# REGULATION OF GENE EXPRESSION BY DIETARY PLANT STEROLS IN CHOLESTEROL ABSORPTION AND METABOLISM

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Dietary plant sterol supplementation has long been used as an effective means to reduce cholesterol absorption efficiency and thus lower plasma low density lipoprotein (LDL) cholesterol concentrations. However, the exact mechanism by which plant sterols reduce cholesterol absorption has not been identified. Several mechanistic possibilities have been proposed and include plant sterol disruption of cholesterol solubility in intestinal micelles, plant sterol-cholesterol cocrystallization, and plant sterol competition with digestive enzymes. With the recent discovery of the sterol transporter, Niemann-Pick C1-Like 1 (NPC1L1), it seems logical that plant sterols could also reduce cholesterol absorption through regulation of the NPC1L1 pathway. We hypothesized that the beneficial effects of plant sterols are, at least in part, due to the altered gene expression of NPC1L1. Male C57BL/6 mice were fed the AIN-93 diet (control), or the AIN-93 diet containing sitosterol or stigmasterol (2 g/2000 kcal) for 4 weeks. While plant sterol supplementation dramatically increased fecal neutral sterol excretion, intestinal expression of genes involved in cholesterol absorption and metabolism were unchanged. Livers of all mice accumulated plant sterols primarily in the form of sitosterol, while plant sterol supplemented mice accumulated significantly more sitosterol and stigmasterol compared to control mice. In addition to the accumulation of plant sterols, livers of mice that consumed diets containing plant sterol exhibited reduced expression of

the transcription factor sterol response element binding protein 2 (SREBP-2) and its target gene HMG CoA reductase. In support of these data, we examined the mRNA expression of genes involved in cholesterol absorption and metabolism from the intestine-derived cell lines FHs 74 Int and CaCo-2 following treatment with plant sterols. In both cell lines there was a parallel reduction in mRNA expression of NPC1L1 and the SREBP-2 target gene HMG CoA reductase when treated with plant sterol. Our findings indicate that plant sterols alter the expression of genes involved in cholesterol transport, thus suggesting an intracellular mechanism—in addition to decreased micellar cholesterol solubility—by which plant sterols inhibit intestinal cholesterol absorption.

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## Introduction

Elevated plasma LDL cholesterol concentration is a primary risk factor for cardiovascular diseases, the leading cause of death in the United States (3). Plasma LDL cholesterol concentration is also directly correlated with intestinal cholesterol absorption in humans (1, 2). In this way, the risk of cardiovascular disease can be reduced by lowering plasma lipid concentrations via lowering cholesterol absorption. Dietary factors known to influence cholesterol absorption include dietary fibers (3), stearic acid (4), and plant sterols (5). Several clinical studies have demonstrated a direct role of dietary plant sterols in reducing cholesterol absorption (6-8). Researchers have reported that inhibition of cholesterol absorption and lowering of plasma cholesterol concentrations occur within one day of the start of consumption of stanol esters (6).



Plant sterols have been used as cholesterol lowering agents for the last 50 years (9). They have been shown to be both effective and safe. In a series of human studies, intakes of up to 8.6 grams per day of plant sterols for four weeks caused no adverse effects (10-13). The FDA has approved the following health claim for plant stanol/sterol esters and reduced risk of heart disease: "Diets low in saturated fat and cholesterol that include at least 1.3 grams of plant sterol esters or 3.4 grams of plant stanol esters, consumed in 2 meals with other foods, may reduce the risk of heart disease." More recently, plant sterols have been made available to consumers in margarines, and with new processes to improve their bioavailability they are now marketed in fruit juice, ice cream and other vehicles. Consumption of plant sterols (2-3 grams/day) reduces LDL cholesterol by 10-

15% (14). It is generally assumed that plant sterols elicit their cholesterol lowering effects by reducing cholesterol absorption within the lumen of the small intestine. We hypothesized that, in addition to their effects within the lumen of the small intestine, plant sterols taken up into the intestinal enterocyte beneficially modulate the gene expression of cholesterol transport proteins, mainly Niemann-Pick C1-Like 1, and further reduce cholesterol absorption.

