.S., Chyou, P.H. & Rodriguez, B.L. (1996) Predictors of myocardial lesions in men with minimal coronary atherosclerosis at autopsy. The Honolulu heart program. *Ann.Epidemiol.* 6: 137-146.

Burr, M.L. (1992) Fish food, fish oil and cardiovascular disease. *Clin.Exp.Hypertens.[A.]* 14: 181-192.

Campos, H., Blijlevens, E., McNamara, J.R., Ordovas, J.M., Posner, B.M., Wilson, P.W., Castelli, W.P. & Schaefer, E.J. (1992) LDL particle size distribution. Results from the Framingham Offspring Study. *Arterioscler.Thromb.* 12: 1410-1419.

Campos, H., Dreon, D.M. & Krauss, R.M. (1995) Associations of hepatic and lipoprotein lipase activities with changes in dietary composition and low density lipoprotein subclasses. *J.Lipid Res.* 36: 462-472.

Campos, H., Genest, J.J.J., Blijlevens, E., McNamara, J.R., Jenner, J.L., Ordovas, J.M., Wilson, P.W. & Schaefer, E.J. (1992) Low density lipoprotein particle size and coronary artery disease. *Arterioscler.Thromb.* 12: 187-195.

Campos, H., McNamara, J.R., Wilson, P.W., Ordovas, J.M. & Schaefer, E.J. (1988) Differences in low density lipoprotein subfractions and apolipoproteins in premenopausal and postmenopausal women. *J.Clin.Endocrinol.Metab.* 67: 30-35.

Carmena, R., Ascaso, J.F., Camejo, G., Varela, G., Hurt-Camejo, E., Ordovas, J.M., Martinez-Valls, J., Bergstom, M. & Wallin, B. (1996) Effect of olive and sunflower oils on low density lipoprotein level, composition, size, oxidation and interaction with arterial proteoglycans. *Atherosclerosis* 125: 243-255.

Castelli, W.P. & Anderson, K. (1986) A population at risk. Prevalence of high cholesterol levels in hypertensive patients in the Framingham Study. *Am.J.Med.* 80: 23-32.

Castelli, W.P., Garrison, R.J., Wilson, P.W., Abbott, R.D., Kalousdian, S. & Kannel, W.B. (1986) Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *JAMA* 256: 2835-2838.

Center for Disease Control and the American Heart Association. (1999) Nationalo Health and Nutrition Examination Survey III, 1988-1994,

Chait, A., Brazg, R.L., Tribble, D.L. & Krauss, R.M. (1993) Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am.J.Med.* 94: 350-356.

Chen, G.C., Liu, W., Duchateau, P., Allaart, J., Hamilton, R.L., Mendel, C.M., Lau, K., Hardman, D.A., Frost, P.H. & Malloy, M.J. (1994) Conformational differences in

human apolipoprotein B-100 among subspecies of low density lipoproteins (LDL). Association of altered proteolytic accessibility with decreased receptor binding of LDL subspecies from hypertriglyceridemic subjects. *J.Biol.Chem.* 269: 29121-29128.

Colditz, G.A., Willett, W.C., Stampfer, M.J., Rosner, B., Speizer, F.E. & Hennekens, C.H. (1987) Menopause and the risk of coronary heart disease in women. *N.Engl.J.Med.* 316: 1105-1110.

Colquhoun, D.M., Moores, D., Somerset, S.M. & Humphries, J.A. (1992) Comparison of the effects on lipoproteins and apolipoproteins of a diet high in monounsaturated fatty acids, enriched with avocado, and a high- carbohydrate diet. *Am.J.Clin.Nutr.* 56: 671-677.

Consensus conference. (1985) Lowering blood cholesterol to prevent heart disease. *JAMA* 253: 2080-2086.

Contacos, C., Barter, P.J. & Sullivan, D.R. (1993) Effect of pravastatin and omega-3 fatty acids on plasma lipids and lipoproteins in patients with combined hyperlipidemia. *Arterioscler.Thromb.* 13: 1755-1762.

Criqui, M.H., Heiss, G., Cohn, R., Cowan, L.D., Suchindran, C.M., Bangdiwala, S., Kritchevsky, S., Jacobs, D.R.J., O'Grady, H.K. & Davis, C.E. (1993) Plasma triglyceride level and mortality from coronary heart disease. *N.Engl.J.Med.* 328: 1220-1225.

Curb, J.D. & Reed, D.M. (1985) Fish consumption and mortality from coronary heart disease. *N.Engl.J.Med.* 313: 821-822.

Davidson, M., Bulkow, L.R. & Gellin, B.G. (1993) Cardiac mortality in Alaska's indigenous and non-Native residents. *Int.J.Epidemiol.* 22: 62-71.

Davidson, S.B., Wander, R.C., Du, S.H. & Riddle, M. (1997) Effect of dietary MUFAs in lipid, lipoproteins, and lipid peroxidation in NIDDM. American Oil Chemists' Society (AOCS) (abs)

Daviglus, M.L., Stamler, J., Orencia, A.J., Dyer, A.R., Liu, K., Greenland, P., Walsh, M.K., Morris, D. & Shekelle, R.B. (1997) Fish consumption and the 30-year risk of fatal myocardial infarction. *N.Engl.J.Med.* 336: 1046-1053.

Davis, C.E., Gordon, D., LaRosa, J., Wood, P.D. & Halperin, M. (1980) Correlations of plasma high-density lipoprotein cholesterol levels with other plasma lipid and lipoprotein concentrations. *Circulation* 62: IV24-IV30

de Graaf, J., Hak-Lemmers, H.L., Hectors, M.P., Demacker, P.N., Hendriks, J.C. & Stalenhoef, A.F. (1991) Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arterioscler.Thromb.* 11: 298-306.

de Lorgeril, M., Renaud, S., Mamelle, N., Salen, P., Martin, J.L., Monjaud, I., Guidollet, J., Touboul, P. & Delaye, J. (1994) Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease *Lancet* 343: 1454-1459.

DeFronzo, R.A. (1992) Insulin resistance, hyperinsulinemia, and coronary artery disease: a complex metabolic web. *J.Cardiovasc.Pharmacol.* 20 Suppl 11: S1-16.

Dejager, S., Bruckert, E. & Chapman, M.J. (1993) Dense low density lipoprotein subspecies with diminished oxidative resistance predominate in combined hyperlipidemia. *J.Lipid Res.* 34: 295-308.

Despres, J.P., Nadeau, A., Tremblay, A., Ferland, M., Moorjani, S., Lupien, P.J., Theriault, G., Pinault, S. & Bouchard, C. (1989) Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. *Diabetes* 38: 304-309.

Dietschy, J.M. (1998) Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. *J.Nutr.* 128: 444S-448S.

Dreon, D.M., Fernstrom, H.A., Campos, H., Blanche, P., Williams, P.T. & Krauss, R.M. (1998) Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men. *Am.J.Clin.Nutr.* 67: 828-836.

Durrington, P.N., Ishola, M., Hunt, L., Arrol, S. & Bhatnagar, D. (1988) Apolipoproteins (a), AI, and B and parental history in men with early onset ischaemic heart disease. *Lancet* 1: 1070-1073.

