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The effects of hypocholesterolemic regimens on regression of the histopathologic manifestations of atherosclerotic lesions

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THE EFFECTS OF HYPOCHOLESTEROLEMIC REGIMENS ON REGRESSION OF THE HISTOPATHOLOGIC MANIFESTATIONS OF ATHEROSCLEROSTIC LESIONS

by

JOSÉ ANIBAL TORRES

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Approved by

First Reader Carol T. Walsh

Carol T. Walsh, PhD
Associate Professor of Pharmacology

Second Reader Selwyn A. Broitman

Selwyn A. Broitman, PhD
Professor of Microbiology

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José Aníbal Torres

Boston University Graduate School, 1989

Major Professsor: C. Walsh, Associate Professor of Pharmacology

This paper summarizes some important aspects of atherosclerotic regression. The morphology of the normal arterial wall and its composition are described briefly. Atherogenesis and the histopathology of atherosclerotic lesions are discussed in detail including observations of structures like foam cells it relationship with oxidized low density lipoprotein. Also some aspects about the physicochemical characteristics of lipids are described and their relationship with the arterial intima. The metabolism of cholesterol and the lipoproteins are discússed and their effect on the emergence of atherosclerotic lesions. An anatomical definition of the process of atherosclerotic regression is given and the steps of the atherosclerotic regression process are briefly described. Lastly, some hypocholesterolemic regimens, namely diets and drugs, are evaluated with respect to evidence from studies in animals and man of their efficacy in inducing atherosclerotic regression.

TABLE OF CONTENTS

Introduction

Atherosclerosis currently represents one of the subjects that has stimulated the most intensive research in medicine. Coronary atherosclerosis is responsible for more deaths each year than any other disease (including deaths from all types of cancers combined). The United States spends over $60 billion each year in direct and indirect health care expenses (Levy 1986).

For a number of years, evidence has been accumulating to indicate that elevated plasma cholesterol levels are the cause for the emergence of coronary atherosclerosis (Anderson et al. 1987). Recent investigations in the development of hypocholesterolemic regimens has resulted in a variety of cholesterol lowering diets and drugs. The effects of such regimens have also brought forth interesting data regarding the state of the atherosclerotic plaque or atheroma. It has been suggested that by lowering plasma cholesterol levels lipid trapped within the atheroma can be mobilized enhancing the rate of cholesterol efflux from the atherosclerotic plaque (Small 1988). The fact that there is a dynamic and constant exchange of lipid between the blood and the endothelium of the arteries demonstrates an equilibrium between the two mediums. This equilibrium could be altered in favor of increased outward flow of lipid from the affected regions where atherosclerotic

lesions are present.

There is still some controversy as to whether atherosclerotic lesions can be made to regress and to what extent this may occur (Clarkson et al. 1981). A series of arterial angiographic studies, histologic and anatomical observations in both animal models and human subjects have brought forth descriptive data demonstrating the atherosclerotic regression.

The following paper will examine the histopathology that leads to the formation of an atherosclerotic plaque in relation to the metabolic and physicochemical characteristics of cholesterol.This paper also examines some of the hypocholesterolemic regimens from which regression of atherosclerotic lesions has been demonstrated in experimental animals and human subjects.

Atherosclerosis Defined

Arteriosclerosis is the generic term used to describe different patterns of vascular disease which cause hardening or thickening of the arteries as well as a loss in their compliance and elasticity. The most characteristic pattern is atherosclerosis. It is a process that develops in the intima of the blood vessels leading to the formation of a series of space-occupying lesions composed of accumulations of fatty and fibrous material, which compromise the patency of the vessel lumen and the anatomical integrity of the media. In 1963, Pickering supported the property and usefulness of the term atherosclerosis, stemming from the greek "athera" which means "porridge-like hardness", as a correct terminology for such an idiopathy (Robbins and Kumar 1987).