The present study was conducted to investigate the effect of plant sterols on gene expression of genes involved in cholesterol absorption and metabolism. To test our hypothesis that plant sterols have an effect on expression of cholesterol-related genes, we used two different approaches; an *in vivo* mouse model and an *in vitro* cell culture model. The mouse is an important model because it exhibits all of the known enzymes and biochemical pathways of cholesterol metabolism present in humans. Additionally, the mouse is widely regarded as an acceptable animal model for laboratory research, particularly in the area of lipid metabolism. Mice were fed experimental diets enriched with plant sterols (sitosterol or stigmasterol) and examined as to how specific dietary plant sterols influence intestinal cholesterol absorption and the genes involved in those processes. We focused our attention on the gene expression of possible cholesterol transporters NPC1L1 and scavenger receptor class B type I (SRBI), the sterol regulatory element binding protein-2 (SREBP-2) and its target gene 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase. In addition, we looked at lipid concentration of several tissues for further insight into the effects of plant sterols' cholesterol lowering abilities.

The *in vitro* cell culture studies used the human small intestine-derived cell line FHs 74 Int, and the widely used human large intestine-derived CaCo-2 for comparison, and examined mRNA abundance following treatment with plant sterols. Both of these cell types were used to mimic the human intestinal tract upon dietary supplementation with various sterols and to examine changes in gene expression. As in the mouse study, we focused our attention on the gene expression of possible cholesterol transporters NPC1L1 and SRBI, SREBP-2 and its target gene HMG CoA reductase.

An additional experiment was performed to specifically examine the role of NPC1L1 or SRBI in cholesterol uptake. To achieve this we transfected NPC1L1 and SRBI into the human embryonic kidney (HEK) 293 cell line, which normally lacks the ability to take up cholesterol as in the intestinal enterocyte, and then quantified their ability to take up cholesterol.

Understanding the cellular and molecular mechanisms by which cholesterol is taken up into the body, as well as the ability of plant sterols to reduce cholesterol uptake, will give us useful insight into the treatment and prevention of atherosclerotic cardiovascular diseases.

# Literature Review

## I. Cardiovascular Diseases

Current mortality data shows that cardiovascular diseases (CVD) are the underlying cause of death in over 32% of all deaths in 2005. CVD claims more lives than the next four leading causes of death combined (cancer, lower respiratory diseases, accidents, and diabetes mellitus) (15). Cardiovascular disease refers to the class of diseases that involve the heart or blood vessels in which the clinical manifestation of atherosclerosis is the underlying cause and includes disease such as coronary heart disease, pulmonary heart disease, stroke, atherosclerosis, and other diseases of the heart and circulatory system. Risk factors for CVD include hypertension, dyslipidemia, diabetes mellitus, cigarette smoking, obesity, and physical inactivity (16). Risk factors for CVD rarely occur alone, but rather tend to be grouped together, which can exacerbate an individuals risk for progression of CVD. Prevention of CVD involves the control of these risk factors through lifestyle modifications such as diet, exercise, and cigarette smoking cessation. Nutrition influences CVD risk in a variety of ways, such as the detrimental effects of excessive energy intake and the beneficial effects of consuming polyunsaturated fatty acids. Consumption of excessive energy intake is thought to have two mechanisms of action that promote CVD progression: obesity and insulin resistance. There is now evidence that suggests insulin promotes the initiation and perpetuation of vascular inflammation, through increased expression of vascular cell adhesion molecule-1 (VCAM-1) and other similar immune cell signaling molecules (17), which could provide some explanation of why persons with insulin resistance or hyperinsulinemia display