Dyerberg, J., Bang, H.O., Stoffersen, E., Moncada, S. & Vane, J.R. (1978) Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* 2: 117-119.

Esterbauer, H., Jurgens, G. & Quehenberger, O. (1988) Modification of human low density lipoprotein by lipid peroxidation. *Basic.Life Sci.* 49:369-73: 369-373.

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

Esterbauer, H., Striegl, G., Puhl, H. & Rotheneder, M. (1989) Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radic.Res.Commun.* 6: 67-75.

Fager, G., Wiklund, O., Olofsson, S.O., Wilhelmsen, L. & Bondjers, G. (1981) Multivariate analyses of serum apolipoproteins and risk factors in relation to acute myocardial infarction. *Arteriosclerosis* 1: 273-279.

Failor, R.A., Childs, M.T. & Bierman, E.L. (1988) The effects of omega 3 and omega 6 fatty acid-enriched diets on plasma lipoproteins and apoproteins in familial combined hyperlipidemia. *Metabolism* 37: 1021-1028.

Feingold, K.R., Grunfeld, C., Pang, M., Doerrler, W. & Krauss, R.M. (1992) LDL subclass phenotypes and triglyceride metabolism in non-insulin- dependent diabetes. *Arterioscler.Thromb.* 12: 1496-1502.

Fox, P.L. & DiCorleto, P.E. (1988) Fish oils inhibit endothelial cell production of platelet-derived growth factor-like protein. *Science* 241: 453-456.

Frankel, E.N. (1998) *Lipid Oxidation*, The oily Press, Dundee.

Fraser, G.E., Sabate, J., Beeson, W.L. & Strahan, T.M. (1992) A possible protective effect of nut consumption on risk of coronary heart disease. The Adventist Health Study. *Arch.Intern.Med.* 152: 1416-1424.

Friday, K.E., Failor, R.A., Childs, M.T. & Bierman, E.L. (1991) Effects of n-3 and n-6 fatty acid-enriched diets on plasma lipoproteins and apolipoproteins in heterozygous familial hypercholesterolemia. *Arterioscler.Thromb.* 11: 47-54.

Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin.Chem.* 18: 499-502.

Frisancho, A.R. (1990) Anthropometric standards for the assessment of growth and nutritional status, pp. Table IV.4University of Michigan Press,

Fujioka, S., Matsuzawa, Y., Tokunaga, K. & Tarui, S. (1987) Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. *Metabolism* 36: 54-59.

Galeano, N.F., Milne, R., Marcel, Y.L., Walsh, M.T., Levy, E., Ngu'yen, T.D., Gleeson, A., Arad, Y., Witte, L. & Al-Haideri, M. (1994) Apoprotein B structure and receptor recognition of triglyceride-rich low density lipoprotein (LDL) is modified in small LDL but not in triglyceride-rich LDL of normal size. *J.Biol.Chem.* 269: 511-519.

Gaziano, J.M., Hennekens, C.H., O'Donnell, C.J., Breslow, J.L. & Buring, J.E. (1997) Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. *Circulation* 96: 2520-2525.

Genest, J.J.J., Bard, J.M., Fruchart, J.C., Ordovas, J.M., Wilson, P.F. & Schaefer, E.J. (1991) Plasma apolipoprotein A-I, A-II, B, E and C-III containing particles in men with premature coronary artery disease. *Atherosclerosis* 90: 149-157.

Gerhard, G.T., Patton, B.D., Lindquist, S.A. & Wander, R.C. (1991) Comparison of three species of dietary fish: effects on serum concentrations of low-density-lipoprotein

cholesterol and apolipoprotein in normotriglyceridemic subjects. *Am.J.Clin.Nutr.* 54: 334-339.

Goldstein, J.L., Schrott, H.G., Hazzard, W.R., Bierman, E.L. & Motulsky, A.G. (1973) Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J.Clin.Invest.* 52: 1544-1568.

Gordon, D.J. & Rifkind, B.M. (1989) High-density lipoprotein--the clinical implications of recent studies. *N.Engl.J.Med.* 321: 1311-1316.

Green, P., Fuchs, J., Schoenfeld, N., Leibovici, L., Lurie, Y., Beigel, Y., Rotenberg, Z., Mamet, R. & Budowski, P. (1990) Effects of fish-oil ingestion on cardiovascular risk factors in hyperlipidemic subjects in Israel: a randomized, double-blind crossover study. *Am.J.Clin.Nutr.* 52: 1118-1124.

Griffin, B.A. (1999) Lipoprotein atherogenicity: an overview of current mechanisms. *Proc.Nutr.Soc.* 58: 163-169.

Grundy, S.M. (1986) Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. *N.Engl.J.Med.* 314: 745-748.

Grundy, S.M., Florentin, L., Nix, D. & Whelan, M.F. (1988) Comparison of monounsaturated fatty acids and carbohydrates for reducing raised levels of plasma cholesterol in man. *Am.J.Clin.Nutr.* 47: 965-969.

Gumbiner, B., Low, C.C. & Reaven, P.D. (1998) Effects of a monounsaturated fatty acid-enriched hypocaloric diet on cardiovascular risk factors in obese patients with type 2 diabetes. *Diabetes Care* 21: 9-15.

Haffner, S.M., Mykkanen, L., Stern, M.P., Paidi, M. & Howard, B.V. (1994) Greater effect of diabetes on LDL size in women than in men. *Diabetes Care* 17: 1164-1171.

Hallberg, L. & Svanborg, A. (1967) Cholesterol, phospholipids, and triglycerides in plasma in 50-year-old women. Influence of menopause, body-weight, skinfold thickness, weight- gain, and diet in a random population sample. *Acta Med.Scand.* 181: 185-194.

Halle, M., Berg, A., Baumstark, M.W., Konig, D., Huonker, M. & Keul, J. (1999) Influence of mild to moderately elevated triglycerides on low density lipoprotein subfraction concentration and composition in healthy men with low high density lipoprotein cholesterol levels. *Atherosclerosis* 143: 185-192.

Hamsten, A., Walldius, G., Dahlen, G., Johansson, B. & De Faire, U. (1986) Serum lipoproteins and apolipoproteins in young male survivors of myocardial infarction. *Atherosclerosis* 59: 223-235.

Harats, D., Dabach, Y., Hollander, G., Ben-Naim, M., Schwartz, R., Berry, E.M., Stein, O. & Stein, Y. (1991) Fish oil ingestion in smokers and nonsmokers enhances peroxidation of plasma lipoproteins. *Atherosclerosis* 90: 127-139.

Harris, W.S. (1989) Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *J.Lipid Res.* 30: 785-807.

Harris, W.S. (1997) n-3 fatty acids and serum lipoproteins: human studies. *Am.J.Clin.Nutr.* 65: 1645S-1654S.

Harris, W.S., Lu, G., Rambjor, G.S., Walen, A.I., Ontko, J.A., Cheng, Q. & Windsor, S.L. (1997) Influence of n-3 fatty acid supplementation on the endogenous activities of plasma lipases. *Am.J.Clin.Nutr.* 66: 254-260.

