

THE EFFECTS OF FLAXSEED ON LIPID PROFILE
IN NATIVE AMERICAN POSTMENOPAUSAL
WOMEN

By

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NOMENCLATURE

ACAT	Acyl CoA: cholesterol acyltransferase
AHA	American Heart Association
ALA	Alpha-linolenic acid
Apo A	Apolipoprotein A-I
Apo B	Apolipoprotein B
BMI	Body Mass Index
BRFSS	Behavioral Risk Factor Surveillance Survey
CRP	C-reactive protein
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
EPA	Eicosapentanoic acid
GI	Glycaemic index
Hb	Hemoglobin
HDL	High-density lipoprotein
HERS	Heart and estrogen/progestin replacement
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
HMGR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
HRT	Hormone replacement therapy
IDL	Intermediate density lipoproteins
LCAT	Lecithin cholesteryl-acyl transferase

LDL	Low-density lipoprotein
Lp-(a)	Lipoprotein-a
MUFA	Monounsaturated fatty acid
n-3	Omega-3
PUFA	Polyunsaturated fatty acid
RBC	Red blood corpuscles
TC	Total cholesterol
TG	Triglyceride
VLDL	Very low-density lipoprotein
WBC	White blood corpuscles
WHI	Women's Health Initiative

CHAPTER I

INTRODUCTION

Cardiovascular disease (CVD) is a major public health concern with an associated health care cost of approximately \$368 billion (Heart Disease and Stroke Statistical Update. AHA, 2005) for the year 2004 and is responsible for approximately 39.4% of all deaths in the US each year (CHD prevalence, AHA, 2005). The prevalence of death due to CVD in Oklahoma is the second highest in the nation with 391.6 deaths per 100,000 population (CHD prevalence, AHA, 2005). Native Americans form approximately 8% of the population in Oklahoma (Hoyert et al., 2005; Sewell et al., 2002). Unfavorable changes in Native American dietary practices and lifestyle along with reduced physical activity put them at higher risk for CVD than other segments of population (Oser et al., 2005; Welty et al., 2002). Moreover, the prevalence of obesity, hypertension, and diabetes, the three main risk factors for CVD is also high in Native Americans (Bursac et al., 2003).

According to the American Heart Association (AHA), the percentage of female deaths related to CVD was 53.5% in 2000 (Heart and Stroke Facts, AHA, 2005) and was the major cause of mortality of more than half million women. Following menopause, the incidence of CVD increases drastically (Raza et al., 2004; Sekuri et al., 2004; CHD prevalence, AHA, 2003; Heart news, 2003; Jensen et al., 1990) and is associated with

elevations in circulating total- and LDL-cholesterol concentrations, placing postmenopausal women at higher risk for CVD (Fukami et al., 1995).

CVD is also the leading cause of death for Native American women and the total deaths due to CVD are higher in Native American women compared to Native American men (Heart and Stroke Facts, AHA, 2005). Sixty one percent of Native American women in the country have one or more CVD risk factors such as high blood cholesterol, diabetes, hypertension (Sekuri et al., 2004; Sjoberg et al., 2004) and obesity (Bursac et al., 2003). The increased incidence of CVD risk factors, such as hypercholesterolemia in combination with menopausal status makes Native American postmenopausal women a high risk population for CVD. To my knowledge, very few intervention studies looking to reduce risk factors of CVD have been conducted in this population. Reducing the risk of CVD in postmenopausal Native American women will have a great health and economic impact on the community.

In addition to drug therapy, which may have some long-term side effects, e.g. liver damage and muscle weaknesses in severe cases (Chang et al., 2004; Kauffman, 2003), lifestyle and nutritional factors play an important role in the maintenance of cardiovascular health. The use of dietary supplements that are safe and feasible can serve as possible alternatives to medications in lowering the risk of CVD.

Among dietary supplements, flaxseed has been reported to effectively lower cholesterol in postmenopausal women (Williams, 2003; Lucas et al., 2002; Jenkins et al., 1999; Arjmandi et al., 1997; Bierenbaum et al., 1993) as well as in animal models (Lucas et al., 2004; Prasad, 1999; Prasad, 1997). The present study was conducted to determine

the extent to which flaxseed intake reduces CVD risks in Native American postmenopausal women.

Hypothesis and Specific Aims

The hypothesis of this study was that the daily consumption of flaxseed improves the lipid profile in Native American postmenopausal women. To test this hypothesis we had three specific aims as follows:

Aim 1: To determine the extent to which an approximately 30 g daily flaxseed regimen reduces serum total cholesterol, low-density lipoprotein-cholesterol and triglyceride levels in Native American postmenopausal women.

- From earlier observations (Lucas et al., 2002; Arjmandi et al., 1998), it was anticipated that flaxseed regimen will reduce total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels without altering the high density lipoprotein cholesterol (HDL-C) concentration.

Aim 2: To investigate the effects of flaxseed consumption on serum levels of apolipoprotein (Apo)-A (HDL-C carrier), apolipoprotein-B (LDL-C carrier) and C-reactive protein a marker of inflammation.

- Our earlier study (Lucas et al., 2002) reported that flaxseed reduces Apo-A and Apo-B, hence we expect to see similar results from this study. It would be interesting to see the effect of omega-3 fatty acid content of flaxseed on acute inflammatory markers such as C-reactive protein (CRP).

Aim 3: To determine whether daily intake of 30 g flaxseed has any deleterious effect on the hematological parameters.

- Daily intake of flaxseed should not alter any of the hematological parameters most importantly total and differential white blood corpuscle (WBC), red blood corpuscle (RBC), platelet count and hemoglobin concentrations (Hb) (Prasad, 2005).

CHAPTER II

REVIEW OF LITERATURE

Prevalence of Cardiovascular Disease among Native Americans in the United States

Native Americans were thought to have inherent protection from CVD with rates even below the Framingham study; however recent data indicates that CVD is now the leading cause of death in this population (CVD prevalence. AHA, 2005). The state of Oklahoma has approximately 8% of its population as Native Americans (Hoyert et al., 2005; Sewell et al., 2002). Significant independent predictors of CVD in women are diabetes, age, obesity, and elevated levels of LDL-C, triglycerides, albuminuria, and hypertension (Zaydun et al., 2005; Welty et al., 2002).

The most likely explanation for the higher incidence of CVD in American Indians is the high prevalence of diabetes in this population (Welty et al., 2002). A study done by Oser et al. (2005) reported that the adverse community-wide CVD risk profile in American Indians in Montana has continued to worsen in recent years. In 1999, over one third of the Native Indians in Montana reported two or more risk factors, a proportion that increased to 44% by 2003. The increases seen in Montana are similar to those described in the Native Indians studied in Strong Heart Study cohort (Welty et al., 2002), where the rates of diabetes, hypertension, and albuminuria increased. The Strong Heart Study, a prospective, population-based epidemiologic survey of cardiovascular disease was

conducted in residents of 13 geographically diverse American Indian communities. Welty et al. (2002) in their 4 year study described changes in the CVD risk factors in older (45-74 years) Native Americans. Welty and colleagues reported that women who entered menopause after the baseline examination, 4 years before, and who did not initiate estrogen therapy had significant increases in total mean cholesterol, LDL-C, and triglyceride levels by 7.3, 10.6, and 7.3 mg/dl, respectively and a significant decrease in mean HDL-C by 4.4 mg/dl. Women who were postmenopausal at the baseline examination and not taking estrogen had significant increases in mean LDL-C by 3.7 mg/dl and triglyceride levels by 10.2 mg/dl and significant decreases in total and HDL-C levels by 2.1 mg/dl and 4.9 mg/dl, respectively.

Among adult Behavioral Risk Factor Surveillance Survey (BRFSS) respondents in the United States, the proportion of people reporting two or more risk factors for CVD also increased from 23.6% in 1991 to 27.9% in 1999 (Oser et al., 2005). A study done by Struthers et al. (2004) examined the response to chest pain in Native American women. In that study (Struthers et al., 2004) almost 32% (264 of 825) took passive action to crushing chest pain, with 23% reporting that they sat down and waited until it passed. Analysis of the results from the study by Struthers and colleagues (2004) revealed that women who reported a passive response were younger in age (under age 45) and had less education (less than a high school education). All of these studies have come to a single conclusion that improvements in management and prevention of CVD risk factors like hypercholesterolemia, hyperglycemia, and obesity are urgently needed in the Native American population.

Risk factors for CVD in postmenopausal women

Diabetes

Diabetes is a condition caused by the body's inability to process glucose, usually due to lack of insulin. Insulin hormone is produced by the beta cells of the Islet of Langerhans in the pancreas. Diabetes can be passed on genetically, but factors other than heredity are also responsible for this disease (American Diabetes Association, 2005). There are two types of diabetes, Type I and Type II. Type I, or insulin-dependent, is the more severe form of the disease. Type II, or adult onset, is the more common form and accounts for more than 85% of all cases (Diabetes statistic, American Diabetes Association, 2005).

CVD is the major cause of morbidity and mortality in patients with diabetes. According to the American diabetes association report for 2005, 18.2 million people in the United States have diabetes. Although an estimated 13 million have been diagnosed, unfortunately, 5.2 million people are unaware that they have the disease (Diabetes statistic. American diabetes association, 2005). The diabetic association further states that diabetes has reached epidemic proportions in Native Americans. On an average, Native Americans are 2.2 times more likely to have diagnosed diabetes as non-Hispanic whites of similar age. There were 107,775 Native Americans and Alaska Natives who have diabetes and are receiving care from Indian Health Services in the year 2002 (Diabetes statistics for Native Americans. American diabetes association, 2005). Complications from diabetes are the major causes of death in this population.

Heart disease strikes people with diabetes twice as often as people without diabetes. Deaths from heart disease in women with diabetes have increased 23% over the

past 30 years compared to a 27% decrease in women without diabetes (Diabetes statistics for Native Americans. American diabetes association, 2005). Management of diabetes includes nutrition, exercise, medication and blood glucose monitoring. Insulin injections are the main medications used by type-I diabetics as their pancreatic beta cells are incapable of producing insulin. Insulin may be administered by injection, or can be given by an external or internal insulin pump, insulin pen, jet injector, or insulin patch. Being a protein, insulin cannot be given in the form of pills as it will be destroyed by the gastrointestinal secretions (Oral medications for diabetes. 2005). Oral hypoglycemic medications may be used alone (mild to moderate hyperglycemia) or in combination with insulin. These hypoglycemic agents are classified according to their mechanism and site of action:

- sulfonylurea drugs, stimulate the production of insulin in the pancreas,
- biguanides, act by decreasing liver gluconeogenesis,
- alpha-glucosidase inhibitors, slow the absorption of starches,
- meglitinides, stimulate the production of insulin in the pancreas, and
- thiazolidinediones, make the body more sensitive to circulating insulin.

Oral medications have their own side effects such as hypoglycemia, nausea, skin rash, decreased appetite, diarrhea (Oral medications for diabetes. 2005).

Weight loss and increased physical exercise reduce the risk of diabetes in people with impaired glucose tolerance. An inter tribal study done by Archer et al. (2004) on the dietary habits of Native Americans diagnosed with diabetes and those not diagnosed with diabetes showed a difference in the eating habits of both of the groups. Participants diagnosed with diabetes had better dietary habits as they received counseling about the

proper foods to eat, whereas the group without counseling ate more fat containing food, placing them at higher risk of diabetes and CVD.

Dietary foods in diabetes are classified according to their glycaemic index (GI) which ranks the foods based on their overall effects on blood glucose (Archer et al., 2004). Slowly absorbed foods have a low GI rating, whereas foods that are more quickly absorbed will have a higher rating. This is important because choosing slowly absorbed carbohydrates can help control blood glucose levels in diabetes. Fruits, beans and pasta are food with low GI. Slow acting carbohydrates will also reduce the peaks in blood glucose that often follow a meal, and this may have a role in helping to prevent or reduce the risk of Type-II diabetes in at risk populations. Low GI foods helps people with hyperglycemia control their appetite by making them feel fuller for a longer time and as a result they may eat less and consequently lose weight. Research findings show that people who have an overall low GI diet have a lower incidence of heart disease (Pawlak et al., 2004). Hence, a change in choice of food will help to improve glucose tolerance to a great extent and prevent or delay the use of hyperglycemic agents or insulin in Type II diabetes.

Obesity and Physical Activity

Due to changing ways of life in modern society and sedentary lifestyle, obesity has become a serious growing problem worldwide. Postmenopausal women and elderly (60-70 yrs) are among the most sedentary and obese segments of the U.S. population and there is strong evidence to indicate obesity as a risk factor for CVD (Carels et al., 2004). According to research in Arizona State University (Richards et al., 2002), obesity in Native Americans can be blamed on “thrifty gene” which is genetical predisposition to

eating more fats and carbohydrates. Obesity in women has been associated with a variety of factors, including genetic predisposition, social class, early age at menarche, lack of exercise, excessive alcohol consumption and diet low in fruits and vegetables (Wasserman et al., 2004). For most women obesity peaks during and after menopause in the fourth and fifth decade of life. These changes in body composition may be due to reduced levels of circulating estrogen, also an increase in the androgen-estrogen ratio is a likely factor for shifting fat distribution (Lovejoy JC, 2003).