increased atherosclerosis. It has been found that obesity itself can induce a proinflammatory state. Studies in mouse models have found that adipose associated macrophage infiltration and accumulation is directly proportional with adipose area (18), along with the up-regulation of inflammatory and macrophage specific genes in models of genetic obesity (19). On the other hand, consumption of n-3 polyunsaturated fatty acids can have a positive effect on CVD risk factors. Several studies examining diets rich in n-3 polyunsaturated fatty acids, such as a Mediterranean-type or Eskimo/Alaskan native diets, have found significant cardio-protective effects of these diets (20, 21). *In vitro* studies have consistently shown that n-3 fatty acids decrease the expression of adhesion molecules on the endothelium and also decrease leukocyte/endothelium interactions (22). In addition to lifestyle modifications, various pharmacological modifications can also be made with treatments such as angiotensin converting enzyme (ACE) inhibitors to treat hypertension or statins to treat elevated plasma lipid levels (23, 24).



Included under the cardiovascular disease umbrella are a number of diseases. The following are a few of the major cardiovascular diseases of which the underlying causes are dyslipidemia and atherosclerosis progression: peripheral arterial disease, stroke, and coronary heart disease. Atherosclerosis can affect arteries anywhere in the body. Atherosclerosis affecting the arteries of the heart results in coronary artery disease; when affecting arteries supplying the brain it is carotid artery disease; and when atherosclerosis affects the arteries supplying the head, limbs, and internal organs it is peripheral arterial disease. Peripheral arterial disease affects 14% of the general population and increases to

20% of persons over the age of 75 (25), resulting in a significant increased risk of cardiovascular morbidity and mortality (26). Screening for peripheral arterial disease is performed using a simple, effective, non-invasive test for the assessment of lower extremity arterial obstruction called the ankle-brachial index. The ankle-brachial index is a ratio of systolic blood pressure in the brachial artery divided by the higher of the lower extremity systolic blood pressure, measured at the dorsalis pedis and the posterior tibial arteries (27). Persons with an ankle-brachial index of 0.90 or less are considered to have peripheral arterial disease. The ankle-brachial index correlates with the extent of coronary artery disease (28), and the risk for cardiac death increases by 12% for every 0.10 decrease in ankle-brachial index ratio (29).

When considered separately from cardiovascular disease, stroke ranks number three among all causes of death, behind diseases of the heart and cancer (30). Stroke, or "brain attack", occurs when a blood vessel in the brain becomes blocked or bursts. The brain relies heavily on a network of blood vessels to supply oxygen and nutrient rich blood, the resulting lack of blood supply from a stroke causes surrounding nerve cells to be cut off from their supply of nutrients and oxygen. When brain tissue is cut off from its blood supply for more than three to four minutes, it will begin to die. Strokes can appear as three types: hemorrhagic, transient ischemic attack, and ischemic. Hemorrhagic stroke occurs when a weakened blood vessel in the brain ruptures, which includes bleeding into (intracerebral hemorrhage) or around (subarachnoid hemorrhage) the brain (31). Transient ischemic attack is generally a brief episode of neurological dysfunction caused by focal brain or retinal ischemia with clinical symptoms typically lasting less than one

hour and without evidence of acute brain infarction (32). Transient ischemic attacks have been described as a "warning stroke" or "mini-stroke" that produces stroke-like symptoms but with no lasting damage. Transient ischemic attacks are important in predicting if a stroke will occur; they can occur days, weeks or even months before a major stroke. In about half the cases, the stroke occurs within one year of the transient ischemic attack. Ischemic stroke occurs when a blood vessel in the brain develops a clot, often resulting from the progression of atherosclerosis. Ischemic stroke is defined as acute onset of neurologic symptoms lasting longer than 24 hours or radiographic evidence of an ischemic event in patients with loss of symptoms within 24 hours. While a transient ischemic attack is an event that lasts less than 24 hours and without evidence of pathology on radiographic studies (33). Ischemic stroke accounts for roughly 83% of all strokes, while the rates of intracerebral and subarachnoid hemorrhage are much lower (30). Risk factors for ischemic stroke are similar to that of all cardiovascular diseases, with 60-80% of all ischemic strokes being attributed to hypertension, dyslipidemia, cigarette smoking, carotid stenosis, and diabetes mellitus (34).