Havel, R. (1994) McCollum Award Lecture, 1993: triglyceride-rich lipoproteins and atherosclerosis--new perspectives. *Am.J.Clin.Nutr.* 59: 795-796.

Hegsted, D.M., McGandy, R.B., Myers, M.L. & Stare, F.J. (1965) Quantitative effects of dietary fat on serum cholesterol in man. *Am.J.Clin.Nutr.* 17: 281-295.

Hjermann, I., Enger, S.C., Helgeland, A., Holme, I., Leren, P. & Trygg, K. (1979) The effect of dietary changes on high density lipoprotein cholesterol. The Oslo Study. *Am.J.Med.* 66: 105-109.

Hodgson, J.M., Wahlqvist, M.L., Boxall, J.A. & Balazs, N.D. (1993) Can linoleic acid contribute to coronary artery disease? *Am.J.Clin.Nutr.* 58: 228-234.

Illingworth, D.R., Harris, W.S. & Connor, W.E. (1984) Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans. *Arteriosclerosis* 4: 270-275.

Iso, H., Folsom, A.R., Wu, K.K., Finch, A., Munger, R.G., Sato, S., Shimamoto, T., Terao, A. & Komachi, Y. (1989) Hemostatic variables in Japanese and Caucasian men. Plasma fibrinogen, factor VIIc, factor VIIIc, and von Willebrand factor and their relations to cardiovascular disease risk factors. *Am.J.Epidemiol.* 130: 925-934.

Jackson, R.L., Kashyap, M.L., Barnhart, R.L., Allen, C., Hogg, E. & Glueck, C.J. (1984) Influence of polyunsaturated and saturated fats on plasma lipids and lipoproteins in man. *Am.J.Clin.Nutr.* 39: 589-597.

Jacobs, D.R.J. & Barrett-Connor, E. (1982) Retest reliability of plasma cholesterol and triglyceride. The Lipid Research Clinics Prevalence Study. *Am.J.Epidemiol.* 116: 878-885.

Kalkhoff, R.K., Hartz, A.H., Rupley, D., Kissebah, A.H. & Kelber, S. (1983) Relationship of body fat distribution to blood pressure, carbohydrate tolerance, and plasma lipids in healthy obese women. *J.Lab.Clin.Med.* 102: 621-627.

Kameda, K., Matsuzawa, Y., Kubo, M., Ishikawa, K., Maejima, I., Yamamura, T., Yamamoto, A. & Tarui, S. (1984) Increased frequency of lipoprotein disorders similar to type III hyperlipoproteinemia in survivors of myocardial infarction in Japan. *Atherosclerosis* 51: 241-249.

Kannel, W.B., Castelli, W.P., Gordon, T. & McNamara, P.M. (1971) Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. *Ann.Intern.Med.* 74: 1-12.

Karpe, F., Tornvall, P., Olivecrona, T., Steiner, G., Carlson, L.A. & Hamsten, A. (1993) Composition of human low density lipoprotein: effects of postprandial triglyceride-rich lipoproteins, lipoprotein lipase, hepatic lipase and cholesteryl ester transfer protein. *Atherosclerosis* 98: 33-49.

Keys, A. (1967) Dietary epidemiology. *Am.J.Clin.Nutr.* 20: 1151-1157.

Keys, A. (1970) Coronary heart diseas in seven countries. *Circulation* 41: 1-211.

Keys, A. (1980) Seven Countries; A multivariate analysis of death and coronary heart disease., pp. 1-381. Cambridge, Mass.

Keys, A. & Parlin, R.W. (1966) Serum cholesterol response to changes in dietary lipids. *Am.J.Clin.Nutr.* 19: 175-181.

Krauss, R.M. (1995) Dense low density lipoproteins and coronary artery disease. *Am.J.Cardiol.* 75: 53B-57B.

Krauss, R.M. (1997) Genetic, metabolic, and dietary influences on the atherogenic lipoprotein phenotype. *World Rev.Nutr.Diet.* 80: 22-43.

Krauss, R.M. & Burke, D.J. (1982) Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J.Lipid Res.* 23: 97-104.

Kromhout, D., Bosschieter, E.B. & de Lezenne, C. (1985) The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N.Engl.J.Med.* 312: 1205-1209.

Kromhout, D., Feskens, E.J. & Bowles, C.H. (1995) The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. *Int.J.Epidemiol.* 24: 340-345.

Kuehl, R.O. (1994) Crossover Design. In: Statistical Principles of Research Design and Analysis Anonymous pp. 529-559. Duxbury Press, Belmont, CA.

Lagrost, L., Florentin, E., Guyard-Dangremont, V., Athias, A., Gandjini, H., Lallemant, C. & Gambert, P. (1995) Evidence for nonesterified fatty acids as modulators of neutral lipid transfers in normolipidemic human plasma. *Arterioscler.Thromb.Vasc.Biol.* 15: 1388-1396.

Lamarche, B., Tchernof, A., Moorjani, S., Cantin, B., Dagenais, G.R., Lupien, P.J. & Despres, J.P. (1997) Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Circulation* 95: 69-75.

Lands, W.E. (1993) Eicosanoids and health. *Ann.N.Y.Acad.Sci.* 676: 46-59.

Lardinois, C.K., Starich, G.H., Mazzaferri, E.L. & DeLett, A. (1987) Polyunsaturated fatty acids augment insulin secretion. *J.Am.Coll.Nutr.* 6: 507-515.

Leaf, A. & Weber, P.C. (1988) Cardiovascular effects of n-3 fatty acids. *N.Engl.J.Med.* 318: 549-557.

Lee, Y.S., Du, S.H., Higdon, J., Wu, T. & Wander, R.C. (1999) The effect of supplementation with n-9, n-6, and n-3 fatty acids on plasma lipid, lipoprotein, apolipoprotein B concentrations, and LDL particle size in postmenopausal women. *Submitted*

Lemieux, S., Prud'Homme, D., Bouchard, C., Tremblay, A. & Despres, J.P. (1996) A single threshold value of waist girth identifies normal-weight and overweight subjects with excess visceral adipose tissue. *Am.J.Clin.Nutr.* 64: 685-693.

Linga, V., Leight, M.A., St.Clair, R.W. & Parks, J.S. (1993) Dietary fish oil modification of cynomolgus monkey low density lipoproteins results in decreased binding and cholesteryl ester accumulation by cultured fibroblasts. *J.Lipid Res.* 34: 769-778.

Lohman, T.G., Roche, A.F. & Martorell, R. (1991) Anthropometric standardization reference manual, human kinetics books,

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measured with folin phenol reagent. *J.Biol.Chem.* 193: 265-275.

Marz, W. & Gross, W. (1986) Analysis of plasma lipoproteins by ultracentrifugation in a new fixed angle rotor: evaluation of a phosphotungstic acid/MgCl2 precipitation and a quantitative lipoprotein electrophoresis assay. *Clin.Chim.Acta* 160: 1-18.