Although no clear link has been documented between genetic predisposition and obesity, it has been suggested that human beta-3 adrenergic receptors induce adipocyte formation and that lipid production can be inhibited significantly by the beta adrenergic receptor antagonist bupranolol (Arch et al., 1996). In the postmenopausal years, women develop a central pattern of fat distribution and an increased risk of developing CVD (Gower et al., 1998). In a cross-sectional study, Gower and colleagues (1998) recruited 141 healthy pre- and postmenopausal women aged 35-65 years to look into the condition if menopause-related differences in lipids are associated with greater estimated intra-abdominal adiposity. The results of the study indicated that postmenopausal women had greater total body fat, central skinfolds and intra abdominal fat and also had higher plasma concentrations of total-C, LDL-C, and triglycerides than premenopausal women. The relationship between central skinfolds and LDL-C differed with menopausal status, being significant in pre- but not postmenopausal women (Gower et al., 1998). Obesity is measured with a Body Mass Index (BMI) which is calculated as weight in kilograms divided by the square of height in meters. Women with a BMI of 25 to 29.9 are considered overweight, while women with a BMI of 30 or more are considered obese

(Oser et al., 2005). Overweight and obesity result from an energy imbalance. This involves eating too many calories and not getting enough physical activity.

According to Thompson and colleagues (2003), physical activity levels among Native American women are low, and few studies have assessed the factors associated with physical activity in this population. The purpose of the study (Thompson et al., 2003) was to determine the relationship among physical activity and various environmental factors such as interaction between the women and community. As part of this multisite study of the Women's Cardiovascular Health Network Project, 350 Native American women, aged 20-50 years, were interviewed. The results emphasized the importance of support from family, friends, communities, and leaders in increasing physical activity among this group of women.

Several studies (Hu et al., 2005; Haapanen-Niemi et al., 2000; Ryan et al., 2000) have supported the theory that increased physical activity reduces obesity and thus has beneficial effects on CVD risk factors. Patalay and colleagues (2005) in their 10 week randomized, double-blind intervention study, concluded that exercise reduces plasma LDL-C and triglycerides in premenopausal women due to increased expression of the LDL receptors and lipoprotein lipase activity. Ryan and colleagues (2000) suggested that fat deposition within midthigh muscle, represented by low-density lean tissue, is associated with increased risk factors for CVD in women. They assigned 24 sedentary, obese (i.e. BMI \geq 32), postmenopausal women (aged 58 ± 1 year) to regimen of, walking 3 times/wk and caloric restriction for a period of 6 mths. The participants were instructed to restrict their energy intake by 1045–1465 kJ (250–350 kcal)/day. The authors report a decrease in body weight by 8% (85.5 ± 2.2 to 78.7 ± 2.0 kg) with the caloric restriction

and walking program. Total body fat decreased by 15%, whereas fat-free mass did not change. Fasting glucose concentrations did not change in the participants, but the glucose tolerance test decreased by 10% (7.1 ± 0.4 compared with 6.2 ± 0.3 mmol/L). HDL-C concentrations increased 8%, from 1.19 ± 0.05 to 1.27 ± 0.04 mmol/L and triacylglycerol concentrations decreased by 19%, from 1.49 ± 0.08 to 1.19 ± 0.06 mmol/L (Ryan et al., 2000). They concluded that increased physical fitness and weight loss improve glucose and lipid profile thus reducing metabolic risk factors for CVD in obese postmenopausal women.

Results from these studies suggest that Native American women, similar to general population, can benefit from increasing physical activity and reducing weight and hence prevent CVD.

Hyperlipidemia

Hyperlipidemia is an elevation of lipids in the bloodstream. It is one of the modifiable risk factors for coronary heart disease. Non-modifiable risk factors such as a strong family history may serve to justify more aggressive treatment. In the 1970s, Fredrickson and colleagues introduced a classification of the primary hyperlipidemias, based on lipoprotein ultracentrifugation and electrophoresis was also accepted by WHO.

- Mixed hyperlipidemia is defined as increase in total cholesterol and triglyceride level with or without decrease in HDL-cholesterol.
- Hypertriglyceridemia is characterized by high serum level triglycerides and also may be associated with high serum LDL-C or low HDL-C levels.
- Hypercholesterolemia is defined as an increase in total cholesterol and low density lipoprotein cholesterol levels.

Most patients who present with hyperlipidemia have a polygenic predisposition to raised blood lipids aggravated by dietary or lifestyle indiscretion. Diets rich in saturated fat tend to raise blood cholesterol levels, more specifically LDL-C, while high carbohydrate intake or excessive alcohol consumption may increase plasma (VLDL) and triglyceride concentrations (Dietary guidelines. AHA 2005). Any treatment plan for hyperlipidemia must exclude common causes of secondary hyperlipidemia such as diabetes and hypothyroidism (Luboshitzky, 2002). Hypothyroidism-induced hyperlipidemia responds to thyroid hormone supplementation. All patients with hyperlipidemia should be managed with a fat-modified diet, initially as the sole form of therapy for 3 to 6 months, before drug therapy is considered (Luboshitzky, 2002). Dietary manipulation should aim to reduce intake of red meat, and refined sugar while increasing the intake of vegetables, fruits. Other sources of protein such as fish should be encouraged, particularly oily fish such as mackerel, salmon or trout. The omega (n)-3 polyunsaturated fatty acid content of fish improves the lipid profile (Dewailly et al., 2002). Docosahexaenoic acid, an n-3 fatty acid, is known to improve endothelial response of vascular system in familial hyperlipidemic patients (Engler et al., 2004). Increased physical activity and attaining ideal body weight, improves glucose, blood pressure and lipid profiles (Hu et al., 2005; Haapanen-Niemi et al., 2000; Ryan et al., 2000).

Prevention and treatment of cardiovascular diseases in postmenopausal women

Cholesterol Metabolism

Cholesterol is a vital constituent of cell membranes, and is a precursor of steroid hormones such as estrogens, androgens, and sterols. In the nervous system it is also needed for myelin sheath formation in neuronal cells and neurotransmitters. Furthermore, it is one of the constituents of lipoproteins. While normal cholesterol level is essential to life, its deposition in arteries has been associated with CVD and stroke. In a healthy organism, an intricate balance is maintained between the biosynthesis, utilization, and transport of cholesterol, keeping its harmful deposition to a minimum (Voet and Voet, 1995).

The biosynthesis of cholesterol is catalyzed by enzymes in the cytosol and enzymes bound to the endoplasmic reticulum. It begins with a condensation in the cytosol of two molecules of acetyl-CoA in a reaction which is catalyzed by thiolase. The next step requires the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) synthase. This enzyme catalyzes the condensation of a third acetyl-CoA with β -ketobutyryl-CoA to yield HMG-CoA which is then reduced to mevalonate by HMG-CoA reductase (HMGR). Regulation of HMGR activity is the primary means for controlling the level of cholesterol biosynthesis. The enzyme HMGR is controlled by four distinct mechanisms: feed-back inhibition, control of gene expression, rate of enzyme degradation and by phosphorylation-dephosphorylation (Voet and Voet, 1995). The first three control mechanisms are regulated by cholesterol itself. Cholesterol acts as a feed-back inhibitor of pre-existing HMGR as well as inducing rapid degradation of the

enzyme. The latter is the result of cholesterol-induced polyubiquitination of HMGR and its degradation in the proteasome. This ability of cholesterol is a consequence of the sterol sensing domain, of HMGR. In addition, when cholesterol is in excess, the amount of mRNA for HMGR is reduced as a result of decreased expression of the gene. HMGR is most active in the dephosphorylated state. Phosphorylation is catalyzed by AMP-activated protein kinase (AMPK) an enzyme whose activity is also regulated by phosphorylation. Hormones such as glucagon and epinephrine negatively affect cholesterol biosynthesis by increasing the activity of the inhibitor of phosphoprotein phosphatase inhibitor-1. Conversely, insulin stimulates the removal of phosphates and, thereby, activates HMGR activity. Additional regulation of HMGR occurs through an inhibition of its activity as well as of its synthesis by elevation in intracellular cholesterol levels. This latter phenomenon involves the membrane-bound transcription factor, sterol regulated element binding protein (Voet and Voet, 1995).

Cholesterol is transported in the plasma predominantly as cholesteryl esters associated with lipoproteins. Essentially, all of the plasma lipids are associated with proteins to form water-miscible lipoprotein complexes. Classification of lipoproteins is generally based on density gradient ultracentrifugation. Each lipoprotein contains different proportions of protein, phospholipids, triglyceride, and cholesterol, thereby giving rise to the different densities of each class (Voet and Voet, 1995).

Five major classes of lipoproteins have been sequentially separated by ultracentrifugation, and are designated as chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins, and high density lipoproteins. Interaction among lipoproteins, between lipoproteins and enzymes

or cells occurs continually even though each plasma lipoprotein is a complex molecule with finite composition and size. An accepted view of lipoprotein structure suggests the existence of an outer shell of polar phospholipid and protein groups, with an inner hydrophobic core of primarily triglycerides and cholesteryl esters (Voet and Voet., 1995).

1. Chylomicrons

The chylomicrons, which are derived from intestinal absorption of triglycerides, have densities of less than 0.95 g/ml and diameters ranging from 750 to 6000 Å. Their primary composition is of triglyceride (80-90% by weight), free and esterified cholesterol (2-7%), phospholipid (3-6%), and protein (1-2%) (Voet and Voet, 1995). The proteins found on the surface of the chylomicrons are Apo A-I, B, and C. Since chylomicrons transport exogenous glycerides from the intestinal mucosa via the thoracic duct to the circulation their formation fluctuates with the load of triglycerides absorbed.

Chylomicrons are cleared rapidly from the blood. Therefore their presence in the post-absorptive state, approximately 12 to 16 hours after the last meal is indicative of defective handling of dietary fat. The composition of chylomicrons is altered in the circulation, with the action of endothelial lipoprotein lipase facilitating triglyceride hydrolysis by extra hepatic tissues. The activity of this enzyme in individual tissues varies with nutritional and physiological states, such as fasting, exercise, pregnancy, and lactation, thereby affecting triglyceride uptake and plasma levels (Redgrave TG, 2004). Chylomicron remnants, with 80% less triglyceride, are then taken up by the parenchymal cells of the liver via receptor-mediated endocytosis after binding to specific remnant receptors (Voet and Voet., 1995).

Chylomicrons and their remnants also contain Apo B-48 which is produced by the intestine as a truncated form of Apo B-100 (only the first 48% of the Apo B-100 molecule is synthesized). Because of these binding specificities, chylomicron remnant receptors are termed Apo E receptors and hepatic LDL receptors are termed Apo B and E receptors (Redgrave TG, 2004).

2. Very Low Density and Intermediate Density Lipoproteins

Very low density lipoproteins are of endogenous origin and are synthesized and secreted primarily by liver and intestine. They are isolated from chylomicron-free plasma at densities less than 1.006 g/ml; and their particles vary in diameter from between 300 and 800 Å. They are composed of 55-65% triglyceride, 10-15% free and esterified cholesterol, 15-20% phospholipid, and 5-10% protein. The proteins found on the surface of VLDL include Apo A-I, Apo B, Apo C, and Apo E. The liver employs Apo B-100 in the formation of VLDL (Voet and Voet, 1995).

Triglycerides carried in VLDL are transported in plasma to utilization sites such as heart, muscle, mammary gland and storage sites such as adipocytes. The key enzyme in VLDL metabolism is the endothelial-bound lipoprotein lipase, which is activated by Apo C-II (Madani et al., 2003). Apo E plays a role in VLDL disposition. Following lipoprotein lipase action, the triglyceride-depleted particles are released into the bloodstream, and are then the primary source of circulating LDL. As VLDLs undergo de-lipidation, the particles change their composition, structure, and biological properties. As VLDL become smaller and denser, percent contribution from the proteins, phospholipids, and cholesterol increases where as proportion of triglyceride decreases. Thus, the

heterogeneity of VLDL represents a dynamic equilibrium of particles from several sources (Madani et al., 2003).

After VLDLs (and possibly chylomicrons) have interacted with lipoprotein lipase at multiple sites, particles of density greater than 1.006 g/ml are formed. These remnant particles are operationally defined as IDL, with a density range of 1.006-1.019 g/ml and a diameter of about 250 Å. Triglycerides contribute only 20% of the mass of IDL, but it is still regarded as triglyceride-rich and may be further reduced to LDL and even HDL (Madani et al., 2003).