Atherosclerosis is the principal cause of death in more than 50% of individuals in developed countries. And almost all of their population has some degree of atherosclerosis (Lickoff 1972). "No disease in the United States is responsible for more deaths, has stimulated more research, and has engendered more controversy about approaches to its control than atherosclerosis" (Robbins and Kumar 1987).

Atherosclerosis can begin early in life and progresses in stages. Shortly after one year of life in humans, lipid

deposits, often referred to as fatty streaks, appear in the intima of the aorta, particularly around the cusps of the heart valves. These deposits usually disappear but by the end of the first decade these are seen again and continue to accumulate for the rest of the individual's life (King et al. 1983). Fatty streaks are considered by some investigators as tissue expression of 'shear stresses' and perhaps as an initial and reversible stage of atherogenesis.

The second stage of the atherosclerotic process is characterized by the formation of a fibrous lesion called the atherosclerotic plaque. This is a "firm mound-like lesion" containing lipids, principally cholesterol, in the intimal and medial layer of the vasculature and found within myoendothelial cells and macrophages and as free cholesterol. Then, macrophages combine with calcium to form a superimposed cellular accumulation that leads to the worsening of the atherosclerotic plaque and reduction of the diameter of the lumen of the vessel (Boucek et al. 1987).

Progression of the atherosclerotic lesion usually results from increased deposition of lipid associated with calcium depositions. The deposition of calcium gives a hardened character to these lesions. The thickened intimal plaque is infiltrated by cells known as monocytes which later become macrophages as they penetrate the lesion. Some

smooth muscle cells "also proliferate and become surrounded by interstitial fibrosis and hyalinization as well as cholesterol crystals" (King et al. 1983).

Although vast information about the atherosclerotic process has been elucidated, the present knowledge of mechanisms involved in atherogenesis are still largely unknown. Current experimental data and further investigation may clear some uncertainties about the disease.

Morphology of the Arterial Wall

"The arteries are dynamic structures capable of adapting to changes quickly as they are influenced by certain hemodynamic variables of blood flow. These are divided into three main categories: the elastic arteries, the muscular arteries, and the arterioles" (Ross and Reith 1985). The latter are the arteries responsible for providing resistance to flow before blood arrives to the capillaries. All three types of arteries share common morphological structures as they are composed of three layers, namely the tunica intima, the tunica media, and the tunica adventitia.

The tunica intima is the closest layer to the vascular lumen. It is composed of a network of endothelial cells and related connective tissue which together form the vessel's endothelium. The intima of the elastic arteries, although it varies with distance from the heart, comprises about 25% of the total thickness of the artery. These flattened simple squamous epithelial cells rest on a basement membrane, underneath which there is a layer of inconspicuous elastic material known as the internal elastic membrane.

The endothelial cells are held together by essentially two types of junctions: zonula occludens (tight junctions), and a nexus or gap junction. The zonula occludens, as seen

through electron microscopy and freeze fracture, is visualized as interlacing ridges around this area. It is not a patch but rather a belt or band that goes around the endothelial cells.

The nexus or gap junction is made of a 20Å space or "gap" between endothelial cells. Functionally, they serve to attach epithelial cells together and for rapid cell-to-cell communication. Both the tight junction and the gap junction not only hold the cells together but also prevent the diffusion of substances between endothelial cells, acting like a chemical seal between environments. Hence, transport of some substances must occur through vesicles across the endothelial membrane through the process known as endocytosis. This phenomenon shall prove of great relevance in our discussion of cholesterol diffusion across the endothelium. In the larger arteries, besides possessing an internal elastic membrane, the subendothelium contains collagen and elastic fibers or ground substance which are manufactured by the main cell type in this layer, the smooth muscle cell. After the arterial lesion is formed macrophages are also present in the endothelium, which together with the smooth muscle cells, share a part in the pathology of cholesterol accumulation in the intima.