Mayer, E.J., Alderman, B.W., Regensteiner, J.G., Marshall, J.A., Haskell, W.L., Baxter, J. & Hamman, R.F. (1991) Physical-activity-assessment measures compared in a biethnic rural population: the San Luis Valley Diabetes Study. *Am.J.Clin.Nutr.* 53: 812-820.

McGowan, M.W., Artiss, J.D., Strandbergh, D.R. & Zak, B. (1983) A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin.Chem.* 29: 538-542.

McNamara, J.R., Campos, H., Ordovas, J.M., Peterson, J., Wilson, P.W. & Schaefer, E.J. (1987) Effect of gender, age, and lipid status on low density lipoprotein

subfraction distribution. Results from the Framingham Offspring Study. *Arteriosclerosis* 7: 483-490.

Mensink, R.P., de Groot, M.J., van den Broeke, L.T., Severijnen-Nobels, A.P., Demacker, P.N. & Katan, M.B. (1989) Effects of monounsaturated fatty acids v complex carbohydrates on serum lipoproteins and apoproteins in healthy men and women. *Metabolism* 38: 172-178.

Mensink, R.P. & Katan, M.B. (1987) Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. *Lancet* 1: 122-125.

Miller, G.J. & Miller, N.E. (1975) Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet* 1: 16-19.

Morris, M.C., Manson, J.E., Rosner, B., Buring, J.E., Willett, W.C. & Hennekens, C.H. (1995) Fish consumption and cardiovascular disease in the physicians' health study: a prospective study. *Am.J.Epidemiol.* 142: 166-175.

Morris, M.C., Sacks, F. & Rosner, B. (1993) Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* 88: 523-533.

National Cholesterol Education Program (1988) Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. The Expert Panel. *Arch.Intern.Med.* 148: 36-69.

National Cholesterol Education Program (1993) Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *JAMA* 269: 3015-3023.

Navab, M., Imes, S.S., Hama, S.Y., Hough, G.P., Ross, L.A., Bork, R.W., Valente, A.J., Berliner, J.A., Drinkwater, D.C. & Laks, H. (1991) Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J.Clin.Invest.* 88: 2039-2046.

Nenseter, M.S., Rustan, A.C., Lund-Katz, S., Soyland, E., Maelandsmo, G., Phillips, M.C. & Drevon, C.A. (1992) Effect of dietary supplementation with n-3 polyunsaturated fatty acids on physical properties and metabolism of low density lipoprotein in humans. *Arterioscler.Thromb.* 12: 369-379.

Nestel, P.J., Connor, W.E., Reardon, M.F., Connor, S., Wong, S. & Boston, R. (1984) Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J.Clin.Invest.* 74: 82-89.

Neuringer, M. & Connor, W.E. (1986) n-3 fatty acids in the brain and retina: evidence for their essentiality. *Nutr.Rev.* 44: 285-294.

Nichols, A.V., Krauss, R.M. & Musliner, T.A. (1986) Nondenaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol.* 128:417-31: 417-431.

Nigon, F., Lesnik, P., Rouis, M. & Chapman, M.J. (1991) Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. *J.Lipid Res.* 32: 1741-1753.

NIH Consensus conference. (1993) Triglyceride, high-density lipoprotein, and coronary heart disease. NIH Consensus Development Panel on Triglyceride, High-Density Lipoprotein, and Coronary Heart Disease. *JAMA* 269: 505-510.

Niki, E., Noguchi, N. & Gotoh, N. (1993) Inhibition of oxidative modification of low density lipoprotein by antioxidants. *J.Nutr.Sci.Vitaminol.(Tokyo.)* 39 Suppl:S1-8: S1-S8

Noguchi, N. & Niki, E. (1994) Apolipoprotein B protein oxidation in low-density lipoproteins. *Methods Enzymol.* 233:490-4: 490-494.

Norell, S.E., Ahlbom, A., Feychting, M. & Pedersen, N.L. (1986) Fish consumption and mortality from coronary heart disease. *Br.Med.J.(Clin.Res.Ed.)* 293: 426

Nydahl, M.C., Gustafsson, I.B. & Vessby, B. (1994) Lipid-lowering diets enriched with monounsaturated or polyunsaturated fatty acids but low in saturated fatty acids have similar effects on serum lipid concentrations in hyperlipidemic patients. *Am.J.Clin.Nutr.* 59: 115-122.

Palinski, W., Rosenfeld, M.E., Yla-Herttuala, S., Gurtner, G.C., Socher, S.S., Butler, S.W., Parthasarathy, S., Carew, T.E., Steinberg, D. & Witztum, J.L. (1989) Low density lipoprotein undergoes oxidative modification in vivo. *Proc.Natl.Acad.Sci.U.S.A.* 86: 1372-1376.

Parks, J.S. & Bullock, B.C. (1987) Effect of fish oil versus lard diets on the chemical and physical properties of low density lipoproteins of nonhuman primates. *J.Lipid Res.* 28: 173-182.

Pearson, T.A., Bulkley, B.H., Achuff, S.C., Kwiterovich, P.O. & Gordis, L. (1979) The association of low levels of HDL cholesterol and arteriographically defined coronary artery disease. *Am.J.Epidemiol.* 109: 285-295.

Pouliot, M.C., Despres, J.P., Lemieux, S., Moorjani, S., Bouchard, C., Tremblay, A., Nadeau, A. & Lupien, P.J. (1994) Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am.J.Cardiol.* 73: 460-468.

Pouliot, M.C., Despres, J.P., Nadeau, A., Moorjani, S., Prud'Homme, D., Lupien, P.J., Tremblay, A. & Bouchard, C. (1992) Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. *Diabetes* 41: 826-834.

Reaven, G.M. (1993) Role of insulin resistance in human disease (syndrome X): an expanded definition. *Annu.Rev.Med.* 44: 121-131.

Reaven, G.M. & Chen, Y.D. (1988) Role of insulin in regulation of lipoprotein metabolism in diabetes. *Diabetes Metab.Rev.* 4: 639-652.

Reaven, G.M., Chen, Y.D., Jeppesen, J., Maheux, P. & Krauss, R.M. (1993) Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. *J.Clin.Invest.* 92: 141-146.

Reaven, P., Parthasarathy, S., Grasse, B.J., Miller, E., Almazan, F., Mattson, F.H., Khoo, J.C., Steinberg, D. & Witztum, J.L. (1991) Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. *Am.J.Clin.Nutr.* 54: 701-706.

Reaven, P.D. (1994) Mechanisms of atherosclerosis: role of LDL oxidation. *Adv.Exp.Med.Biol.* 366: 113-128.

Reaven, P.D., Grasse, B.J. & Tribble, D.L. (1994) Effects of linoleate-enriched and oleate-enriched diets in combination with alpha-tocopherol on the susceptibility of LDL and LDL subfractions to oxidative modification in humans. *Arterioscler.Thromb.* 14: 557-566.

Reaven, P.D. & Witztum, J.L. (1996) Oxidized low density lipoproteins in atherogenesis: role of dietary modification. *Annu.Rev.Nutr.* 16:51-71: 51-71.

Reeves, R.A. (1995) Does this patient have hypertension? How to measure blood pressure. *JAMA* 273: 1211-1218.