Some of the circulating VLDL remnants are removed directly by the liver in a process mediated by Apo E. The surface of liver cells contains receptors that recognize and bind to the Apo E; the remnant lipoprotein is then internalized and removed from the circulation (Knouff et al., 2004). However, another fraction of VLDL remnants remains in the circulation and this is the fraction that is converted to LDL. Usually, about two-thirds of IDL is taken up by the liver, and one-third goes to LDL. Thus, the proportion of VLDL fraction converted to IDL and then to LDL greatly influences the concentrations of serum LDL. If a greater fraction of VLDL remnant is transformed to IDL and then to LDL, the concentrations of latter will rise (Knouff et al., 2004).

3. Low Density Lipoproteins

Low density lipoprotein, a spherical particle, contains mostly cholesteryl ester in its nonpolar core; its surface apolipoprotein consists almost exclusively of one Apo B-100 molecule. Apo B-100 contains 4563 amino acids and is synthesized in the liver (Voet and Voet, 1995). Low density lipoproteins are the end product of a chain of de-lipidation steps of VLDL and chylomicron particles involving lipoprotein lipase, hepatic

lipase, and lecithin: cholesterol acyltransferase (LCAT). Studies suggest that direct hepatic secretion of LDL is a normal process, although its magnitude varies from species to species and may be markedly heightened in disease states, as in familial hypercholesterolemia (Koeijvoets et al., 2005).

Low density lipoproteins normally transport 60 to 75% of the total plasma cholesterol. They are isolated from chylomicron-free plasma in the density range 1.019-1.063 g/ml and have a diameter of about 220 Å. They are composed of about 50% free and esterified cholesterol, 20% phospholipid, 20% protein, and 10% triglyceride (Voet and Voet, 1995). The small size and high cholesterol content of LDL may be the reason for its high atherogenic property. Apolipoprotein-B serves as a marker for LDL turnover as it is the only protein found on the surface of LDL.

Although polyacrylamide gel electrophoresis identified several subtypes of LDL, two major subclasses are usually compared in terms of their association with CHD (Voet and Voet, 1995). The larger, more buoyant LDL has a peak diameter > 25.5 nm; the smaller LDL has a peak diameter < 25.5 nm. Serum LDL concentration is affected by the fractional removal rate of LDL from the circulation, and this rate is determined in large part by the availability of cell-surface receptors for LDL. In normal cells, the LDL is internalized via endocytosis. The endocytotic vesicle containing LDL then fuses with a primary lysosome, where the protein moiety is degraded completely to amino acids and the cholesteryl esters are hydrolyzed by cholesterol esterase. Re-esterification of excess cholesterol for storage is catalyzed by acyl CoA: cholesterol acyltransferase (ACAT). Endogenous cholesterol synthesis within the cells is suppressed by HMGR when exogenous cholesterol is delivered via LDL to the tissues. The need for cellular

cholesterol for membrane and steroid hormone synthesis regulates the number of LDL membrane receptors (Voet and Voet, 1995).

In addition to the high-affinity receptor pathway, LDL can be degraded by less efficient receptor-independent pathways. When the plasma concentrations of LDL are markedly elevated due to the lack of high-affinity LDL receptors, uptake of LDL by receptor-independent pathways is markedly increased. Originally, it was thought that the LDL receptor was specific for ApoB, but it has since been shown that certain lipoprotein fractions rich in ApoE, but without ApoB, also bind to the LDL receptor. About 75% of LDL degradation occurs intrahepatically and the remaining extrahepatically (Voet and Voet, 1995).

4. High Density Lipoproteins

High density lipoproteins are isolated in the density range 1.063-1.21 g/ml and are the smallest of the lipoproteins, with a diameter of 70-120 Å (Voet and Voet., 1995). They normally account for 20-25% of total plasma cholesterol and are composed of about 50% protein, 25% phospholipid, 20% free and esterified cholesterol, and 5% triglyceride. The greater density of HDL compared with other lipoproteins reflects its greater protein and lesser lipid content. Their cholesteryl ester to free cholesterol ratio is approximately 3:1 (Voet and Voet., 1995). HDLs have been divided essentially into two classes: HDL₂ (density range 1.063-1.125 g/ml) composed of about 60% lipid and 40% protein and HDL₃ (density range 1.125-1.21 g/ml) contains about 55% proteins (Voet and voet, 1995).

Several different apolipoprotein species exist within HDL, the major one being Apo A, containing Apo A-I and Apo A-II. HDLs may be separated into specific

subpopulations on the basis of their apolipoprotein composition. One subpopulation consists of HDLs that contain Apo A-I (A-I HDLs), while another contains both Apo A-I and Apo A-II (A-I/A-II HDLs) (Voet And Voet., 1995). Most of the A-I/A-II HDLs are found in the HDL-3 density range, while A-I HDLs are prominent components of both HDL-2 and HDL-3. Plasma levels of ApoA-I and ApoA-II correlate with HDL cholesterol.

Liver is the primary site for HDL and ApoA biosynthesis and secretion, while intestine is considered secondary site of secretion. More than 95% of total HDL proteins from the livers of normal rats are comprised of ApoE, ApoA-I, and ApoC. Apolipoprotein C is synthesized only in the liver and is transferred to intestinal HDL when it enters the plasma, whereas the synthesis and secretion of ApoA-I is found in both the intestine and liver in mammals (Bouchard et al., 2005).

Nascent HDL (HDL precursor complexes) formed in vivo may be transformed in the plasma to mature spherical particles. The nascent HDL particles are converted to mature spherical circulating HDL through the action of plasma LCAT. This enzyme is responsible for removing phospholipids from HDL and replacing them with cholesteryl esters, as well as facilitating the loss of Apo E from nascent HDL. The end result of these processes appears to be the net movement of cholesterol from peripheral tissues to hepatic tissue for removal and breakdown (Voet and Voet, 1995).

LCAT is an extra cellular enzyme that is synthesized and secreted by the liver, circulates in the plasma, and acts on plasma HDL. This enzyme also promotes non-enzymatic transfer of cholesteryl esters from HDL to VLDL and LDL. The cholesteryl esters formed by the LCAT reaction are distributed among HDL, VLDL, and LDL, which

increases their total cholesterol content. This process may serve to increase the rate of cholesterol removal by a receptor-mediated process, and may in addition provide a mechanism for directing cholesterol transport toward specific tissues. Patients with familial LCAT deficiency show an accumulation of plasma unesterified cholesterol, with all lipoproteins having an abnormal lipid and apolipoprotein composition.

Liver and intestine are the only organs with the capacity to effectively remove cholesterol from the circulation. The transfer of HDL cholesteryl esters to the liver for elimination could theoretically be achieved by: 1) the uptake and catabolism of whole HDL particles; 2) selective removal of cholesteryl esters from mature HDL, with its subsequent recycling; and/or 3) initial transfer of cholesteryl ester to lower density lipoproteins, with subsequent hepatic removal (Brousseau et al., 2005). The transfer of cholesteryl esters from HDL to LDL is facilitated by the cholesteryl ester transfer protein. The increased net transfer of cholesteryl esters by the cholesteryl ester transfer protein results in decreased HDL concentration (Brousseau et al., 2005).

5. Lipoprotein (a) [Lp (a)]

Lipoprotein (a) is a LDL-like particle with a cholesterol rich core and a molecule of Apo B-100 linked by a disulphide bridge to the glycoprotein Apo A. The Lp (a) component varies in size ranging from 200 to 700 kD and has structural similarities with plasminogen (Voet and Voet., 1995) which is involved in dissolving blood clots. Lipoprotein (a) competes with plasminogen for binding sites on the cell surface, thereby decreasing plasminogen activation and inhibiting clot lysis, thus promoting wound healing.

Lipoprotein (a) is the most atherogenic lipoproteins but little is known about its physiological functions and metabolism. Metabolism of Lp(a) is thought to be due to specific kringle IV domains in Apo A, mainly T-6 and T-7, binding to circulating LDLs, which is followed by a second step in which stabilization of the newly formed Lp (a) complex is achieved by a disulfide bridge (Kostner and Kostner, 2002). Circulating Lp (a) is excreted via urine due to possible interactions specifically with kidney cells, or other tissues, causing cleavage of 2/3-3/4 of the N-terminal part of Apo A by a collagenase-type protease (Kostner and Kostner, 2002).

Cholesterol is excreted in the bile as free cholesterol or as bile salts. Most of the cholesterol in the liver is converted to bile salts and excreted via bile duct. The end products of cholesterol utilization are the bile salts, synthesized in the liver. Synthesis of bile salts is one of the predominant mechanisms for the excretion of excess cholesterol. Bile salts are synthesized from cholesterol by the liver with the help of enzyme 7 α -hydroxylase which hydroxylates cholesterol to 7-hydroxycholesterol (Voet and Voet, 1995). This substrate undergoes several transformations to form bile salts, cholyl Co-A and chenodeoxycholyl Co-A. The most abundant bile acids in human are chenodeoxycholic acid (45%) and cholic acid (31%). These are referred to as the primary bile acids. Within the intestines the primary bile acids are acted upon by bacteria and converted to the secondary bile acids, identified as deoxycholate (from cholate) and lithocholate (from chenodeoxycholate). Both primary and secondary bile acids are reabsorbed by the intestines and delivered back to the liver via the portal circulation. Within the liver the carboxyl group of primary and secondary bile acids is conjugated via an amide bond to either glycine or taurine before their being resecreted into the bile

canaliculi. These conjugation reactions yield glycoconjugates and tauroconjugates, respectively (Voet and Voet, 1995). Bile acids are carried from the liver through these ducts to the gallbladder, where they are stored for future use. The ultimate fate of bile acids is secretion into the intestine, where they aid in the emulsification of dietary lipids. In the gut the glycine and taurine residues are removed and the bile acids are either excreted (only a small percentage) or reabsorbed by the gut and returned to the liver. This process of secretion from the liver to the gallbladder, to the intestines and finally reabsorption is termed the enterohepatic circulation. Bile acids have physiologically significant functions such as 1) their synthesis and excretion in the feces is the significant mechanism for the elimination of excess cholesterol, 2) bile acids deoxycholate and lithocholate and phospholipids solubilize cholesterol in the bile, thereby preventing the precipitation of cholesterol in the gallbladder, 3) bile acids facilitate digestion of triacylglycerols by acting as emulsifying agents which makes fats accessible to pancreatic lipases, 4) also bile acids facilitate absorption of fat soluble vitamins due to their emulsifying property.

Cholesterol lowering drugs

Drug therapy is considered for patients who in spite of adequate dietary therapy, regular physical activity and weight loss need further treatment for elevated blood cholesterol levels. There are five main categories of cholesterol lowering drugs: statins, bile acid resins, nicotinic acid, fibrates and ezetimibe.

Statins

These drugs block the action of HMGCR which converts HMG-CoA to mevalonate and hence reduce de novo synthesis of cholesterol. Drugs in this group include:

atorvastatin (Lipitor); cerivastatin (Baycol); fluvastatin (Lescol); lovastatin (Mevacor); pravastatin (Pravachol); simvastatin (Zocor); and rosuvastatin (Crestor) (Ikeda et al., 2001). These drugs cause significant reduction in cholesterol levels when accompanied with dietary changes like reducing fat intake. Potential beneficial effects of these agents include enhancement of nitric oxide production in vasculature and the kidney. Statins have been shown to stabilize plaques, improve vascular relaxation, and promote new vessel formation (Ikeda et al., 2001). These actions are mediated, in part, by the effects on small G-proteins, modulation of signaling cascades, transcription, and gene expression. In particular, the inhibition of small GTP-binding proteins: Rho, Ras, and Rac, whose proper membrane localization and function are dependent on isoprenylation, may play an important role in mediating the direct cellular effects of statins on the vascular wall (Ikeda et al., 2001). A recent study by Mraiche and colleagues (Mraiche et al., 2005) showed that statin drugs potentially inhibit both prepro-endothelin-1 (ET-1) mediated contraction in the vascular smooth muscles (VSM) and also inhibited DNA synthesis in quiescent VSM cells.

Statins are usually well tolerated, but like other medications they also have side effects. The most common side effects nausea, gastrointestinal disturbance and muscle ache which if severe is a cause of concern as it may be a sign of rhabdomyolysis or muscle cell breakdown, which is one of the rare side-effects of statin drugs. Breakdown of muscle cells release myoglobin which can impair kidney function and hence cause renal failure. The possible breakdown of cells is due to lack of coenzyme Q10 (CoQ10). HMGR enzyme which statin drugs block is necessary for CoQ10 synthesis. CoQ10 is necessary for energy production in the mitochondria via the electron transfer chain.

Hence inhibition of CoQ10 synthesis will lead to cell death (Golomb et al., 2004). Cerivastatin had a high incidence of rhabdomyolysis cases and was discontinued as a drug for hypercholesterolemia (Salarieh et al., 2004). Certain drugs such as gemfibrozil, erythromycin, cyclosporine and niacin when taken along with statins increase the risk of rhabdomyolysis (Chang et al., 2004). As the drugs are metabolized in the liver regular liver enzyme checkups are needed to ensure safety. Not many researchers have looked into the fate of the substrates such as acetyl Co-A and HMG Co-A of cholesterol synthesis. Acetyl Co-A can be used in other mechanisms such as ketone body formation, in citric acid cycle for ATP synthesis and in turn energy production, and protein synthesis. The fate of HMG Co-A is not known. It is also not known whether continuous statin treatment for a long period of time approximately 20-30 years will lead to irreversible suppression of HMGR.