Today coronary heart disease is the single largest killer of American men and women. Every 26 seconds an American experiences a coronary event and about every 60 seconds a person will die from a coronary event (35). The category of coronary heart disease includes acute myocardial infarction, acute ischemic (coronary) heart disease, angina pectoris, atherosclerotic cardiovascular disease, and all other forms of chronic ischemic heart disease. While diseases of the heart includes coronary heart disease, it is also comprised of rheumatic heart disease, hypertensive heart disease, pulmonary heart

disease and others. Today, diseases of the heart account for about three-fourths of total CVD mortality. As with most cardiovascular diseases, atherosclerosis is an underlying cause in the initiation and progression of coronary heart disease. Patients with coronary artery disease typically become symptomatic after age 40 years, yet 17 % of teenagers show coronary atherosclerosis and lesion formation (36). Thus, atherosclerosis progresses beneath clinical detection for decades, evolving from early fatty streaks in teenagers to a complex unstable plaque that causes a clinical cardiovascular event. Cardiovascular events, such as myocardial infarction or heart attack, are caused by a critical arterial narrowing from plaque rupture and not just lesion occlusion of the arterial lumen. Angiogram data indicates that extreme narrowing of the arteries occurs in the prior weeks and months before a heart attack in only 15% of cases (37). Additionally, coronary arteries have been shown to enlarge and compensate for the lesion in order to preserve the flow of blood to the heart tissue, but this mechanism becomes overwhelmed when the stenosis occupies greater than 40% of the arterial lumen (38). Plaque rupture, most often from disruption of the fibrous cap that protects the lipid core from contacting the circulating blood, and subsequent thrombosis causes most acute coronary syndromes. The strength and stability of the plaque's fibrous cap comes from interstitial collagen, which can become compromised by various inflammatory events. Inflammation can interfere with the stability of the fibrous cap in two ways: blocking collagen synthesis and enhancing collagen degradation. Collagen is primarily synthesized by smooth muscle cells, and is stimulated by transforming growth factor-β, platelet derived growth factor, and interleukin 1. Conversely, T lymphocytes within the plaque produce interferon γ, which inhibits both basal collagen synthesis and the stimulatory effects of transforming

growth factor β, platelet derived growth factor, and interleukin 1 (39). T lymphocytes also promote the synthesis of a family of matrix metalloproteinase's (40), which act to degrade existing collagen and weaken plaques. Inflammation is involved in many aspects of cardiovascular disease from the initiation of atherosclerosis and plaque formation, to weakening of the fibrous cap and rupture.

## II. Atherosclerosis

The underlying cause of coronary heart disease is atherosclerosis, which is defined as atheromatous deposits in and fibrosis of the inner layer of the arteries (41). We now know that atherosclerosis is a complex series of events involving endothelial dysfunction, subendothelial inflammation and various immune responses, although it was initially thought simply to be the accumulation of lipids in the arteries. In one of the earliest published papers on atherosclerosis, a researcher at the Military Medical Academy in St. Petersburg found that feeding rabbits a purified diet supplemented with cholesterol dissolved in sunflower oil induced vascular lesions resembling fatty streaks found in humans(42, 43). This would mark the beginning of research in the field of atherosclerosis.



The main principle of atherosclerosis is that atheroma is initiated by the accumulation of cholesterol within the arterial intima following the diffusion of lipoproteins across the endothelium (44). In addition to the accumulation of lipids in the arterial intima, progression of atherosclerosis involves the proliferation of smooth muscle cells and

macrophages, along with increased production of connective tissue within the smooth muscle cells. Atherosclerosis has many risk factors that contribute to its development, including dyslipidemias, hypertension, diabetes mellitus, smoking, thrombogenic factors, age, male gender, family history and genetic predisposition.