Renaud, S. & Ruf, J.C. (1994) The French paradox: vegetables or wine [letter; comment]. *Circulation* 90: 3118-3119.

Rowland, M.L. (1989) Reporting bias in height and weight data. *Stat.Bull.Metrop.Insur.Co.* 70: 2-11.

Rudel, L.L., Bond, M.G. & Bullock, B.C. (1985) LDL heterogeneity and atherosclerosis in nonhuman primates. *Ann.N.Y.Acad.Sci.* 454:248-53: 248-253.

Rudel, L.L., Parks, J.S. & Sawyer, J.K. (1995) Compared with dietary monounsaturated and saturated fat, polyunsaturated fat protects African green monkeys from coronary artery atherosclerosis. *Arterioscler.Thromb.Vasc.Biol.* 15: 2101-2110.

Rudel, L.L., Pitts, L.L. & Nelson, C.A. (1977) Characterization of plasma low density lipoproteins on nonhuman primates fed dietary cholesterol. *J.Lipid Res.* 18: 211-222.

Sakai, N., Matsuzawa, Y., Hirano, K., Yamashita, S., Nozaki, S., Ueyama, Y., Kubo, M. & Tarui, S. (1991) Detection of two species of low density lipoprotein particles in cholesteryl ester transfer protein deficiency. *Arterioscler.Thromb.* 11: 71-79.

Schaefer, E.J., Levy, R.I., Ernst, N.D., Van Sant, F.D. & Brewer, H.B.J. (1981) The effects of low cholesterol, high polyunsaturated fat, and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholesterolemic subjects. *Am.J.Clin.Nutr.* 34: 1758-1763.

Sedlis, S.P., Schechtman, K.B., Ludbrook, P.A., Sobel, B.E. & Schonfeld, G. (1986) Plasma apoproteins and the severity of coronary artery disease. *Circulation* 73: 978-986.

Selby, J.V., Austin, M.A., Newman, B., Zhang, D., Quesenberry, C.P.J., Mayer, E.J. & Krauss, R.M. (1993) LDL subclass phenotypes and the insulin resistance syndrome in women. *Circulation* 88: 381-387.

Shen, M.M., Krauss, R.M., Lindgren, F.T. & Forte, T.M. (1981) Heterogeneity of serum low density lipoproteins in normal human subjects. *J.Lipid Res.* 22: 236-244.

Shepherd, J., Packard, C.J., Grundy, S.M., Yeshurun, D., Gotto, A.M.J. & Taunton, O.D. (1980) Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. *J.Lipid Res.* 21: 91-99.

Shimokawa, H. & Vanhoutte, P.M. (1989) Dietary omega 3 fatty acids and endothelium-dependent relaxations in porcine coronary arteries. *Am.J.Physiol.* 256: H968-H973

Sirtori, C.R., Gatti, E., Tremoli, E., Galli, C., Gianfranceschi, G., Franceschini, G., Colli, S., Maderna, P., Marangoni, F. & Perego, P. (1992) Olive oil, corn oil, and n-3 fatty acids differently affect lipids, lipoproteins, platelets, and superoxide formation in type II hypercholesterolemia. *Am.J.Clin.Nutr.* 56: 113-122.

Soderhjelm, L., Wiese, H.F. & Blondheim, S.H. (1970) The role of polyunsaturated fatty acid in human nutrition and metabolism. In: Prog chem fats and other lipid (Holman, R.T., ed.), pp. 555-585. Pergamon Press, Oxford.

Song, J. & Wander, R.C. (1991) Effects of dietary selenium and fish oil (MaxEPA) on arachidonic acid metabolism and hemostatic function in rats. *J.Nutr.* 121: 284-292.

Sorensen, N.S., Marckmann, P., Hoy, C.E., van Duyvenvoorde, W. & Princen, H.M. (1998) Effect of fish-oil-enriched margarine on plasma lipids, low-density-lipoprotein particle composition, size, and susceptibility to oxidation. *Am.J.Clin.Nutr.* 68: 235-241.

Sparrow, C.P., Parthasarathy, S. & Steinberg, D. (1989) A macrophage receptor that recognizes oxidized low density lipoprotein but not acetylated low density lipoprotein. *J.Biol.Chem.* 264: 2599-2604.

Sperling, R.I., Robin, J.L., Kylander, K.A., Lee, T.H., Lewis, R.A. & Austen, K.F. (1987) The effects of N-3 polyunsaturated fatty acids on the generation of platelet-activating factor-acether by human monocytes. *J.Immunol.* 139: 4186-4191.

Stampfer, M.J., Krauss, R.M., Ma, J., Blanche, P.J., Holl, L.G., Sacks, F.M. & Hennekens, C.H. (1996) A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA* 276: 882-888.

Stampfer, M.J., Sacks, F.M., Salvini, S., Willett, W.C. & Hennekens, C.H. (1991) A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N.Engl.J.Med.* 325: 373-381.

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C. & Witztum, J.L. (1989) Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N.Engl.J.Med.* 320: 915-924.

Steinbrecher, U.P., Witztum, J.L., Parthasarathy, S. & Steinberg, D. (1987) Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL. Correlation with changes in receptor-mediated catabolism. *Arteriosclerosis* 7: 135-143.

Storlien, L.H., Kraegen, E.W., Chisholm, D.J., Ford, G.L., Bruce, D.G. & Pascoe, W.S. (1987) Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* 237: 885-888.

Subcommittee on the Tenth Edition of the RDAs. (1989) Food and Nutrition Board,Commission on Life Sciences,National Research Council. Recommended Dietary Allowances, Ed.10, National Academy Press, Washington, D.C.

Sullivan, D.R., Sanders, T.A., Trayner, I.M. & Thompson, G.R. (1986) Paradoxical elevation of LDL apoprotein B levels in hypertriglyceridaemic patients and normal subjects ingesting fish oil. *Atherosclerosis* 61: 129-134.

Suzukawa, M., Abbey, M., Howe, P.R. & Nestel, P.J. (1995) Effects of fish oil fatty acids on low density lipoprotein size, oxidizability, and uptake by macrophages. *J.Lipid Res.* 36: 473-484.

Thiery, J. & Seidel, D. (1987) Fish oil feeding results in an enhancement of cholesterol-induced atherosclerosis in rabbits. *Atherosclerosis* 63: 53-56.

Thind, I.S. & Sandhu, R.S. (1981) Signification of high density and total cholesterol and triglycerides in acute myocardial infarction -- a case-control study. *Clin.Biochem.* 14: 57-60.

Thomas, M.J., Thornburg, T., Manning, J., Hooper, K. & Rudel, L.L. (1994) Fatty acid composition of low-density lipoprotein influences its susceptibility to autoxidation. *Biochemistry* 33: 1828-1834.

Trevisan, M., Krogh, V., Freudenheim, J., Blake, A., Muti, P., Panico, S., Farinaro, E., Mancini, M., Menotti, A. & Ricci, G. (1990) Consumption of olive oil, butter, and vegetable oils and coronary heart disease risk factors. The Research Group ATS-RF2 of the Italian National Research Council. *JAMA* 263: 688-692.