Bile acid Resins

Enzyme 7 α -hydroxylase converts cholesterol to bile acids in the liver (Voet and Voet, 1995). Bile acids help in absorbing fat and cholesterol from the small intestine via a process called entero-hepatic circulation. Bile acids bind to cholesterol in the small intestine and then pass into the portal vein and back to liver. This process though is useful in a normal healthy person, but for a hypercholesterolemic patient is detrimental. Bile acid resins such as gemfibrosil and cholestyramine block the uptake of cholesterol by bile acids which are then excreted via feces. The liver produces more bile acids in response to loss of bile acids in the feces. This effectively reduces LDL-cholesterol levels. These drugs work well in combination with statin drugs to reduce blood cholesterol levels (Chang et al., 2004).

Nicotinic acids

Nicotinic acid or niacin, the water-soluble B vitamin, improves all lipoproteins when given in doses well above the vitamin requirement of 35 mg daily for adults. Nicotinic acid lowers total cholesterol, LDL-C, and triglyceride levels, while raising HDL-C levels. There are three types of nicotinic acid supplements: immediate release, timed release, and extended release (Birjmohun et al., 2005). Nicotinic acid is inexpensive and widely accessible to patients without a prescription, but must not be used for cholesterol lowering without proper monitoring because of the potential side effects. Patients on nicotinic acid are usually started on low daily doses and gradually increased to an average daily dose of 1.5 to 3 grams per day for the immediate release form, and 1.5 to 2 grams per day for the other forms. Nicotinic acid reduces LDL-C levels by 10 to 20 percent, reduces triglycerides by 20 to 50 percent, and raises HDL-C by 15 to 35 percent (Birjmohun et al., 2005).

A common and troublesome side effect of nicotinic acid is flushing or hot flashes, which is a result of dilatation of blood vessels. Most patients develop a tolerance to flushing and, in some patients it can be decreased by taking the drug during or after meals or by the use of aspirin or other similar medications prescribed by his/her physician. The extended release form may cause less flushing than the other forms. A variety of gastrointestinal symptoms including nausea, indigestion, gas, vomiting, diarrhea, and the activation of peptic ulcers have been seen with the use of nicotinic acid. Three other major adverse effects include increased hepatic enzymes, gout, and high blood sugar. Risk of the latter three increases as the dose of nicotinic acid is increased.

Hence, nicotinic acid is not always the drug of choice to control cholesterol levels (Birjmohun et al., 2005).

Ezetimibe

Ezetimibe is a new class of agents approved by the FDA in 2002, which specifically block the absorption of cholesterol from the gastrointestinal tract. Ezetimibe has a metabolic pathway involving enterohepatic circulation that allows for once a day administration due to a prolonged half-life (Manhas et al., 2004). Ezetimibe, blocks the intestinal absorption of dietary and biliary cholesterol, a mechanism of action complementary to that of statins, which inhibit hepatic cholesterol synthesis, however, the absorption of triglycerides or fat-soluble vitamins is not hampered. Ezetimibe co-administered with statins produces significant incremental reductions in LDL-C compared with statin monotherapy (Bennett et al., 2004). Two studies testing the effectiveness of a combination therapy of ezetimibe with statins showed that a combination of ezetimibe and simvastatin (Bays et al., 2004) was effective in reducing LDL-C better than a combination of ezetimibe and rosuvastatin (Ballantyne et al., 2004) . The bile acid resin drug, cholestyramine, blocks the absorption of ezetimibe and hence reduces its effectiveness. Also cyclosporins increase the toxicity of this drug by delaying its excretion. Diarrhea, abdominal pain, back pain, joint pain, and sinusitis were the most commonly reported side effects, occurring in 1 in every 25 to 30 patients. Rarely, severe hypersensitivity reactions such as fluid accumulation (angioedema), skin rash, pancreatitis and nausea may occur (Manhas et al., 2004).

Using drugs to lower cholesterol may reduce deaths from coronary heart disease or the risk of it, but is probably dependent on patients' level of risk: those at low risk of

coronary heart disease are unlikely to benefit from treatment. A study done by de Lorgeril M et al. (2005) which looked into the effects of lipid-lowering drugs (LLD) namely simvastatin (statin drug) and fenofibrate on the metabolism of essential n-6 and n-3 fatty acids in patients with established coronary heart disease (CHD). They report that LLDs significantly alter the metabolism of essential fatty acids, especially the two major n-3 fatty acids, alpha-linolenate and docosahexanoate, that are critically important for the pathogenesis and prevention of CHD. Hence, widespread use of drugs for lowering cholesterol in older people should not be promoted, unless they are at particularly high risk of developing, or already have, coronary heart disease.

Hormone Replacement Therapy

Hormone or estrogen replacement therapy (ERT) may prevent cardiovascular disease in postmenopausal women by reducing serum cholesterol. Estrogen therapy has been shown to improve lipid profiles, bone quality due to action of estrogen receptor-linked calcium transport in the intestine (Arjmandi et al., 1993), blood pressure through direct action on vascular endothelium and also slows buildup of atherosclerotic plaque (Nanda et al., 2003). According to the American heart association (2005) atherosclerosis is responsible for the majority of CHD. Atherosclerosis is an inflammatory condition characterized by the development of plaque in the walls of blood vessel. Stroke or myocardial infarction results when plaque becomes dislodged which blocks the blood flow to the brain or heart muscles. Benefits of estrogen replacement is seen in all stages of the plaque formation but its role in the early stages like reducing serum cholesterol levels have been extensively studied. Nanda et al. (2003) report estrogens ability to reduce serum LDL-C levels and increase serum HDL-C levels. There are reports that

estrogens replacement therapy increases triglyceride levels through increase in very low-density lipoprotein (VLDL) particles (Anderson et al., 2004; Knopp, 1996). VLDL particles are known to be atherogenic but those produced due to use of exogenous estrogen are low in cholesterol concentration and are believed to be less likely to enter the arterial walls because of their buoyancy (Knopp, 1996). Estrogen increases all major cholesterol transport pathways and causes up-regulation of LDL receptors thus allowing more LDL uptake by the liver which is used for production of steroids or bile salts. Estrogen also increases Apo A and HDL-C levels this is probably due to decrease in hepatic lipase activity. Though ERT was shown to have promising results in alleviating postmenopausal symptoms the results from recent follow up studies have been surprising and all together unexpected. The Women's Health Initiative study started in 1993 had two components, the estrogen only treatment arms (0.625 mg/day of conjugated equine estrogen) which enrolled 10,739 postmenopausal women, aged 50-79 years, with prior hysterectomy, and the estrogen plus progestin component (0.625 mg/day conjugated equine estrogens plus 2.5 mg/day medroxyprogesterone acetate) in which 16,608 postmenopausal women aged 50-79 years with an intact uterus at baseline were recruited by 40 US clinical centers in 1993-1998. Both treatment were stopped in the 2002 after only a 5-6 yr follow-up as the results obtained showed that neither of the treatments reduced the CHD incidence in postmenopausal women but were increasing the risk of stroke in the estrogen only group and invasive breast cancer in the estrogen plus progestin group (Anderson et al., 2004; Rossouw et al., 2002).

The Heart and Estrogen/Progestin replacement (HERS) therapy which gave the same dose of hormones as WHI study to 2763 postmenopausal women with CHD and

followed them for 4 yrs found no advantage of the therapy for these women. The HERS trial found that taking estrogen plus progestin for up to 4 years did not prevent further heart attacks or death from previous heart disease in postmenopausal women who already had a previous heart attack or known heart disease. This neutral finding occurred even though there was a positive effect of treatment on cholesterol (Shlipak et al., 2003). This increased incidence of CHD may be due to prothrombotic or proinflammatory effects of exogenous estrogen hormones given (Herrington and Klein, 2001). In the Postmenopausal Hormone Replacement Against Atherosclerosis Trial (Angerer et al., 2002), 321 postmenopausal women with increased carotid intima-media thickness based on ultrasound examination were randomly assigned to receive placebo or HRT regimens. After 48 weeks, there was no difference between placebo and active treatment groups in progression of atherosclerosis measured as a change in carotid (neck) or femoral (leg) artery intima-media thickness (Hodis et al., 2001).

Less than 30% of postmenopausal women in the United States receive HRT and of these women most discontinue use after one year (Barrett-Connor et al., 2000). Most women stop taking HRT after severe symptoms of menopause like hot flashes are relieved and some stop due to financial reasons. The main reasons for stopping estrogen therapies by most women are due to side-effects such as irregular and break out bleedings, and the increased incidence of development of cancers and thrombosis. Hormone replacement therapy is therefore now recommended for women who have osteoporosis and severe menopausal symptoms like hot flashes and mood swings (Barrett-Connor et al., 2000).

Dietary factors that alter lipid profile

The increased awareness of the role that diet plays in human health has led to the recognition that some non-nutrient components of foods may provide protection against many western health-related problems. The rates of death due to heart disease, cancers especially colon cancers, have been shown to be reduced due to incorporation of fiber in the diet (Dietary guidelines. AHA, 2005). The American Heart Association endorses Step I and Step II diets created by the National Heart, Lung, and Blood Association's National Cholesterol Education Program (NCEP). Both diets were designed to reduce risk of cardiovascular disease by reducing high blood cholesterol levels.

The Step I diet restricted total fat to no more than 30 percent of total calories, saturated fat to no more than 10 percent of total calories, and cholesterol to less than 300 mg/day. This diet was intended as the starting point for patients who had high cholesterol levels.

The Step II diet goals were lower for saturated fat (less than 7 percent) and cholesterol (less than 200 mg/day). They were intended for people who had attained Step I diet goals or for patients with a high-risk cholesterol level (240 mg/dL or higher) or who had had a heart attack (Dietary guidelines. AHA, 2005).

For people at high risk or who have known cardiovascular disease, the Therapeutic Lifestyle Changes (TLC) diet is preferred. This is the "next generation" of the Step II diet recommended in May 2001. The essential components of TLC are saturated fats less than 7% of total calories and dietary cholesterol less than 200 mg/day which is similar to Step II diet. In addition it provides therapeutic options for LDL-lowering agents such as intake of plant sterols at least 2 g/day and increased intake of

soluble fiber to at least 10-25 g/day. Along with the caloric restriction it also includes moderate exercise to expend at least 200 kcal/day (Dietary guidelines. AHA, 2005).

Hence, we conclude that healthy food habits can help to reduce risk factors for CVD such as high blood cholesterol, high blood pressure and excess body weight.

Fiber

Dietary fiber is a part of the plant which is not completely broken down during digestion. Fiber has been widely accepted as playing a significant role in reducing total blood cholesterol particularly LDL-C (Lamarche et al., 2004, Arjmandi et al., 1998), thereby decreasing the risk of coronary heart disease, and in helping to alleviate numerous bowel disorders. Studies done both in humans (Behall et al., 2004; Arjmandi et al., 1998; Everson et al., 1992) and animals (Li et al., 2004; Arjmandi et al., 1997; Arjmandi et al., 1992a) suggest that dietary fiber lowers cholesterol by enhancing bile acid synthesis and their fecal excretion which results in increased hepatic cholesterol synthesis. Also, gut bacteria ferment soluble fiber to form short chain fatty acids (SCFA). These SCFA are readily absorbed and at concentrations of 15-30 mmol/L inhibit *in vivo* hepatic cholesterol and fatty acid synthesis (Arjmandi et al., 1992b).

Researchers (Jenkins et al., 2005; Behall et al., 2004) have reported moderate, but significant, reductions of plasma cholesterol when subjects consumed increased amounts of mixed vegetables and fruits with fat intake kept constant for long periods of time. Perhaps the beneficial effects of soluble fiber can be seen most clearly when it is consumed as part of a balanced, or prudent, diet. A study by Jenkins and colleagues (2005) compared the cholesterol-lowering potential of food with that of a statin, in the same subjects. Thirty-four hyperlipidemic participants underwent three 1-month

treatments in random order, a very-low-saturated fat diet, the same diet plus 20 mg lovastatin (statin diet), and a diet high in plant sterols (1.0 g/1000 kcal), soy-protein foods (21.4 g/1000 kcal), almonds (14 g/1000 kcal), and viscous fibers from oats, barley, psyllium, and the vegetables okra and eggplant (10 g/1000 kcal). LDL-C concentrations decreased by 8.5%, 33.3%, and 29.6% after 4 wk of the control, statin, and vegetable diets, respectively. The statin and the vegetable diets did not differ significantly ($P=0.288$) in their ability to reduce LDL-C. Hence, the authors concluded that dietary combinations are similar in potency to statin drugs in improving the lipid profile (Jenkins et al., 2005). A study by Behall et al. (2004) used whole-grain foods containing 0, 3, or 6g beta-glucan/day from barley, a rich source of soluble fiber, for 5 weeks, in a hyperlipidemic population of men, pre- and postmenopausal women. The mean LDL-C was significantly lower when the diet contained 3 or 6 g beta-glucan/day from barley than when it contained no beta-glucan and the greatest change was observed in men and postmenopausal women (Behall et al., 2004).