The progression of lesion development is a gradual occurrence with eight histological classifications of lesion (45). Type I, II, and III early lesions are classified as having lipid and macrophage foam cell accumulation, but no disorganization of the arterial wall. Early lesions are harmless and do not require treatment. Lesion types IV, V, VI, VII, and VIII are classified as advanced, displaying changes in the contour of the artery and disorganization of the intima.



Endothelial dysfunction was initially thought of as impaired vasodilation, while later proinflammatory and prothrombic states were also found to be associated with endothelial dysfunction. Imbalance between vasodilation and vasoconstriction, caused by damaged endothelium, results in a number of events that exacerbate atherosclerosis. These events include, but are not limited to: endothelial permeability, platelet aggregation, leukocyte adhesion, and generation of cytokines (46). One proposed mechanism of endothelial dysfunction is reduced nitric oxide (NO) production. NO synthesis is a two step reaction in which NO synthase (NOS) catalyzes a five electron oxidation of L-arginine via N-hydroxy-L-arginine to citrulline and NO (47). Inhibition of this pathway, resulting in reduced NO production, rapidly accelerates lesion formation and size. Apo-E knockout mice fed a chow diet were treated with N-nitro-L-arginine

methyl ester, a NOS inhibitor, displayed reduced vascular responses to NO and increased aortic atherosclerotic plaque area (48). The inhibition of NO elicits several atherosclerotic effects, such as inducing platelet aggregation, leukocyte adhesion and smooth muscle cell proliferation, all leading to accelerated atherosclerosis. Furthermore inducing NO production inhibits multiple contributing factors that accelerate atherosclerosis. Rabbits maintained on a 1% cholesterol diet for 11-13 weeks to induce atherosclerosis underwent carotid artery NOS gene therapy. Rabbits rapidly and significantly reduced lipid accumulation, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and lymphocyte infiltration as early as 24 hours after gene therapy, compared with control animals (49). These data suggest NO plays a significant role in the progression rate of atherosclerotic lesions by regulating various cell adhesion molecules, monocyte infiltration, and lipid accumulation. Inhibitors of 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, the rate-limiting enzyme in cholesterol synthesis, have previously been shown to effectively reduce plasma lipids concentrations, and thus are effective pharmaceutical agents in reducing the risk of heart attack. Recently, HMG CoA reductase inhibitors have been shown to have anti-inflammatory roles, enhancing their effectiveness in reducing risk of heart attack. One such study found that treatment of mice with HMG CoA reductase inhibitor Simvastatin selectively up-regulated endothelial NOS, resulting in increased blood flow, inhibition platelet aggregation and leukocyte adhesion (50).

In general, normal endothelium does not support the binding of white blood cells, but after the initiation of an atherogenic diet, endothelial cells begin to express adhesion

molecules on their surface to bind various classes of leukocytes that penetrate the vascular cell wall. This marks the beginning of subendothelial inflammation in atherosclerosis. Triggers of atherosclerosis can initiate the expression of cell adhesion molecules by endothelial cells, allowing for the attachment of leukocytes to the surface artery wall and pass between the endothelial cells lining. The process of extravasation by leukocytes into regions of inflammation is a multistep process in which cascades of adhesion molecule interactions occur. First, selectins and their carbohydrate ligands bind to mediate the tethering and rolling process. At this point chemokines work to activate leukocyte integrins on the cell surface. Once activated leukocyte integrins bind to their ligands, which includes cell adhesion molecules, to introduce firm adhesion (51). Lastly, chemoattractants act to govern leukocyte adhesion and migration across the endothelium and through the extracellular matrix into the damaged tissue.