Tribble, D.L., Holl, L.G., Wood, P.D. & Krauss, R.M. (1992) Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis* 93: 189-199.

Tribble, D.L., Krauss, R.M., Lansberg, M.G., Thiel, P.M. & van den Berg, J.J. (1995) Greater oxidative susceptibility of the surface monolayer in small dense LDL may contribute to differences in copper-induced oxidation among LDL density subfractions. *J.Lipid Res.* 36: 662-671.

Tribble, D.L., Thiel, P.M., van den Berg, J.J. & Krauss, R.M. (1995) Differing alpha-tocopherol oxidative lability and ascorbic acid sparing effects in buoyant and dense LDL. *Arterioscler.Thromb.Vasc.Biol.* 15: 2025-2031.

Tribble, D.L., van den Berg, J.J., Motchnik, P.A., Ames, B.N., Lewis, D.M., Chait, A. & Krauss, R.M. (1994) Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and alpha-tocopherol content. *Proc.Natl.Acad.Sci.U.S.A.* 91: 1183-1187.

Trichopoulou, A., Kouris-Blazos, A., Vassilakou, T., Gnardellis, C., Polychronopoulos, E., Venizelos, M., Lagiou, P., Wahlqvist, M.L. & Trichopoulos, D. (1995) Diet and survival of elderly Greeks: a link to the past. *Am.J.Clin.Nutr.* 61: 1346S-1350S.

U.S.Department of Agriculture and U.S.Department of Health and Human Service. (1995) Nutrition and your health, Dietary guidelines for Americans, 4th ed.,

Wander, R.C., Du, S.H., Ketchum, S.O. & Rowe, K.E. (1996) alpha-Tocopherol influences in vivo indices of lipid peroxidation in postmenopausal women given fish oil. *J.Nutr.* 126: 643-652.

Wander, R.C., Du, S.H., Ketchum, S.O. & Rowe, K.E. (1996) Effects of interaction of RRR-alpha-tocopheryl acetate and fish oil on low-density-lipoprotein oxidation in postmenopausal women with and without hormone-replacement therapy. *Am.J.Clin.Nutr.* 63: 184-193.

Wander, R.C., Hall, J.A., Gradin, J.L., Du, S.H. & Jewell, D.E. (1997) The ratio of dietary (n-6) to (n-3) fatty acids influences immune system function, eicosanoid metabolism, lipid peroxidation and vitamin E status in aged dogs. *J.Nutr.* 127: 1198-1205.

Whitman, S.C., Fish, J.R., Rand, M.L. & Rogers, K.A. (1994) n-3 fatty acid incorporation into LDL particles renders them more susceptible to oxidation in vitro but not necessarily more atherogenic in vivo. *Arterioscler.Thromb.* 14: 1170-1176.

Wolfe, M.S., Sawyer, J.K., Morgan, T.M., Bullock, B.C. & Rudel, L.L. (1994) Dietary polyunsaturated fat decreases coronary artery atherosclerosis in a pediatric-aged population of African green monkeys. *Arterioscler.Thromb.* 14: 587-597.

Zhang, J., Sasaki, S., Amano, K. & Kesteloot, H. (1999) Fish consumption and mortality from all causes, ischemic heart disease, and stroke: an ecological study. *Prev.Med.* 28: 520-529.

APPENDICES

A.1 Phone screening form

Date _____ Time _____ Name of Screener_____

How did you hear about the study?

_____

Purpose: to determine the effect of supplement olive oil, fish oil, and safflower oil on LDL cholesterol (the bad cholesterol)

Commitment: Total time = 27 weeks. 2 weeks-normal diet, (5 weeks taking oil supplement, 5 week washout period-no supplement) x 3. Fasting, A.M. blood draw the first and last week of each supplementation period. Come in for breakfast and supplement refill during the third week of each period. 3 day diet record during each supplementation period.

Personal Information

Name _____ Date of birth _____Age _____

Address _____ _____

zip co

Phone: Daytime _____Evening _____Best time to call_____

Height _____ Weight _____ Blood pressure_____ Any Hx of high blood pressure? _____

Recent laboratory values: (normal/abnormal if values aren't known)

Cholesterol _____ Triglycerides _____ Blood glucose (sugar) _____

Date of last menstrual period _____

Have you had a hysterectomy? • Yes • No When? _____ Oophorectomy?

(ovaries removed) • Yes • No When?_____

Any other major surgery(s)? • Yes • No _____ date

Are you presently taking any hormone replacement therapy (HRT)? • Yes • No

_____date last taken

Estrogen _____ Progesterone ___

type dose type dose

Note: If they know they use HRT but not the type or dose, ask them the color and size of the pill and write that down.

Other types of medications and the reason for their use

_____

_____

Non-prescription medications and the reason for their use_____

_____

_____

Any other health problems? • Yes • No If yes, explain

_____

_____

Personal Habits

Do you smoke? • Yes • No What do you smoke? _____
Amount smoked _____

Do you drink alcohol? • Yes • No What do you drink? _____ How often do you drink a week?_____

Are you presently following any special diet? • Yes • No
Explain_____

How often do you eat fish? • Never • 1-2 servings/month • 1-2 servings/week

• 3+ servings/week

Would you be willing to stop eating fish during the study? • Yes • No
Have you taken any vitamin or mineral supplements regularly in the past 6 months?

• Yes • No Variety
Would you be willing to stop taking the vitamin or mineral supplement during the study?

• Yes • No

Do you exercise? • Yes • No Kind of exercise? _____ Minutes per session _____
Sessions per week? _____

A.2 Informed consent document

Department of Nutrition and Food Management
Oregon State University

I have received an oral explanation of this study and I understand the following:

1. This is a research study designed to investigate the susceptibility of low density lipoprotein (a carrier of cholesterol in the blood) to oxidation. I am being screened to see if my medical history, diet history, and blood chemistry values make me a possible candidate for the study. If this information proves satisfactory, I will be asked to be a participant in a study in which I will be given various dietary oils.

   My diet, medical history, and blood values must indicate an overall healthy condition.

2. To participate in the screening, I am required to come to the Metabolic Feeding Unit at OSU one time. During this visit I will provide a medical history, be weighed, be instructed in the correct way to complete diet records and have 10 mL (about 2 teaspoons) of blood drawn from a forearm vein by a certified medical technologist or phlebotomist. The blood will be drawn in a fasted state, i.e. no food or beverage except water will be consumed from 6 P.M. on the day before the blood is to be drawn until after the blood is taken. The maximum time that I will be involved in this screening procedure is about 3 months. I need to visit the Unit only once during this time. The screening phase will last 3 months to give the investigators sufficient time to identify other participants.

3. After being instructed in the correct way to keep diet records, I will keep one for two week days and one weekend day during the week following my visit to the Metabolic Feeding Unit on the forms that I am given. I will return the record within two weeks of my visit by mail in the stamped envelope with which I am provided.