The Strong Heart Dietary Study compared diets of 10 Native American tribes in Arizona, Oklahoma, North and South Dakota to examine the possible contribution of diet to cardiovascular and other chronic diseases. Participants reported diets higher in fats and cholesterol which were comparable to high levels of cholesterol observed in this population (Zephier et al., 1997). The American Dietetic Association (2002) recommends 20-35 g/day of fiber for healthy adults and also states that these recommendations are not being met, because intakes of good sources of dietary fiber, fruits, vegetables, whole and high-fiber grain products, and legumes are low both in general and Native American population (Marlett et al., 2002).

Dietary intervention programs are necessary to educate people about dietary modifications to reduce the risk of cardiovascular and other nutrition-related disorders. A diet adequate in fiber-containing foods is also usually rich in micronutrients and nonnutritive ingredients that have additional health benefits. By means of increasing variety in the daily food pattern, the dietetics professional can help most healthy adults achieve adequate dietary fiber intakes (Marlett et al., 2002).

Omega-3 fatty acids

Omega (n)-3 fatty acids are polyunsaturated fatty acid and are classified as essential fatty acids as they cannot be synthesized by the body. The term "omega-3" signifies that the first double bond in the carbon backbone of the fatty acid, from the carboxyl end, occurs in the third carbon-carbon bond. Like all polyunsaturated fatty acids, the n-3 fatty acids have a minimum of 2 and a maximum of 6 double bonds in a carbon chain that ranges from 18 to 22 carbon atoms. Common n-3 fatty acids in the body are alpha-linolenic acid (ALA) (18:3), eicosapentaenoic acid (EPA) (20:5), and docosahexaenoic acid (22:6) (DHA). Arachidonic acid, an n-6 fatty acid (C20:4), is one of the most important precursors of inflammatory prostaglandins. Since the metabolism of n-3 and n-6 fatty acids shares common enzymes (elongase and desaturase enzymes), it is widely accepted that both types of polyunsaturated fatty acids behave as competitive substrates (Thompson and Cunnane, 2003). EPA supplementation inhibits or attenuates the pro-inflammatory cascade that follows on the enzymatic release of free arachidonic acid from the cell membrane (Li et al., 2005).

A study by Dewailly et al (2002) on the James Cree Bay adult population (18-74 years) who traditionally follow a high fish intake diet (~ 60 g/day) had relative

concentrations of 0.65% EPA and 2.80% DHA. These levels were positively associated with plasma HDL-C levels and inversely associated with triacylglycerols (Dewailly et al., 2002). Docosahexaenoic acid supplementation, 1.2 g/d for 6 weeks, has been reported to restore endothelial-dependent flow-mediated dilation in hyperlipidemic children suggesting that the endothelium must be site of action for DHA (Engler et al., 2004).

The dietary consumption of nuts such as walnuts has been studied for their cholesterol lowering properties. Walnuts are a rich source of both antioxidants and ALA. In an 8-week crossover feeding trial conducted by Ros et al. (2004) on subjects aged 20-75 years, with moderate hypercholesterolemia (serum LDL cholesterol ≥ 3.36 mmol/L or 130 mg/dL and, triglycerides ≤ 2.82 mmol/L), substitution of walnuts for $\sim 32\%$ of the energy from monounsaturated fatty acid in a cholesterol-lowering Mediterranean diet improved vascular endothelial function and also improved lipid profile. The walnut diet significantly reduced total cholesterol from 6.93 ± 0.70 mmol/L to 6.43 ± 0.69 mmol/L ($-4.4 \pm 7.4\%$) and LDL-C from 4.75 ± 0.62 mmol/L to 4.33 ± 0.47 mmol/L ($-6.4 \pm 10.0\%$) (Ros et al., 2004). The walnut allowances used in the study provided daily amounts of ALA ranging from 3.7 to 6.0 g. Apart from walnut, almonds, olives and flaxseed are also good plant sources of n-3 fatty acids (Thompson and Cunnane, 2003). Good health requires a ratio of 2:1 for omega-3 to omega-6 fatty acid where as the average American diet has an average ratio of 1:20 to 1:50. Flaxseed has a ratio of 4:1 for omega-3 to omega-6 fatty acid and studies have shown this ratio to be ideal for cholesterol lowering and in turn reducing the risk factors for CVD (Thompson and Cunnane, 2003). Since flaxseed is rich source of n-3 fatty acids, it should increase the ratio between n-3 versus

n-6 fatty acid intakes which would be considered a positive influence on maintaining CVD health.

Phytoestrogens

Phytoestrogens are naturally occurring plant-derived phytochemicals. Their common biological role is to act as a part of a plant's defense mechanism. Structurally they are similar to endogenous steroidal estrogens and thus have the ability to bind to estrogen receptors and elicit hormonal responses in certain mammalian tissues. They have both agonist and antagonist estrogenic effects. Phytoestrogens include mainly isoflavones (IF), coumestans, and lignans (Anderson and Garner, 1997). These compounds are known to be present in fruits, vegetables, and whole grains commonly consumed by humans. Isoflavones are found in legumes, mainly soybeans, whereas flaxseed is a major source of lignans, and coumestans are significantly present in clover, alfalfa and soybean sprouts.

Phytoestrogens exist in plants as glucose conjugates, or glycones. These glucose molecules must be removed before phytoestrogens can be absorbed in the gastrointestinal tract (Anderson and Garner, 1997). In the jejunum glucosidases catalyze the hydrolysis reaction to produce aglycones (Setchell and Cassidy, 1999). These aglycones are further metabolized by intestinal bacteria to more estrogenically potent compounds. Production of these metabolites varies greatly between individuals depending on their gastrointestinal bacterial flora, antibiotic use and constituents of diet (Setchell and Cassidy, 1999; Anderson and Garner, 1997). These aglycones and their bacterial metabolites are absorbed and distributed throughout the body as fat soluble molecules

and also through entero-hepatic circulation. Phytoestrogen metabolites are stored in liver and adipocytes and excreted in urine (Anderson and Garner, 1997).

Phytoestrogens are structurally similar to estrogen hormone and hence bind competitively with estrogen receptors specifically to estrogen receptor beta (ER β). This receptor is found in certain mammalian tissue such as breast, uterus, prostate, bladder, brain and bone (Setchell and Cassidy, 1999).

Studies have reported decrease in postmenopausal symptoms such as hot flashes, mood swings and also decrease in osteoporosis and breast cancer incidence after phytoestrogen intake (Setchell and Cassidy, 1999). A probable difference in the results obtained in clinical trials is attributed to variability in phytoestrogen metabolism in study participants (Setchell and Cassidy, 1999).

Flaxseed

Flaxseed is one of the oldest known annual crops, and is found as both a cultivated and semi-wild plant throughout temperate and tropical regions as well as Canada and Dakotas (Thompson and Cunnane, 2003). Flaxseed was traditionally used for the production of industrial linseed but not for edible grain. However, the use of flaxseed has been gaining popularity because of its potential health benefits. The four major components of flaxseed are fiber, oil, protein, and lignan precursor (Thompson and Cunnane, 2003).

Flaxseed provides high amounts of dietary fibers, 28% present by weight, of which 75% is insoluble and 25% is viscous fiber, called mucilage (Thompson and Cunnane, 2003). The outer coat of the seed contains both viscous soluble and insoluble fiber. Viscous fiber has more potential to reduce cholesterol (Arjmandi et al., 1992b) than

insoluble fibers, as soluble fiber are fermented by intestinal bacteria to form short chain fatty acids (SCFA). These SCFA inhibit hepatic cholesterol and fatty acid synthesis (Arjmandi et al., 1992b).

The content of oil by weight in flaxseed is approximately 41%, and the most abundant fatty acid is n-3 fatty acid ALA (i.e. 57%) (Thompson and Cunnane, 2003) which has a main action of preventing platelet aggregation (Yao et al., 2004; Allman et al., 1995). Alpha-linolenic acid has also been shown to reduce coronary heart disease (CHD) probably due to its beneficial effect on serum lipid profile (Ander et al., 2004).

Flaxseed is the richest sources of the lignan precursor's secoisolariceresinol diglycoside (SDG) and materesinol, which are converted to enterolactone and enterodiol, respectively. These lignan precursors possess potential antioxidant activity. Lignans are diphenolic compounds having a 2, 3-dibenzylbutane structure and have been shown to have cholesterol lowering properties and weak estrogenic activities. Their cholesterol lowering properties are thought to be due to modulating activities of enzymes 7α -hydroxylase and acyl Co-A cholesterol transferase (Arjmandi et al., 1998). A part of their cardioprotective effects has been proposed to result from their ability to protect LDL from oxidation and prevent the production of the more atherogenic particles (van der Schouw et al., 2002). These lignan precursors are phytoestrogens, structurally similar to estrogens (Branca and Lorenzetti et al., 2005). They are thought to have an estrogenic effect on lipid metabolism and are also being studied for their possible anti-cancer action, especially breast cancer (Bylund et al., 2005; Dabrosin et al., 2002; Thompson et al., 1996).

It is not clear whether the hypocholesterolemic actions of flaxseed are due to consumption of whole flaxseed or due to its individual components, such as lignans, fiber, and omega-3 fatty acid. There are a number of studies that support both views. For instance studies by Lucas et al. (2004), Yao et al.(2004), Lucas et al.(2002), Nestel PJ (2002) have demonstrated that whole flaxseed reduces the risk of CVD while a study by Prasad (1999) demonstrated secoisolariciresinol diglucoside (SDG), isolated from flaxseed, reduced atherosclerotic plaque as well as lowered total cholesterol and LDL-cholesterol concentrations in rabbits. Fibers both soluble and insoluble have been shown to reduce total cholesterol in various animal (Li et al., 2004; Arjmandi et al., 1997; Arjmandi et al., 1992) and human studies (Jenkins et al., 2005; Behall et al., 2004; Arjmandi et al., 1998).

Flaxseed has been shown to lower cholesterol in different population groups from healthy young adults (Cunnane et al., 1995), men both young and elderly (Stuglin and Prasad, 2005; Demark-Wahnefried et al., 2004) and postmenopausal women (Dodin et al., 2005; Lucas et al., 2002; Jenkins et al., 1999). Daily consumption of 50 g of flax seed with high-linolenic acid content has been shown to be safe and potentially beneficial by raising n-3 fatty acids in plasma and erythrocytes (Jenkins et al., 1999).

The n-3 fatty acid component of flaxseed has also been shown to have a major role in cholesterol metabolism (Gamez et al., 2005; Zhao et al., 2004). Therefore in part, the antiatherogenic property of flaxseed maybe due to its n-3 fatty acid content (Lucas et al., 2002; Lucas et al., 2004). Flaxseed has also been reported to improve arterial compliance in obese subjects (Nestel et.al., 1997) and reduce Lp (a) concentrations in

postmenopausal women (Arjmandi et al., 1998). In a study done by Allman et al. (1995) the platelet aggregation decreased in hyperlipidemic patients fed flaxseed.

Thus incorporation of flaxseed in the daily diet can improve lipid profile and reduce risk factors for heart disease, especially by improving lipid profile in both the general and Native American populations.

CHAPTER III

MATERIALS AND METHODS

Subject Recruitment

A total of fifty-five mild to moderate hypercholesterolemic (total cholesterol level $\geq 5.1 \leq 9.8$ mmol/L) Native American postmenopausal women between age 47 to 63 yrs of age who were not on hormone replacement therapy (HRT), for at least six months prior to study, were enrolled for this study. The study protocol was approved by the Institutional Review Board (IRB) at Oklahoma State University. Volunteers were accepted into the study by meeting the following criteria: being postmenopausal as determined by at least 1 year of amenorrhea, total cholesterol levels of more than 200 mg/dL (5.1 mmol/L) but less than 380 mg/dL (9.8 mmol/L), not taking any medications known to alter lipid metabolism, no history of hypo- and hyperthyroidism, liver or kidney diseases. Potential candidates were recruited from health fairs conducted by Native American tribes and advertisement at large. Participants were provided with a verbal and written explanation of the study. They were assured that their participation in the study was completely voluntary and their information would be kept confidential. After signing the consent form approved by the IRB, detailed medical and diet histories were obtained.