Atherosclerotic lesions show increased expression of various leukocyte adhesion molecules in response to inflammatory stimuli which include P-selectin, E-selectin, intercellular adhesion molecule (ICAM), and vascular cell adhesion molecule (VCAM) (52-55). P-selectin has been reported in platelets, endothelial cells, and vascular smooth muscle cells in response to injury. Apolipoprotein (Apo) $E^{-/-}$ mice, in which mice spontaneously develop lesions in the arterial vasculature, deficient in P-selectin exhibits a 45% reduction in lesion area at 20 weeks of age (56). This suggests that P-selectin, and possibly E and L selectin, play a pivotal role in the initiation and progression of atherosclerotic lesions. In addition to the selectins, adhesion molecules of the immunoglobulin superfamily, especially ICAM-1 and VCAM-1, have been shown to be

critical in the progression of atherosclerosis. Post mortem immunohistochemistry of human atheroma demonstrated expression of ICAM-1 on endothelial cells, macrophages and smooth muscle cells, while normal arterial endothelial cells exhibited a much lower expression of ICAM-1 (54). In another study, LDL receptor $^{-/-}$ mice with disrupted VCAM-1 expression had significant reductions in the area of early atherosclerotic lesions, while ICAM-1 disruption did not alter early lesion formation (57), thus suggesting that VCAM-1 is involved in early lesion formation, and ICAM-1 is involved in the progression of mature atherosclerotic lesions (56, 58).

Once firm adhesion of monocytes occurs, they penetrate the endothelial lining and enter the intima of the vessel wall by a process in which a chemoattractant gradient is essential. The first chemokine implicated in the development of atherosclerosis was monocyte chemoattractant protein-1 (MCP-1), also known as CCL2 (59). Early research in a model of dietary induced hypercholesterolemia in non-human primates found clear evidence of MCP-1 induction in the hypercholesterolemic animals, whereas the animals fed a control diet found little or no expression of MCP-1. Examination of human carotid arteries revealed elevated expression of MCP-1 in 33% of organizing thrombi as well as 24% other macrophage rich areas bordering the lipid core. While in arteries of normal patients less than 0.1% of cells expressed MCP-1 (60). In addition to hypercholesterolemia, modified LDL have been shown to accelerate atherosclerosis and macrophage foam cell formation. Cushing et al. has demonstrated the effect of minimally oxidized LDL on MCP-1 production; exposure of endothelial cells and smooth muscle cells to minimally oxidized LDL showed a 2 to 3 fold increase in monocyte chemotactic activity compared

to cells incubated with freshly isolated LDL (61). Deletion of CCR2, the functional receptor for MCP-1, in apo E null mice showed significant protection from lesion formation while having no effect on plasma lipid profiles (62). Taken together these data indicate a role for MCP-1 in the progression of early atherosclerosis in response to hypercholesterolemia and minimally oxidized LDL.

In addition to the recruitment of monocytes by MCP-1, macrophage colony stimulating factor (M-CSF) plays a key role in the development of atherosclerosis. M-CSF regulates the survival and proliferation of bone marrow derived macrophages (63), as well as increasing the synthesis of various cytokines and growth factors (64). An osteopetrotic (op) mouse model was developed to study the effect of M-CSF and atherosclerosis, in which a mutation in the gene for M-CSF was bred into apo E deficient mice. Mutant op/apoE deficient mice fed a low fat chow diet displayed a significantly smaller lesion size compared with their apoE littermates. In addition, op/apoE mice had decreased blood monocytes as well as increased plasma cholesterol levels, relative to their apoE littermates (65). These data suggest that M-CSF plays a key role in the development and function of macrophages, as well as progression of atherosclerotic lesions.



**III. Plasma Lipids**

The major carrier of cholesterol in the circulation of humans is LDL, containing a single apo B100 molecule that renders the particle soluble in water, and is implicated as the major contributor to the generation of atherosclerotic plaques. The Atherosclerosis Risk

in Communities (ARIC) Study examined 12,339 subjects and evaluated various cardiovascular risk factors to provide coronary heart disease prediction. Researchers found the lowest incidence of coronary heart disease events occurred in participants in the lowest LDL quintile, with medians of 88 and 95 mg/dL for women and men, respectively. Risk of coronary event accelerated with increasing LDL levels, the group in the highest quintile for LDL observed a relative risk of 2.7 and 2.5 for women and men compared to the lowest quintile, respectively. They also found increased predictive value from HDL-cholesterol, triglycerides in women but not men, and lipoprotein a (66). Additional LDL based risk factors for coronary heart disease include oxidized LDL and LDL particle size.