The tunica media is the thickest of the three arterial layers. The tunica media is very rich in elastic fibers which are arranged in bundles or concentric lamellae with

extracellular matrix and ground substance scattered around it. The medial layer is also referred to as the muscular layer because it is the section in which smooth muscle cells are more abundant. It is worth noting that due to the absence of fibroblast in the intima and media, the smooth muscle cells are responsible for the production of collagen and elastic fibers. This function of the smooth muscle cells is exacerbated in atherosclerosis.

The tunica adventitia is the outermost layer composed of connective tissue which becomes continuous with the surrounding connective tissues forming the stroma of the organ. In the elastic arteries, the tunica adventitia comprises only 50% that of the thickness of the tunica media. At the boundary of the tunica media and the tunica adventitia, elastic fibers become interlaced to form the external elastic membrane. The dynamic structure of the elastic membrane is due mainly to the high concentration of collagenous fibers which expand during systole and then recoil to their original state providing a pulse or wave of blood transit through the arteries. In contrast to the cellular composition of the tunica media and most certainly the tunica intima, the tunica adventitia is composed mainly of fibroblast and macrophages. Since the tunica adventitia is furthest from the main circulation, in major arteries diffusion of oxygen and nutrients from the lumen does not meet the need of the tissue. Hence, a separate set of blood

vessels known as the *vasa vasorum*, provides the necessary nutrients and the removal of metabolites for the proper maintenance of the outer layers of the arterial wall. However, such structure is not found in the coronary arteries.

Histopathology and Atherosclerosis

In the last few years atherosclerosis has been approached as an ailment whose etiology is based on the process of lipid or fat accumulation. This understanding has led to the development of useful and valuable means to treat this ailment.

As mentioned previously, atherosclerosis is a process that starts early in life and continues to develop throughout life. Researchers have pointed to an increasing rigidity of the major arteries, like the aorta and the coronary arteries, due to a decrease in the elastin/collagen ratio (Finch & Hayflick 1977). "This rigidity results in a progressive decrease of the capacity of the aorta to dilate and accommodate the systolic volume, and a decrease of the elastic recoil of the aorta, which helps push the expelled blood volume toward the periphery during diastole" (Gotto & Paoletti 1986). This increase in vessel rigidity can lead to progressive fragmentation of the internal elastic membrane and increasing collagen deposition in the lacerated endothelium.

The concept of the endothelium as a barrier of "selective permeability" has been elucidated from many studies, especially from those performed by Schwartz and colleagues, who experimented on problems with the transendothelial passage of macromolecules in the large

arteries. (Schwartz, Gerrity and Lewis 1977). Their data suggests that the permeability of the endothelium to macromolecules like lipids, may show significant regional variation and that this variation is mediated through hemodynamic factors. Additionally, "the areas of greatest permeability [to lipids] like the coronary arteries, aorta and femoral arteries appear to be most at risk for the subsequent development of an atherosclerotic lesion" (Woolf 1982).

Morphology of the development of an atherosclerotic lesion is mainly characterized by three stages: the fatty streak, the intermediate lesion or fibrous plaque, and the complicated or gruel plaque.

Although the visibility of a very first lesion on the endothelial wall has been somewhat controversial among histopathologists, "the general consensus has been to consider the fatty dot or streak as the best recognizable structure leading the process of atherogenesis. The fatty streaks are found mainly throughout the large arteries and are routinely encountered in infants and young children" (Small 1988). These generally cause no or minimal obstruction of the vascular lumen and often show no clinical symptoms as they go unnoticed. Schwartz (1967) noticed that the incidence of fatty streaks in 43 out of 100 infants (between the ages of 1-12 months), that were coming to necropsy, were mostly localized to the aortic

valve, the ductus scar and distal to the ostia of the intercostal arteries. During puberty, fatty streaks appear mostly in the left coronary arteries like round yellowish patches minimally elevated around the endothelium averaging 2 by 10mm in size. Histologically, all of these lesions contain cells that have considerable lipid accumulation known as "foam cells". These are derived from monocytes that wander in the circulation and smooth muscle cells that are present in the media of the arterial wall which invade the intima in the case of excess lipid deposition (Robbins and Kumar 1987). Foam cells which shall be discussed in detail later on, are overloaded with lipid droplet occlusions and are visualized as regional elevations of the intima.