Experimental Design

The experimental design was a randomized controlled design. Eligible subjects were randomly assigned to one of three treatment groups (n=20 per treatment group): 1) control regimens, 2) flaxseed, and 3) flaxseed+fiber for a period of 3 months. Thirty grams of flaxseed was provided to each of the subjects in the treatment groups in the form of bread, muffins, and powder as part of their daily diet. The treatment regimens were distributed to the subjects on a biweekly basis. The participants in the flaxseed and flaxseed+fiber treatment groups were asked to consume 2 muffins (~10 g of flax), 2 slices of bread (~3 g of flax) and 2 tablespoon of flax powder (~16 g) per day, whereas the participants in control group consumed 2 oat muffins and 2 slices of bread per day. Additional ~ 8 g oat bran soluble fiber was added to the flaxseed+fiber treatment group because soluble fiber has already been shown by previous studies (Behall et al., 2004; Arjmandi et al., 1997) to reduce cholesterol levels. The flaxseed+fiber treatment regimen was included to determine if the combination of these two components will have synergistic or additive effect in improving lipid profile compared to flaxseed alone. The treatment regimens provided similar amounts of calories, and protein content. Participants were advised to adjust their daily food intake in order to maintain their caloric intake. Composition of the study products is presented in Table 1.

Dietary assessment and anthropometric measurement

Height, weight, hip and waist circumferences were measured and the waist to hip ratio was calculated at baseline and at the end of the study. The total body fat, and lean mass content was measured using bioelectric impedance (Biodynamic Model 310e, Biodynamics corp., Seattle) which measures the impedance to the electric current through the body. The higher the impedance, higher the body fat (Stang and Story, 2004). With the exception of height, all other measurements were repeated at each monthly visit. If weight gain was observed during the course of the study, the participants were counseled regarding their daily food intake.

To ensure compliance, the following steps were taken 1) a monthly calendar was given to the participants to record their intake of regimens daily; 2) participants were asked to return unused dietary regimens and were given new supplies and a new calendar during their monthly visits; 3) a bi-monthly 24 hr food recall over the phone was done on a random basis to check for compliance; 4) after three months of treatment blood draw, anthropometric measurements, physical activity and dietary assessment was repeated. An additional 7-day physical activity questionnaire enquiring about the activities of the participants was also completed.

Blood collection and processing

Fasting venous blood samples, approximately 20 mL, were obtained from each participant in EDTA and non-EDTA vacutainer tubes at the beginning and at the end of study. All blood samples were placed on ice until processing. Plasma and serum were separated by centrifuging the samples at $2500\times g$ for 20 minutes at 4 °C. Serum and

plasma were aliquoted and stored at -80 °C until analyses. At the end of the study all samples were run together to minimize variability.

Serum analyses

Total cholesterol, HDL-C, triglycerides, LDL-C, apolipoprotein A-I, apolipoprotein B, and C-reactive protein levels from serum were analyzed. All lipid and other clinical parameters such as albumin (ALB), alkaline phosphates (ALP), blood urea nitrogen (BUN), calcium, creatinine, magnesium, phosphorus, total protein, excluding LDL-C, were measured using ACE Clinical Analyzer (Monclair, NJ). The clinical analyzer was calibrated using Gemcal reference serum (Alfa Wassermann, Inc; West Caldwell, NJ) before each test. Alfa Wassermann quality control (QC)-1 Normal Chemistry Control and QC-2 Abnormal Chemistry Control were used as control in all tests.

The reagent used for measuring total cholesterol concentrations in serum contains cholesterol esterase, which releases cholesterol from its esters. This released cholesterol and endogenous free cholesterol are both oxidized by cholesterol esterase to produce hydrogen peroxide. A red colored quinoneimine complex is produced when hydrogen peroxide (H_2O_2) is combined with 4-aminoantipyrine (AAP) and p-hydroxybenzoic acid, which is measured photometrically at 505 nm and is directly proportional to cholesterol concentration. In our assays, intra and inter assay coefficient of variation (CV) were 1.9 and 1.3 percent for total cholesterol, respectively.

The triglyceride reagent works similarly to cholesterol assay. The triglycerides are converted to produce H_2O_2 through a series of enzymatic reactions and this reacts with p-chlorophenol and AAP in a reaction catalyzed by peroxidase to produce a red colored

quinoneimine complex which is absorbed strongly at 505 nm to a level directly proportional to the triglyceride concentration. The intra and inter test assay CV were 1.3 and 1.9%, respectively, for triglyceride.

The HDL-C assay utilizes two reagents, the second containing a unique detergent. This detergent solubilizes only the HDL lipoprotein particles, thus releasing HDL cholesterol to react with cholesterol esterase and cholesterol oxidase, in the presence of a chromogen to produce color. The detergent also inhibits the reaction of the cholesterol enzymes with LDL, VLDL and chylomicron lipoproteins by adsorbing to their surfaces, the amount of chromogen formed, determined by measuring the increase in absorbance bichromatically at 592/692 nm, is directly proportional to the HDL-C concentration. The intra and inter test assay CV were 2.6% and 3.8%, respectively, for HDL-C.

The Apo-A and Apo-B reagents contain antiserum which forms a precipitate with Apo-A or Apo-B and the turbidity is measured at 340 nm. The intra and inter test assay CV for Apo-A were 1.6 and 5.1 respectively. The intra and inter test assay CV were 2.5 and 2.6%, respectively, for Apo-B.

Lipoprotein-A containing sample mixes with the anti-serum reagent and forms a turbid antigen-antibody complex. After an incubation period of 10 minutes the turbidity is measured at 540 nm. The intra test precision assay was 4.0 and inter test precision assay was 6.8.

C-reactive protein in the sample reacts with the anti-CRP sensitized latex particles in the hsCRP antibody reagent to form antigen-antibody complexes that agglutinate the latex particles. The increase in absorbance, measured monochromatically at 592 nm, during a fixed time interval, is directly proportional to the amount of CRP in the sample.

LDL-C was calculated using the Friedewald equation: (Friedewald et al., 1972)

$$\text{LDL} = (\text{TC}) - (\text{HDL}) - (\text{TG}/5)$$

The intra test precision assay was 1.8 and inter test precision assay was 3.6.

Hematological analyses

Overnight fasting venous blood was collected in vacutainer tubes with EDTA as anti-coagulant (Fischer Scientific, St. Louis, MO) at the beginning and end of the study. Samples were analyzed for white blood cells (WBC), red blood cells (RBC), percentage and actual number of differential white blood cells (i.e., lymphocytes: LYM, monocytes: MON, neutrophils: NEU, eosinophils: EOS, basophils: BAS), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width index (RDW), platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW) by an automated combined impedance-light focusing hematology counter (Pentra 120 Retic Hematology Instrument, ABX Diagnostics, Irvine, CA).

Hormonal analyses

To confirm the postmenopausal status of the subjects, serum follicle stimulating hormone (FSH) and 17 β -estradiol (E₂) levels were measured at the initial and at end of the treatment period. Ultra-sensitive estradiol radioimmunoassay (RIA) kit and FSH immunoradiometric assay (IRMA) kits from Diagnostic System Laboratories were used for the analyses.

The estradiol test followed the basic principle of radioimmunoassay where there is competition between a radioactive and a non-radioactive antigen for affixed number of antibody binding sites. The amount of [I-125]-labeled estradiol bound to the antibody is inversely proportional to the concentration of the estradiol present in the sample.

The FSH immunoradiometric assay is a non-competitive assay in which the analyte to be measured is "sandwiched" between two antibodies. The first antibody is immobilized to the inside walls of the tubes. The other antibody is radio labeled for detection. The analyte present in the samples, Standards and Controls is bound by both of the antibodies to form a "sandwich" complex. Unbound materials are removed by decanting and washing the tubes.

Data management and statistical analysis

All data obtained about the subjects were kept in lock cabinets with limited accessibility. Upon completion of the study the data from the serum analyses and questionnaires were entered into spreadsheets.

The experiment was a completely randomized design with repeated measures. Treatment is the main plot factor and was applied to the subject, while baseline and final were the repeated measures factor. Results are expressed as mean \pm SE. The significance of percentage differences between and within treatments was assessed with Student's *t*-test for paired data (two-tailed). Data were analyzed using PC SAS version 8.0 (SAS Inst., Cary, NC) using PROC GLM MIXED. The primary outcome variables were the change from baseline in total cholesterol, HDL-C, triglycerides, LDL-C, C-reactive protein. Analysis of variance technique was used to assess treatment (flaxseed vs.

flaxseed with additional fiber vs. control) differences. Significant differences were determined using alpha level as 0.05.

CHAPTER IV

RESULTS

Subject participation

Out of the 55 participants recruited, 13 dropped out of the study. Nine women completed the control regimen, 17 completed the flaxseed regimen and 16 completed flaxseed + additional fiber regimen. One woman dropped out of flaxseed treatment group complaining of gastrointestinal discomfort. Subjects complaining of gastrointestinal problem and unpalatability of study food were given suggestions to incorporate the study food into their diet to reduce these problems. Four of the participants from the control group relocated and hence could not continue with the study. Eight subjects, four from control regimen, and two each from the flaxseed treatment groups did not give any reason for dropping out of the study.

Nutrient intake

The baseline daily nutrient intake was determined using a food frequency questionnaire approved by the National Institutes of Health (NIH). There were no differences in the baseline caloric intake, protein, CHO, fiber, total fat, saturated fat, polyunsaturated fat and calcium intake (Table. 2) among the groups. The nutrient intake of the subjects during the study was monitored by a 24-hr food recall conducted over the phone. There was no change in caloric intake, CHO and polyunsaturated fat

intake among the groups while they were consuming the study regimen (Table. 3). There were a tendencies for a increases protein ($P=0.074$), total fat ($P=0.062$), and saturated fat ($P=0.099$) intakes in the flaxseed groups. Dietary fiber and calcium ($P=0.015$) intakes were significantly increased in the flaxseed group.

Anthropometric measurements

The results of the anthropometric measurement are shown in Table 4. Age range for the study participants was 47 to 62 yrs of age. There were no significant changes in body weight after 3 months of consuming the dietary regimens. However, body weight of subject in the control group tended ($P=0.066$) to decrease after 3 months on the study regimen. The body mass index (BMI) did not change among the subjects in any treatment groups but as compared to the baseline values there was a decrease in weight in all the treatment groups. There was no significant change in the mean waist circumference in any of the groups. The hip circumference was decreased significantly from baseline in the flaxseed group ($P= 0.03$) while the other groups showed no change. The percent body fat increased significantly among women in the flaxseed with additional fiber treatment group.

Serum analyses

The lipid parameters are presented in Table 5 There was a significant drop of approximately 7% in the mean total cholesterol level (TC) after 3 month consumption of the flaxseed, and the flaxseed with additional fiber regimen. There was no change in total cholesterol for women in control regimen. Both flaxseed groups significantly reduced LDL-C by approximately 10%. HDL-C significantly decreased by 17% in the control group while there were no changes in both the flaxseed treatment groups. The triglyceride

levels increased by approximately 18% in the control and by 3% in the flaxseed group but it decreased by 8.5% in the flaxseed with additional fiber group but these changes were not significant. No significant change was seen in the Apo-A and Apo-B levels. The lipoprotein (a) levels tended to be lower in the flaxseed without additional treatment but not in the control or flaxseed plus fiber group.

Hormone levels and markers of inflammation are presented in Table 6. C-reactive protein (CRP) levels were not altered by any of the study regimen. Serum E₂ and FSH values were within range for postmenopausal status and neither of the treatment groups changed these hormone levels.

Results of other clinical parameters are presented in Table 7. A significant increase (P=0.009) in serum creatinine level was seen in the flaxseed with additional fiber treatment as compared to the baseline. The total protein (P=0.006) and calcium (P=0.039) levels showed significant decreases in only the flaxseed treatment group. The serum levels of other clinical parameters such as albumin, alkaline phosphatase, blood urea nitrogen, magnesium and phosphorus did not change.

Hematological analyses

The results of hematological analysis are presented in Table 8. No significant changes from the baseline levels were observed in the white blood corpuscles (WBC), red blood corpuscles (RBC) and the hemoglobin concentration (Hb) in either of the flaxseed group. A significant decrease was seen in the mean platelet volume (MPV) in the control group (P = 0.023) whereas in both the flax groups an increase in MPV was seen. The platelet distribution width (PDW) was reduced in the control group (P = 0.003) but an approximate increase of 2% and 1.5 % in PDW was seen in both the flaxseed and flax

with additional fiber groups. A significant increase ($P = 0.022$) was seen in the percentage of monocytes in only the flaxseed group. No significant change was observed in neutrophil, lymphocyte and basophil percentage values. The other hematological parameters such as hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width and platelet counts were not affected by any of the treatments.