The oxidative modification hypothesis suggests that LDL in its native form is not atherogenic, but chemically modified LDL is readily taken up into macrophages and degraded through the scavenger receptor pathway (67). More recently, Sawamura and others discovered the lectin-like oxidized LDL receptor (LOX-1) pathway in vascular endothelial cells, separate from the macrophage scavenger receptor pathway (68). Recent reports show a strong association of circulating oxidized LDL with documented coronary artery disease in patients under the age of 60 years. Subjects in the highest quartile for oxidized phospholipid had an odds ratio of 3.12 for coronary artery disease, compared to those subjects in the lowest quartile (69). Previous studies have shown a relationship between oxidized LDL and the progression of the inflammatory process (70).

Treatment of endothelial cells with minimally oxidized LDL caused a significant increase of monocyte chemotactic factor, along with a three to five fold increase in monocyte binding to endothelial cells (71). Further research from the same group identified two molecules present in oxidized LDL that induce monocyte-endothelial interactions. The study found that specific oxidized derivatives of arachidonic acid containing phospholipids may be important in monocyte adherence and progression of atherosclerosis (72). Other research supporting the role of oxidized LDL in the inflammatory response found that oxidized LDL activates platelet activating factor. Injection of lipids from oxidized LDL into the pleural cavity of mice produced rapid accumulation of monocytes, neutrophils, and eosinophils. The injection of lipids from oxidized LDL induced mRNA expression of monocyte chemoattractant protein-1 and chemokines, synthesis of monocyte chemoattractant protein-1, and leukotriene B-4. These data suggest that the lipids from oxidized LDL induce an inflammatory response through the platelet activating factor receptor to induce transcription of chemokines and lipid body formation (73).



Small dense LDL has been reported as being more susceptible to oxidation than large buoyant LDL. The data shows increased oxidative susceptibility with increasing lipoprotein density (intermediate density lipoproteins < large buoyant LDL < small dense LDL). Additionally, subjects having a small dense LDL phenotype showed greater oxidative susceptibility of intermediate density lipoproteins compared to subject with the large, buoyant LDL phenotype (74, 75). These data suggest that the lipoprotein precursor have different oxidative susceptibility, and may contribute to the increased risk of

coronary artery disease associated with small, dense LDL. Others report that the size of the particle, but not oxidative susceptibility, is independently associated with cardiovascular disease (76).

The relationship between small dense LDL and coronary artery disease is gaining a large body of evidence indicating LDL particle size is an independent risk factor for the development of coronary artery disease. The Quebec Cardiovascular Study was the first prospective study to suggest a relationship between LDL particle diameter and risk of developing ischemic heart disease. The study consisted of more than 2100 male patients initially free of ischemic heart disease. Over a 5 year follow up 114 men developed ischemic heart disease and these men were matched to healthy control subjects for analysis. They found men in the first tertile (smallest diameter LDL) had a 3.6 fold increased risk of ischemic heart disease compared to men in the third tertile (largest diameter LDL) (77). Additionally, after adjustment for LDL cholesterol, triglycerides, HDL cholesterol, and apo B concentrations, there was essentially no impact on the relationship between LDL particle diameter and risk of ischemic heart disease.



While much of the atherosclerosis research has been looking at the role of LDL in the progression of coronary arterial disease (CAD), dyslipidemias in plasma levels of triglycerides and high density lipoproteins (HDL) may affect premature development of CAD. While still controversial, many epidemiological studies have found a positive correlation with triglycerides and CAD, the controversy is due to the limited significance