4. If I am selected to participate in the study, I will be asked to take three supplements: fish oil, safflower oil, and sunflower oil. I will take one supplement for five weeks (Period 1) followed by a seven-week washout period with no supplement. I will then take the second supplement (Period 2) for five weeks followed by a seven-week washout period. I will then take the third supplement for five weeks (Period 3). I will not know which supplement I am taking during these five-week intervals. The total time that I will be involved in the supplementation part of the study is 30 weeks.

During each Period, I will come to the Metabolic Feeding Unit five times, on Monday and Thursday of the first and sixth week of each period and on Thursday of the third week. I will visit the Metabolic Feeding Unit a total of fifteen times during the course of the study. At the visits during the first and sixth week, I will come to the Unit fasted. While there I will be weighed, empty supplement containers collected and/or new supplements provided, blood drawn, and breakfast provided. I will give about 30 ml of blood (about 2 tablespoons) at each visit. Over the course of the entire study I will give about 360 ml (about 1.5 cups) blood. This is less than the two cups usually given in one setting during a blood drive. On the visits during the third week I will be weighed, given new supplements, and provided breakfast.

I will be asked to keep a 3-day diet record one time during each phase.

5. I incur no medical risks from my participation except for the possibility of a small bruise or slight bleeding when blood is drawn.

6. I derive benefits from my participation is this study. They are as follows. During the screening part of the project, I will have a thorough analysis made of the nutritional content of my diet, have a chemical profile of my blood measured at Good Samaritan Hospital, and have my lipid profile (cholesterol, triglycerides, high density lipoprotein cholesterol, and low density cholesterol) measured. During the supplementation part of the study my diet will be analyzed three more times, my chemical profile measured six more times, and my blood lipids will be measured six times. All of these data are available to me. If I am asked to participate in the study and complete it, I will be given $100. If I withdraw from the study before it is completed, the amount of money that I receive will be prorated.

7. Any information obtained from me will be confidential. A code number will be used to identify my diet history, weight, blood analysis, and any other information that I provide. The only persons who have access to this information will be the personnel involved with this project.

8. I understand that the University does not provide a research subject with compensation or medical treatment in the event the subject is injured as a result of participation in a research project.

9. My involvement in this study is voluntary and I can withdraw at any time.

10. If I do not adhere to the requirements of the study, I can be dropped as a participant.

11. I understand that questions about the research should be directed to Rosemary C. Wander at 737-0972 and that questions about my rights or research-related injuries should be directed to Mary Nunn, OSU Research Office, at 737-0670.

_____ _____

Name Date

_____

Present Address

A.3 Medical history screening form

Oregon State University
Department of Nutrition and Food Management

This page will be filed separately from your responses to this questionnaire. Your records, laboratory data and any other information we obtained from you will be identified with a code number which is assigned to you.

Name _____

Current address _____

Telephone number _____

DO NOT WRITE BELOW THIS LINE
OFFICE USE ONLY

Code number _____

Medical History

Date _____ Code No. _____

Name _____

1. Personal Data

Age _____

Are you on hormone replacement therapy? • Yes • No

Have you had a surgical hysterectomy? • Yes • No

Date of last menstrual cycle: _____

Your height: _____

Your weight: _____

Your blood pressure: _____

II. Past Illnesses and Present Medical Problems

1. Have you ever had any of the following? If so, when?

	Yes	No	When?
Diabetes	•	•	_____
Asthma, Emphysema or Bronchitis	•	•	_____
High Blood Pressure	•	•	_____
Thyroid Trouble	•	•	_____
Diseases of other endocrine glands (*Cushings, Disease, Addison's Disease, Parathyroid or Pituitary Disease*)	•	•	_____
Coronary Heart Disease	•	•	_____
High Blood Lipids (*Cholesterol or Triglycerides*)	•	•	_____
Gallstones or Gallbladder Disease	•	•	_____
Ulcer	•	•	_____
Liver Disease	•	•	_____
Colitis or Diverticulitis	•	•	_____
Intestinal Malabsorption	•	•	_____
Kidney Disease	•	•	_____
Cancer	•	•	_____
Stroke	•	•	_____
Arthritis or other musculoskeletal problems	•	•	_____
Multiple Sclerosis or other neurological disorders	•	•	_____
Bleeding Disorder	•	•	_____
Peripheral Vascular Disease	•	•	_____
Varicose Veins or Phlebitis	•	•	_____

2. Are you presently using any prescription drugs? • Yes • No

Please list the drugs you are taking, the dosage, the number of times you take them per day, and the number of months you have been taking them.

Drug Name	Dosage	Number Per Day	Number Months Taken

3. Are you in good health at present? • Yes • No

If not, please explain:

4. Do you frequently get sick? • Yes • No

If yes, please explain:

5. Are you under the care of a physician or clinical JH for any reason?

• Yes • No

If yes, please explain:

6. How often do you take aspirin or anti-inflammatory drugs (i.e., Motrin, Advil, Aleve)?

• Never • 1 to 2 times/week • 5 to 10 times/week • Other

What anti-inflammatory drugs do you use?

_____ _____

_____ _____

III. Personal Habits

1. Do you smoke? • Not at all • Cigarettes • Pipe • Cigars

   How much?

2. Do you drink alcohol? • Yes • No at all

   Type of beverage _____ Average oz.consumed

   _____

   How many drinks per week? _____

3. Have you been using any supplements such as vitamins or minerals regularly for the last six months?

   • Yes • No

   Please list any supplements you are taking, the dosage, the number of times you take them per day, and the number of months that you have been taking them:

Supplement Name	Dosage	Number Per Day	Number Months Taken

4. Do you exercise? • Yes • No

How often? • None • less than 20 minutes • 3x per week
• more than 20 min. 3x/week

IV. Family History

Do you have any blood relatives with the following?

	Yes	No
Heat attack before age 40	•	•
Diabetes	•	•
Stroke before age 60	•	•
Kidney failure	•	•
Known high cholesterol or high triglycerides	•	•

V. **Please use this space for any additional comments you may have.**

A.4 Subject entry schedule form

RED = Subjects 01xx, 02xx, 03xx, 04xx
YELLOW = Subjects 05xx, 06xx, 07xx, 08xx
BLUE = Subjects 09xx, 10xx, 11xx, 12xx
GREEN = Subjects 13xx, 14xx, 15xx, 16xx

SHADED = BLOOD Draw
RI = Run-in
W = Week

	SUNDAY	MONDAY	TUESDAY	WED	THURSDAY	FRIDAY	SAT
MAY	4	5 Phone	6 Phone	7 Phone	8 Phone	9 Phone	10
	11	12 Phone	13 Phone	14 Phone	15 Phone	16 Phone	17
	18	19 Phone	20 Phone	21 Phone	22 Phone	23 Phone	24
	25	26 Phone	27 Phone	28 Phone	29 Call back	30 Call back	31
JUNE	1	2 RED RI	3	4	5	6	7
	8	9 BLUE RI	10	11	12	13	14
PERIOD 1	15	16 RED W1 GRE RI	17	18 RED W1	19	20	21
	22	23 BLUE W1 YEL RI	24	25 BLUE W1	26	27	28
	29	30 GRE W1 RED W3	1	2 GRE W1	3	4 HOLIDAY	5
JULY	6	7 YEL W1 BLUE W3	8	9 YEL W1	10	11	12
	13	14 GRE W3	15	16	17	18	19
	20	21 RED W6 YEL W3	22	23 RED W6	24	25	26
	27	28 BLUE W6	29	30 BLUE W6	31	1	2
AUGUST	3	4 GRE W6	5	6 GRE W6	7	8	9
	10	11 YEL W6	12	13 YEL W6	14	15	16
	17	18	19	20	21	22	23
	24	25	26	27	28	29	30
	31	1	2	3	4	5	6
SEPT **PER 2**	7	8 RED W1	9	10 RED W1	11	12	13
	14	15 BLUE W1	16	17 BLUE W1	18	19	20
	21	22GRE W1 RED W3	23	24 GRE W1	25	26	27
	28	29 YEL W1	30	1 YEL W1	2	3	4