CHAPTER V

DISCUSSION

The findings of the present study indicate that daily consumption of approximately 30 g of whole ground flaxseed incorporated into bread, muffins, and energy drink lowers total cholesterol in postmenopausal Native American women. These findings are consistent with our earlier observations (Lucas et al., 2002; Arjmandi et al., 1998) in which similar amounts of flaxseed lowered total cholesterol in Caucasian postmenopausal population. In the present study, total cholesterol and LDL-C were decreased by approximately 7% and 10%, respectively in both groups that received flaxseed and flaxseed with added fiber. The cholesterol lowering effect of flaxseed was more pronounced in the present study when compared to our earlier findings (Lucas et al., 2002) in which total cholesterol levels were lowered by 4.7%. In that study, postmenopausal women consumed whole ground flaxseed rather than in the form of baked products. According to Thompson and Cunnane report (2003), the cholesterol lowering effects of flaxseed may be enhanced by baking, perhaps due to the influence of high temperature on bioavailability of some of the flaxseed phytochemicals. Therefore, the better results seen in this study may be due to the intake of whole flaxseed incorporated into bread and muffins, rather than consumption of raw flaxseed.

In a study by Jenkins et al. (1999), though higher amounts of flaxseed were used, 50g vs 30g, serum LDL-C was higher than in the present study. The differences in findings may have been due to the use of defatted flaxseed rather than whole ground flaxseed and the inclusion of both men and women in the study by Jenkins et al. (1999). The LDL-C lowering role of flaxseed is thought to be due to the up-regulation of LDL receptors and enhanced lipoprotein lipase activity (Thompson and Cunnane, 2003). In addition to the positive effects of flaxseed in lowering total and LDL-cholesterol, flaxseed was able to maintain HDL cholesterol levels. The control regimen on the other hand, elevated total cholesterol with an accompanying decrease in HDL cholesterol. Though we cannot offer an explanation for these observations, it should be pointed out that the sample size in this group was nearly half of the other two treatment groups, which may have influenced the results. The findings of study by Stuglin and Prasad (2005) are also comparable to our observations. They reported that while flaxseed did not alter serum HDL-C concentration in healthy adults, it decreased total cholesterol levels.

Since the LDL-C decreased in the present study a comparable decrease in serum levels of LDL-C carrier protein, Apo-B was anticipated. Our previous study (Lucas et al., 2002) had reported a significant decrease of 7.5% in the Apo-B consistent with decrease in serum LDL-C concentrations. We cannot offer an explanation at this time as to why Apo-B levels did not decrease as anticipated.

Although not significant, we observe a numerical increase in triglyceride concentrations after flaxseed supplementation which was decreased in the flaxseed with additional fiber group. This finding may be due the effect of 8 g/d additional fiber intake in flaxseed with additional fiber group. Stuglin and colleagues (2005) have reported

increase in triglyceride levels in men who consumed 32.7 g of flaxseed for 4 weeks. Animal studies (Lucas et al., 2004; Prasad, 1997) have also reported a high triglyceride levels in flaxseed fed hamsters. These findings imply that n-3 fatty from flaxseed, unlike that of fish oil, is ineffective in lowering triglyceride levels in humans (Dewailly et al., 2002).

Flaxseed has been reported to reduce Lp (a), a strong independent predictor of atherosclerosis. In our previous study, (Arjmandi et al., 1998), we have reported a 7.4% decrease in Lp (a) as compared to the baseline, in hypercholesterolemic (5.85-9.05 mmol/L) postmenopausal women on 38 g/day flaxseed supplements. Similarly, in the present study, we report a decrease of approximately 22% in the Lp (a) levels in the flaxseed treatment group, albeit not significantly. However, we did not find such an effect when postmenopausal women were provided with ground flaxseed as reported by Lucas et al. (2002). The data in the current study provided further support that heat processing of flaxseed does not affect the active components of flaxseed (Thompson and Cunnane, 2003). Lipoprotein (a) lowering effect of flaxseed is probably due to activity of omega-3 component of flaxseed, especially EPA which has been shown to reduce Lp (a) concentration (Shinozaki et al., 1996) though other components such as fiber and non-protein constituents present in these seeds may also be responsible.

Incorporation of daily flaxseed into the diet of Native American postmenopausal women had no effect on serum levels of CRP, a marker of inflammation. These finding indicate that neither whole flaxseed nor its components such as lignans and n-3 fatty acids have any effect on this marker. The lack of effect of flaxseed on CRP could be due to the fact that participants in this study had no overt inflammation or CRP may not be a

sensitive marker. Based on the CRP finding alone in this study, it can be suggested that flaxseed or its components may not have measurable anti-inflammatory effect in Native American postmenopausal women.

Concerns related to ipriflavone, a synthetic phytoestrogen that it causes lymphocytopenia (Alexandersen et al., 2001) raised the issue of safety with naturally occurring phytoestrogens, such as those found in flaxseed. Flaxseed has become a popular supplement among postmenopausal women because of their potential health benefits, e.g. cholesterol lowering (Dodin et al., 2005; Lucas et al., 2002; Arjmandi et al., 1998) and its anticancer effects (Bylund et al., 2005; Dabrosin et al., 2002; Thompson et al., 1996). Therefore, it was necessary to determine if consumption of flaxseed had any deleterious effects on hematological parameters including total and differential leukocyte counts using whole blood. Our findings indicate that flaxseed has no effect on the hematological parameters such as white blood corpuscle, red blood corpuscles and hemoglobin concentrations during the course of the study. Nonetheless, there was a significant increase in the monocyte percentage in the flaxseed treatment group. Monocyte count is a better independent predictor of atherosclerosis and plaque formation than IL-6, CRP, fibrinogen, and WBC (Chapman et al., 2004). Monocytes lead to a higher level of tissue macrophages, which then results in more uptake of cholesterol from plasma to form foam cells (Chapman et al., 2004). Nonetheless, the effect of flaxseed treatment on monocyte adherence or activity, rather than count, is important as atherosclerosis is more associated with enhanced monocyte adherence (Huang et al., 1996). A recent study done by Horne and colleagues (2005) indicates that apart from monocyte count, high neutrophil ($> 6.6 \times 10^3 \mu\text{l/L}$) and low lymphocyte counts are independent risk factors for cardiovascular

disease. However, in our study no significant changes were seen in average neutrophil or lymphocyte cell count. Another animal study by Prasad (2005) which specifically looked at the chronic intake of the lignanic component of flaxseed on the hematopoietic system, found no adverse effect on any of the immunological parameters and hemoglobin concentrations. In our study, there was a decrease in the hemoglobin concentration in the flaxseed with additional fiber group probably due to excess fiber intake which can impair the iron absorption in the gastrointestinal tract.

In the present study, flaxseed did not alter estrogen and follicle stimulating hormone levels, suggesting that flaxseed or its lignanic compounds lack any estrogenic property. This effect is similar to our previous studies (Lucas et al., 2002; Arjmandi et al., 1998) which also reported lack of effect of flaxseed treatment on hormone levels. Results concerning the effects of phytoestrogen supplementation on serum hormones are conflicting. Although the present study reports no change in serum hormone concentrations, another study reported that consumption of 5 or 10 g ground flaxseed/day for 7 wk significantly reduced serum estradiol concentrations in postmenopausal women (Hutchins et. al., 2001). This observation (Hutchins et. al., 2001) was linked to the structural similarities of flaxseed phytoestrogen final metabolites, enterolactone and enterodiol, to that of estrogen, which might interfere with estrogen metabolism.

Similar to a number of flaxseed studies (Dodin et al., 2005; Brooks et al., 2004; Lucas et al., 2002), the mean body weight remained unchanged after three months consumption of the dietary regimens. This is perhaps due to feeling of satiety (Dodin et al., 2005). In the present study, flaxseed had no effect on other clinical parameters such as serum albumin, alkaline phosphatase, blood urea nitrogen. A significant decrease,

however, was seen in the serum calcium and total protein in the flaxseed treatment group. These observations were unexpected and we cannot offer an explanation for these findings.

In summary, the results of the present study suggest that dietary changes, like incorporating approximately 30 g of flaxseed into the daily diet of Native American postmenopausal women, is effective in lowering total and LDL-C concentrations, thus providing them with a safe and relative inexpensive alternative for reducing cholesterol and the risk of cardiovascular disease.

Table 1:- Nutrition facts of the study products

	Bread			Muffin			Powder	
Treatment	A	B	C	A	B	C	B	C
Serving size	1 slice			1 piece			2 Tbsp	
Energy (kcal)	70	80	70	150	160	130	55	
Flaxseed (g)	-	1.5	1.5	-	5	5	16	
Carbs (g)	12	16	7	25	22	21	9	
Protein (g)	2	3	8	5	4	4	5	
Fat (g)	1	1	4	4.5	6	4.5	3	
Fiber (g)	0.5	3	4	1	2	5	6	

A= Control treatment group, B= Flaxseed treatment group, C= Flaxseed + fiber treatment group.
Values are as mentioned on the product labels provided by Natural oven bakery, Manitowic, WI.

Table 2:- Baseline food intake measured with food frequency questionnaire

Measures	Control (A)	Flaxseed (B)	Flaxseed with additional fiber (C)	P Value
Total energy (kcal)	1123± 104	1683± 300	1784 ±684	0.541
<i>Nutrients (g)</i>				
Protein	47 ±14	65± 47	59± 25	0.718
Carbohydrates	136± 42	195±106	229±91	0.285
Dietary fiber	13 ±2	18 ±13	16 ±6	0.658
Total fat	45±16	77± 67	74± 31	0.613
Saturated fat	11± 3	23±17	23±11	0.399
Polyunsaturated fat	10± 2	17± 16	17± 8	0.685
<i>Minerals (mg)</i>				
Calcium	366 ±129	661± 417	648± 449	0.529

Data represent least square mean \pm SE. Differences were considered significant at *P* value <0.05.

Table 3:- Food intake during the study by 24hr Food recall

Measures	Control (A)	Flaxseed (B)	Flaxseed with additional fiber (C)	P Value
Total energy (kcal)	1511± 193	1994± 161	1967± 161	0.195
<i>Nutrients (g)</i>				
Protein	46± 8	67± 5	69± 6	0.074
Carbohydrates	195± 32	252± 26	218± 19	0.325
Dietary fiber	12± 5 ^c	22± 8 ^b	30± 10 ^a	<0.0001
Total fat	49± 27	78± 24	71± 28	0.062
Saturated fat	12± 3	20 ±2	20± 3	0.099
Polyunsaturated fat	5± 2	8± 1	10± 2	0.230
<i>Minerals (mg)</i>				
Calcium	476± 72 ^c	814 ±68 ^a	654± 66 ^b	0.015

Data represent least square mean ± SD. Differences were considered significant at P value <0.05.

Table 4:-Subject characteristics and Anthropometric measurements

	Control (A)			Flaxseed(B)			Flaxseed+Fiber (C)		
	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value
n	17	9	-	20	17	-	18	16	
Age, yrs	50.8 ±3.1	-	-	57.0±2.2	-	-	60.4±2.5	-	-
Weight, kg	75.0±5.5	72.1±7.0	0.06	82± 3.6	81.5 ±4.3	0.61	79.1±4.2	78.5 ±4.0	0.51
BMI	28.9±1.9	28.1±1.9	0.17	30.8±1.4	31.1±1.4	0.42	30.8±1.4	30.5±1.4	0.39
Hip*, inches	42.4 ±1.2	43.3±1.4	0.30	45.2±1.3 ^a	43.8±1.5 ^b	0.03	43.5±1.2	42.7 ±1.2	0.16
Waist*, inches	36.7± 2.3	36.3± 2.6	0.62	39.8± 1.4 ^a	38.4±1.5 ^b	0.01	39.3 ±1.1	39.1 ±1.1	0.60
Waist/ hip ratio	0.86	0.83	0.47	0.88	0.88	0.45	0.95	0.91	0.38
Body Fat*, %	38.2 ±1.6	35.7 ±2.5	0.20	39.9 ±1.2	39.1 ±1.5	0.56	40.1± 1.4 ^b	43.3± 1.5 ^a	0.02
Fatbody wt	29.2 ±2.3	26.5± 2.6	0.10	33.5 ±1.45	32.7 ±1.5	0.50	32.3 ±1.1	34.2 ±1.1	0.12
Lean Body Wt	45.7± 2.7	45.8 ±3.0	0.93	48.8 ±1.7	49.0 ±2.0	0.77	45.6± 2.1	44.9 ±2.6	0.31

Data represent least square mean ± SE. Differences were considered significant at *P* value <0.05. In each group, values that do not share the same superscript letters are significantly different. BMI=Body mass index.