In Pathology of Atherosclerosis, Woolf describes, form the work of Mitchell and Schwartz (1965), the distribution pattern of these fatty streaks in adults. He mentions that "the streaks were distributed in a fan-shaped pattern made up of discrete confluent streaks which distally become concentrated on the posterior aspect of the aorta". Again, due to the hemodynamic of blood flow in the arteries, a pattern emerges from the intima of the affected vessels, giving an overall structure to this atherosclerotic lesion.

Lipid found in the fatty streaks is mainly concentrated in the endothelial and smooth muscle cells. The lipid accumulated in these lesions is similar in

appearance to the uniform infiltrations of lipid found normally in clear vacuoles. Any difference in the histological observations of accumulated lipid in the fatty streaks arise as a result of differences in the chemical composition of the lipids present in this lesion. Although most of the lipid hereby described is present principally in these cells, in patients with hyperlipoproteinemic disorders there can also be accumulation of lipid in the extracellular spaces (Woolf 1982).

The role of the fatty streak as a precursor of atherosclerosis has been an issue of considerable discussion. The fatty streak differs from atherosclerotic plaque in that most of the lipid is intracellular and very little lipid in the extracellular matrix. The immediate tissue about the accumulated lipid is not necrotic. Thus it is suggested that due to their minimal effect on the arterial wall, the fatty streak lesions are readily reversible (Robbins and Kumar 1987).

Small (1984) suggests that the physicochemical nature of lipids is what makes their accumulation characteristic. The three different densities of the lipid accumulated in these lesions can be represented in a graph which accounts for each one of these lipids at its axes: a cholesterol axis, a phospholipid axis, and a cholesterol-ester axis. According to the concentration of each lipid in the specific atherosclerotic lesion stage (that is, fatty

streak, intermediate plaque and gruel plaque) one can find how many lipid phases there are in a specific atherosclerotic lesion stage as plotted in the triangular coordinate with the defined axes. Ordinary fatty streaks consists primarily of two lipids, that is phospholipid and cholesterol. The mixture of these two lipids is considered to lie in what Small refers to as zone III because these two lipids separate into a phase of cholesterol (zone II) that floats in water, and a phospholipid or "lamellar liquid-crystalline phase" (zone I) which when centrifuged appears at the bottom of the phase separation. This lipid mixture is characteristic of a fatty streak where cholesterol ester droplets can be identified (Small 1977).

The second stage of atherogenesis is the formation of the fibrous or intermediate plaque. Whereas the fatty streak is a yellow fan-like protrusion into the arterial lumen, the fibrous plaque is white and clearly invades the vessel lumen. The lesion contains cholesterol esters which accumulate in the smooth muscle cells in the intima of the arteries. Woolf comments that the fibrous or intermediate plaque are very much elevated(as it is filled with lipid deposits) above the surface of the non-affected intimal layer and that even if the media shows no loss of thickness, the edematous intima may be greater or equal in thickness to the underlying media and intima. Endothelial cells and the extracellular matrix, also intruded with

lipid, form the fibrous plaque that covers a deeper deposit of cell debris, free extracellular lipid and other plasma-derived constituents. Stary (1987) mentions that after staining with Sudan IV, he found that the elevations had a pink periphery. "The coloration reflected the microscopic observation of superficially located foam cells at the thinner periphery, while the thick midportion remained unstained because both intracellular and extracellular lipid were submerged well below the surface of the fibrous plaque". Stary often referred to the intermediate plaque as a "pre-atheroma".