		BLUE W3					
OCTOBER	5	6 GRE W3	7	8	9	10	11
	12	13 RED W6	14	15 RED W6	16	17	18
		YEL W3					
	19	20 BLUE W6	21	22 BLUE W6	23	24	25
	26	27 GRE W6	28	29 GRE W6	30	31	1
NOVEMBER	2	3 YEL W6	4	5 YEL W6	6	7	8
	9	10	11	12	13	14	15
	16	17	18	19	20	21	22
	23	24	25	26	27 HOLIDAY	28HOLIDAY	29
PERIOD 3	30	1 RED W1	2	3 RED W1	4	5	6
DECEMBER	7	8 BLUE W1	9	10 BLUE W1	11	12	13
	14	15 GRE W1	16	17 GRE W1	18	19	20
	21	22	23	24 HOLIDAY	25 HOLIDAY	26	27
	28	29	30	31	1 HOLIDAY	2	3
JANUARY	4	5 YEL W1	6	7 YEL W1	8	9	10
		RED W6		RED W6			
	11	12 BLUE W6	13	14 BLUE W6	15	16	17
	18	19 GRE W6	20	21 GRE W6	22	23	24
	25	26	27	28	29	30	31
FEBRUARY	1	2	3	4	5	6	7
	8	9 YEL W1	10	11 YEL W1	12	13	14
	15	16	17	18	19	20	21
	21	23	24	25	26	27	28

A.5 Physical Activity Screening form

A. Self-assessed physical activity rank for work and leisure time

What best describes your level of physical activity connected with work? If you are a homemaker, consider homemaking your work. Check the appropriate choice.

- Sedentary (involves mostly sitting)
- Work that mainly involves standing and walking, but does not require other physical activity
- Work that includes standing and walking and also some heavy lifting and carrying
- Heavy physical work
- Currently not working

In the past year, how much physical activity did you get during your free or leisure time (time spent not working)?

- Practically none
- A little
- A moderate amount
- Quite a bit
- A great amount

B. Participation in vigorous activity for work and leisure time

Vigorous activity is activity you consider to be strenuous. It will result in fatigue, rapid heart rate, or sweating. If you are a homemaker, consider homemaking your work.

In your work, do you perform some vigorous activity? • Yes • No

If yes, how often does this occur? _____ times per week _____ times per month _____ times per year

How long does this activity last ? _____ hours _____ minutes

Do you engage in any vigorous activity in your free or leisure time,? Include the past year. • Yes • No

If yes, how often does this occur? _____ times per week _____ times per month _____ times per year

How long does this activity last? _____ hours _____ minutes

A.6 The method of measuring LDL particle size

Principle

LDL subpopulations range of 21.5-27.5 nm. Their subpopultions can be separated by gradient gel electrophoresis. Gradient gel electrophoresis involves the migration of charged particles through a matrix (comprised of increasing concentrations of polyacrylamide gel. The pore size of the matrix is progressively reduced as the gel concentration increase, resulting in differential retardation of the migrating charged particles as a function of the size. Calibration is usually performed by use of standards of protein mixtures of known size or molecular weight.

Procedure

1. ***Solution-***You need to prepare four solutions.

a. Running buffer-0.09 M Tris (Base), 0.08M Boric acid, 0.0025M EDTA, pH 8.4(+/- 0.1). Add 21.81 g of Tris, 9.90 of Boric acid and 1.93 g of EDTA to 200 ml beaker. Add distilled water to bring the volume up to 2 liters, The pH should be 8.4(±0.1).
b. Fixing solution-Wear gloves since TCA solution is very irritating. Add 30 g TCA to a beaker, bring volume up to 200 ml with distilled water. Stir until the TCA is dissolved.
c. Staining solution – Add 40 mL Glacial Acetic Acid and 120 mL Ethanol to 340 ml distilled water. To this solution add 0.55 g Brilliant Blue G-250. Completely dissolve the solution.

c. Destaining Solution- Add 80 ml Glacial Acetic Acid and 240 mL Ethanol to 680 ml distilled water.

2. ***Gel Preparation***- Wear gloves since not completely polymerized PAGE gel can be carcinogenic. Take out the gel from the refrigerator. Cool to room temperature. Examine the gel. If any air bubbles are present between the gel and the glass plate, gently press them by pressing on the cassette.

   Insert a gel to our home-made electrophoresis chamber. Insert the sample applicator into the gel cassette. Push the sample applicator down until it makes contact with the gel surface. Completely seal the space between the gel and chamber with silicon. Soak bottom of gel by adding buffer to bottom electrophoresis chamber. Add buffer into each wells of sample applicator by using syringe. Leave the gel for 30 min to dry silicon.

3. ***Sample preparation***- Increase the sample density by adding sucrose or glycerol (one-quarter of the sample volume). Add 0.1% bromophenol blue solution (one-tenth of the sample volume) to aid in the observation of the sample during application.

4. ***Pre-electrophoresis***- Add buffer to upper electrophoresis chamber as much as it hold. If any bubbles are in the wall of sample applicator, remove it. Pre-electrophoresis the gel at 125 Volts for 15 minutes.

5. ***Applying the sample***- Turn off the power supply. Apply the samples (3 uL plasma, 6 uL LDL) into well of the sample Applicator. Do not use #1 and #12 well. Apply 6 uL standards (two calibration standards from Krauss lab) into the well at the middle of the gel.

6. ***Electrophoresis***- Set the power supply to constant voltage and run the gel for 30 min at 70 Volts. After 30 min increase Volt up to 125 Volt for 24 hours at 4 °C.

7. ***Fixing and Staining the gel***

a. Wear gloves. After the electrophoresis run is complete, turn the power supply off and remove the gel from the electrophoresis unit. Remove the side seal of the gel, pull out the plastic spacer and open the cassette and transfer gel to 50 ml of TCA Fixing solution. Rock the gel for 15 min.
b. Discard the TCA Fixing solution and pour on 100 ml of the Staining solution. Rock the gel for 1 hour.
c. Discard the staining solution and pour on 100 mL of Destain solution to remove excess stain. Repeat this step until the background is clear (overnight).

d. After destaing discard Destaing solution and soak the gel into distilled water for 2 hours to bring gel to normal size.

8. ***Scan the gel***

9. ***Calibration***