Table 5:- Effects of three-month flaxseed supplementation on serum lipid parameters in postmenopausal women

	Control (A)				Flaxseed (B)				Flaxseed+Fiber (C)			
	Baseline	Final	<i>P</i> Value	% Change from baseline	Baseline	Final	<i>P</i> Value	% Change from baseline	Baseline	Final	<i>P</i> Value	% Change from baseline
TC*, <i>mg/dL</i>	213.8 ± 5.8	218.2 ± 7.9	0.63	+2.0	231.4 ± 8.6 ^a	215.7 ± 5.7 ^b	0.02	-7.3	231.5 ± 10.1 ^a	215.3 ± 9.1 ^b	0.02	-7.5
LDL-C*, <i>mg/dL</i>	134.6 ± 6.6	141.2 ± 5.02	0.36	+4.6	150.2 ± 8.2 ^a	135.8 ± 5.7 ^b	0.009	-10.6	154 ± 8.4 ^a	139.5 ± 6.9 ^b	0.01	-10.4
HDL-C*, <i>mg/dL</i>	47.2 ± 4.9 ^a	40.3 ± 4.5 ^b	0.007	-17.1	47.8 ± 3.4	45.5 ± 3.4	0.20	-5.0	47.6 ± 3.1	46.6 ± 3.4	0.61	-2.1
TG, <i>mg/dL</i>	154.2 ± 29.3	183.1 ± 40.3	0.12	+15.7	166.1 ± 17.6	171.4 ± 13.9	0.69	+3.1	152.1 ± 13.8	145.5 ± 14.8	0.63	-4.5
Apo A, <i>g/L</i>	523.7 ± 170	478.3 ± 154	0.60	-9.5	647.8 ± 141	713.9 ± 161	0.30	+9.3	449.4 ± 151	378.4 ± 127	0.28	-18.7
Apo B, <i>g/L</i>	409 ± 136	426.3 ± 140	0.82	+4.1	541.9 ± 108.3	539.8 ± 113	0.97	-0.39	430.2 ± 141	340.2 ± 92	0.13	-26.4
Lp (a)	22.5 ± 9.2	22.3 ± 8.3	0.89	-0.9	9.2 ± 3.1	7.2 ± 2.7	0.08	-22	6.4 ± 2.2	6.0 ± 1.8	0.72	-6.6

Data represent least square mean ± SE. Differences were considered significant at *P* value <0.05. In each group, values that do not share the same superscript letters are significantly different; n= 9 for control group, n= 17 for flaxseed group, n= 16 for flaxseed with additional fiber group. TC= Total cholesterol, LDL-C= low density lipoprotein cholesterol, HDL-C= high density lipoprotein cholesterol, TG= triglyceride, Apo= Apolipoprotein, Lp (a) = lipoprotein a.

Table 6:- Effects of three-month flaxseed supplementation on 17 β -estradiol, Follicle stimulating hormone, and C-reactive protein concentration in postmenopausal Native American women

	Control(A)			Flaxseed(B)			Flaxseed+Fiber(C)		
	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value
<i>Hormone</i>									
<i>E₂, pg/ml</i>	27.9± 6.4	38.5±12.4	0.29	22.8± 4.4	20.8± 3.8	0.79	17.2± 4.7	11.8± 0.6	0.52
<i>FSH, mIU/ml</i>	20.2± 4.2	25.8± 1.7	0.15	30.2 ±7.4	31± 7.5	0.77	20.5± 2.6	17.3 ±2.3	0.27
<i>Inflammation</i>									
<i>CRP, mg/L</i>	0.36 ± 0.1	0.23± 0.08	0.06	0.38± 0.07	0.37±0.06	0.81	0.24±0.05	0.18±0.04	0.27

Data represent least square mean \pm SE. Differences were considered significant at *P* value <0.05. In each group, values that do not share the same superscript letters are significantly different; n= 9 for control group, n= 17 for flaxseed group, n= 16 for flaxseed with additional fiber group. E₂= estradiol, FSH= follicle stimulating hormone, CRP = C-reactive protein

Table 7:-Effects of flaxseed on other clinical parameters

	Control (A)			Flaxseed(B)			Flaxseed+Fiber (C)		
	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value
ALB, g/dL	4.2± 0.1	4.0 ± 0.1	0.10	4.0± 0.06	3.9± 0.05	0.09	4.1± 0.1	4.1 ± 0.09	0.60
ALP, U/L	105.7±8.1	102.7±10.1	0.53	137.6±42.6	131.1 ±40.3	0.07	103.3±5.4	97.5± 6.0	0.12
BUN, mg/dL	12.8 ± 2.0	12.7± 1.3	0.94	15.5± 1.4	15.4± 1.1	0.91	14.6± 0.8	15.3 ± 0.7	0.58
Calcium*, mg/dL	9.2 ± 0.1	9.1± 0.1	0.19	9.2± 0.1 ^a	9.0 ± 0.1 ^b	0.03	9.1± 0.1	9.1± 0.1	0.68
Creatinine*, mg/dL	0.7±0.02	0.7 ± 0.04	0.68	0.8± 0.02	0.8± 0.03	0.14	0.7±0.05 ^b	0.8 ± 0.06 ^a	0.009
Magnesium, mEq/L	2.0± 0.09	1.9± 0.05	0.57	2.1± 0.03	1.9± 0.04	0.19	1.9 ± 0.06	1.9 ± 0.02	0.53
Phosphorus, mg/dL	3.5 ± 0.2	3.3± 0.2	0.32	3.0 ± 0.1	3.0 ± 0.1	0.19	3.1± 0.1	2.9± 0.09	0.23
Totalprotein*, g/dL	7.4± 0.2	7.3± 0.2	0.44	7.4± 0.1 ^a	7.1 ± 0.07 ^b	0.006	7.3 ± 0.2	7.2 ± 0.4	0.23

Data represent least square mean ± SE. Differences were considered significant at *P* value <0.05. In each group, values that do not share the same superscript letters are significantly different; n= 9 for control group, n= 17 for flaxseed group, n= 16 for flaxseed with additional fiber group. ALB= albumin, ALP= alkaline phosphatase, BUN= blood urea nitrogen.

Table 8:-Effects of flaxseed on hematological parameters

	Control (A)			Flaxseed (B)			Flaxseed+Fiber (C)		
	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value
WBC									
(x 10 ⁹ /L)	7.2 ±1.1	7.1 ±0.7	0.91	6.0 ±0.3	6.7 ±0.4	0.35	6.9 ±0.6	5.8 ±0.4	0.08
RBC									
(x10 ¹² /L)	4.6± 0.08	4.8 ±0.3	0.60	4.6 ±0.3	4.6± 0.1	0.84	4.5± 0.2	4.3 ±0.1	0.65
Hb (g/dl)	14.4±0.4	14.5±0.7	0.94	14.3±0.8	14.1 ±0.4	0.80	15.1 ±0.7	13.7 ±0.4	0.05
Hct (%)	40.1± 0.8	42.0± 2.0	0.49	41.1± 2.2	39.9 ±1.1	0.58	42.0 ±1.9	38.4 ±1.3	0.08
MCV (flit)	87.1 ±1.2	88.4 ±1.4	0.38	87.9 ±0.9	87.3 ±0.8	0.58	87.5 ±1.0	89.4 ±0.9	0.06
MCH (pg)	31.3 ±1.2	31.7 ±0.6	0.74	30.6 ±0.3	30.9± 0.4	0.77	31.6 ±0.4	32.0 ±0.4	0.54
MCHC (%)	35.9± 0.7	35.6± 0.3	0.68	34.8 ±0.3	35.3±0.3	0.50	36.1± 0.4	35.8± 0.4	0.59
RDW (%)	15.0± 0.6	14.2± 0.3	0.24	13.3 ±0.2	14.2 ±0.3	0.10	13.7± 0.3	14.2± 0.2	0.29
PLT(x10 ⁹ /L)	241.4±29.5	291.3±43.4	0.23	275.0±21.2	259.5± 15.1	0.63	236.7±18.3	244.6 ±18.5	0.78
MPV*	9.6 ±0.2 ^a	8.7 ±0.3 ^b	0.02	8.9± 0.1	9.1 ±0.3	0.59	9.2 ±0.2	9.2 ±0.3	0.96
PCT	0.2± 0.02	0.2± 0.03	0.86	0.2± 0.01	0.2 ±0.01	0.63	0.2± 0.01	0.22± 0.01	0.92
PDW*	18.7± 0.5 ^a	15.3± 1.1 ^b	0.003	16.7±0.6	17.1± 0.7	0.67	17.5 ±0.5	17.7± 0.9	0.72
LYM %	30.3± 2.8	30.3±2.5	0.99	33.7± 2.3	30.9 ±1.8	0.25	30.8± 2.6	31.5± 1.9	0.75
MON%*	6.7± 1.7	6.7± 0.8	0.96	3.9 ±1.0 ^b	7.2± 0.6 ^a	0.02	4.7± 1.2	6.7 ±0.7	0.12

Table 8:- Effects of flaxseed on hematological parameters continued...

	Control (A)			Flaxseed (B)			Flaxseed+Fiber (C)		
	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value	Baseline	Final	<i>P</i> Value
NEU%	59.9±3.6	56.9±3.6	0.43	58.1± 2.8	58.1±2.5	0.99	60.3± 2.6	58.4±2.8	0.51
EOS%	3.3 ±0.6	2.8±0.6	0.37	2.8±0.5	3.3 ±0.4	0.32	3.2±0.5	3.4 ± 0.5	0.60
BAS%	0.7±0.2	0.9±0.2	0.59	0.8±0.2	0.8±0.1	0.98	0.8±0.1	0.9 ±0.1	0.68
ALY%*	1.3±0.2	1.0±0.2	0.39	1.1±0.1	1.3 ±0.1	0.58	0.7±0.1 ^b	1.2 ±0.1 ^a	0.04
LIC%	0.6±0.1	0.7±0.1	0.77	0.4±0.1	0.6±0.09	0.10	0.5± 0.1	0.59 ±0.10	0.75

Data represent least square mean ± SE. Differences were considered significant at *P* value <0.05. In each group, values that do not share the same superscript letters are significantly different; n= 9 for control group, n= 17 for flaxseed group, n= 16 for flaxseed with additional fiber group. WBC= white blood corpuscles, RBC =red blood corpuscles, Hb = hemoglobin, Hct = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width index, PLT = platelet count, MPV = mean platelet volume, PCT = plateletcrit, PDW = platelet distribution width, LYM % = percentage of lymphocytes, MON % = percentage of monocytes, NEU%= percentage of neutrophil, EOS%= percentage of eosinophil, BAS%= percentage of basophil, ALY%= percentage of alymphocyte, LIC%= percentage of large immature cells

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APPENDIX

Oklahoma State University
Institutional Review Board

Protocol Expires: 1/22/2005

Date : Friday, January 23, 2004

IRB Application No HE0345

Proposal Title: DOES FLAXSEED REDUCE THE RISK OF CVD IN NATIVE AMERICAN WOMEN?

Principal Investigator(s) :

✓
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Reviewed and Processed as: Expedited

Continuation

Approval Status Recommended by Reviewer(s) : Approved

Signature

Carol Olson
Carol Olson, Director of University Research Compliance

Friday, January 23, 2004
Date

Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modifications to the research project approved by the IRB must be submitted for approval with the advisor's signature. The IRB office MUST be notified in writing when a project is complete. Approved projects are subject to monitoring by the IRB. Expedited and exempt projects may be reviewed by the full Institutional Review Board.

VITA

Anagha B. Patade

Candidate for the Degree of

Master of Science

- Thesis: THE EFFECTS OF FLAXSEED ON LIPID PROFILE IN
NATIVE AMERICAN POSTMENOPAUSAL WOMEN
- Major Field: Nutritional Sciences
- Biographical:
- Education: Graduated from Sathaye Junior College, Mumbai, India in
1995; received Bachelor of Medicine and Bachelor of
Surgery from University of Mumbai, Mumbai, 2002.
Completed the requirements for the Masters of Science
degree with a major in Nutritional Sciences at Oklahoma
State University in July, 2005.
- Professional Membership: Maharashtra Medical Council, Mumbai, India.

Name: Anagha B. Patade

Date of Degree: July, 2005

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: THE EFFECTS OF FLAXSEED ON LIPID PROFILE IN NATIVE AMERICAN POSTMENOPAUSAL WOMEN

Pages in Study: 81

Candidate for the Degree of Master of Science

Major Field: Nutritional Science

Scope and Method of Study: There is a need to explore the health benefits of functional foods, e.g. their cardiovascular protective effects in Native American Postmenopausal population. A large number of Native American women have one or more risk factors for cardiovascular disease (CVD) such as hypercholesterolemia, hypertension, diabetes, and obesity. This study was designed to investigate whether incorporation of 25 to 30 g of flaxseed per day, a rich source of lignans, omega-3 fatty acids, and fiber for a period of 3 months into the diet of Native American postmenopausal women positively affect lipid profiles. Forty-two mild to moderately hypercholesterolemic ($\geq 5.1 \leq 9.8$ mmol/L) Native American postmenopausal women were randomly assigned to the control (A), flaxseed (B) or flaxseed + additional fiber (C) groups.

Findings and Conclusions: Both the flaxseed groups reduced total cholesterol and low density lipoprotein cholesterol by approximately 7% and 10%, respectively, without altering the high density lipoprotein and triglyceride levels. The findings of this study indicate that daily consumption of flaxseed is beneficial in improving the lipid profiles of Native American postmenopausal women.

ADVISER'S APPROVAL: Dr. Bahram H. Arjmandi