In order for the fibrous plaque to come about, invasion of the medial layer of the vessel wall does not need to be involved. However if it does happen it could result in the hypertrophy of the smooth muscle cells lying underneath the affected intimal region. There is a tendency for these plaques to be located at specific vessels. It is worth noting that "the predilection of the emergent areas [for the formation of atheroclerostic plaques] of the left anterior descending artery (LAD) and the circumflex artery (CxA) and the left coronary artery (LCA), in particular, represent the sites in the heart where the greatest extent and severity of involvement is found for the generation of the fibrous plaques" (Boucek et al. 1984). Free fatty acids that accumulate in the fibrous lesion combine with calcium to form "calcium soaps" which

lay on the arterial wall contributing to what is commonly known in atherosclerosis as hardening of the arteries. Upon hypertrophy of the immediate underlying myointimal cells, collagen is overproduced and the internal elastic membranes is disrupted with the reduction of the elastic fibrils in the media (King et al. 1983). Intermediate plaques are most commonly found lying in the same plane as the intimal surface resulting in lesion formation and decreased elastic content of the intima (Robbins and Kumar 1987). Although the transition between the fatty streak and the intermediate plaque have not been well investigated, increases in cholesterol accumulation renders the cholesterol esterifying enzyme, acyl cholesterol CoA: acyltransferase (ACAT), insufficient in activity to the point where it is unable to esterify fat, thereby leading to further accumulation of unesterified cholesterol in the atherosclerotic plaque. Experiments perfomed in rat hepatocytes have shown to contain cholesterol droplets representing the intermediate plaque (Adams 1973; Bleich 1977).

The third stage of atherosclerosis is the formation of the complicated or gruel plaque. The complicated plaque comes forth as a result of worsening of the fibrous plaque as it becomes calcified due to the deposition of calcium soaps, degeneration of the smooth muscle, ulceration of the arterial lesion and hemorrhage. The surface of the lesion

exposes its content to the arterial lumen leading to platelet aggregation and thrombus formation which become encrusted on the atherosclerotic plaque or atheroma (Robbins and Kumar 1987; King et al. 1983).

In contrast to the fatty streak and the fibrous plaque, the media is very much involved in the necrotic process as its thickness is markedly reduced. The hypertrophy of the smooth muscle cells as they become engorged with lipid leads to the disruption of the composition of the media by decreasing the elastin/collagena ratio. The proliferation of these cells reduces the thickness of the media as smooth muscle cells migrate into the intima, while the remaining elastic fibers become diluted in collagen that has been oversynthesized by the surrounding myointimal cells(Woolf 1983).

Moreover, the ulceration of the complicated plaque leads to further reduction of the diameter of the lumen of the artery. This condition may allow the blood from the arterial lumen to gain access into the atherosclerotic plaque precipitating the rupture of the atherosclerotic lesion (Woolf 1983). This partial reduction in the arterial lumen may not cause significant occlusion of big arteries like the abdominal artery and the aorta, but it can cause great damage to the media of the smaller coronary vessels which may lead to "atherosclerotic aneurysm" (Robbins and Kumar 1987). Complicated atherosclerotic lesions affect

blood flow by reducing the availability of blood to the heart. The development of ischemic heart disease (IHD), due to decrease blood flow, may develop leading to recurrent episodes of angina pectoris (Boucek et al. 1984).

The lipid contained in the atheroma or complicated plaque exists in three chemical states, each with different densities. These are phospholipid, cholesterol ester, and free cholesterol. Small (1988) separated the lipid contained in the complicated plaque using a sucrose medium. The uppermost layer consisted mainly of cholesterol ester lipid droplets. If one visualizes the point at which the cholesterol ester separates in the sucrose preparation in the context of the gruel or complicated plaque it follows that since these cholesterol droplets are less dense, they will be at the crest of the atherosclerotic plaque. However, the presence of cholesterol ester together with phospholipid indicates that as more cholesterol ester accumulates in the complicated plaque, the lipid droplets tend to penetrate deeper into the atherosclerotic lesion and hence unavailable to efflux into the arterial lumen. This intermediate phospholipid layer gives way to the next layer of lipid which is rich in cholesterol monohydrate crystals. These cholesterol crystals come from the cholesterol ester that has made its way through the plaque's crest and the phospholipid present in the atherosclerotic lesion. These cholesterol crystals tend to

be very stable, and their location furthest from the crest of the plaque prevents their efflux into the blood stream (Small 1988). However, these can be dissolved in cholesterol ester which, under specific experimental conditions (size of the crystals, temperature) serves as a solvent of cholesterol monohydrate crystals (North et al. 1978). North, Katz and Small (1978) suggest that, even though cholesterol crystals seem to be inert, the dissolution of cholesterol monohydrate crystals into cholesterol ester oil is not a rate-limiting step in the reversal of an atherosclerotic plaque, but it is rather the transport of cholesterol ester out of the endothelium. If transport of dissolved cholesterol could be enhanced, cholesterol monohydrate crystals could be rapidly dissolved facilitating the reversal of atherosclerotic lesions (North, Katz and Small 1978). The transport of cholesterol out of the endothelium may well be augmented by the use of low cholesterol diets and/or hypocholesterolemic agents which lower serum cholesterol.

Due to the high stability of the cholesterol found in the complicated plaque or atheroma, Small and other researchers (Small et al. 1984; Katz et al. 1982) concluded that the turnover rate of cholesterol in the atheroma is very slow, when compared to the turnover rate of cholesterol in muscle, skin, and even in tendon. This is attributed to the fact that the atheroma has three phases

of cholesterol content and each lipid phase has different physicochemical characteristics. The turnover rate of cholesterol in the liquid phase (cholesterol ester droplets) is not as slow as the phospholipid and the crystalline phases where cholesterol appears to be almost inert for lipid mobilization (Small 1988). However, it does not mean that cholesterol cannot be mobilized, but rather that the actual mobilization of cholesterol from atherosclerotic lesions is a very slow process.

The properties of these morphologically distinct stages of atherosclerotic development may give us clue to understand which treatments may be more useful for a successful approach to alter the atherogenic process.

Lipid and Cholesterol Metabolism

Lipids are, by definition, chemical compounds that are water insoluble as a result of the nonpolar hydrocarbon regions that make a sizable proportion of their structure. The lipids are classified in three basic groups: the triglycerides, the phospholipids, and cholesterol. All three have biological importance in that they serve for different purposes in our body (Kirk 1980).

The triglycerides are an important lipid source for the production and storage of energy in different metabolic processes. Phospholipids are the principal components of plasma membranes forming stable bilayers. Cholesterol plays an important role throughout the body as it regulates the fluidity of the plasma membrane and serves as the template for the biosynthesis of important molecules like steroids.

Cholesterol is obtained from two main pathways, namely the exogenous pathway and the endogenous pathway. The exogenous pathway of cholesterol begins in the intestine where dietary fat is absorbed by the intestinal microvilli and incorporated into particles called chylomicrons and then delivered to the liver and the adipose tissue. Cholesterol is readily absorbed in the intestine with the help of hepatic bile which is composed of cholesterol, bile salts, lecithin and other inorganic salts helping in the

emulsification process of fats (Ganong 1987). The chylomicron particle has specific apoproteins, apoprotein B-48 and apoprotein E, which are cleared by the liver through a receptor mediated endocytosis clearance pathway. The endocytosed cholesterol is esterified in the liver by the enzyme acyl cholesterol CoA: acyltransferase (ACAT) and is stored in the ester form until needed (Grundy 1986). The liver, in turn, delivers the necessary cholesterol, phospholipid and triglyceride in association with the lipoproteins to the rest of the body.

An integral part of the metabolism of lipids is their transport in the blood. Lipids are insoluble in water. Although 91% of the plasma in which blood cells are suspended is water (Woolf 1983), the total lipid concentration in our blood achieves levels of 300-750 mg/dl! The dilemma is resolved by the fact that lipids are part of a complex with specific proteins at different concentration percentages. The proteins that are associated with lipid are referred to as lipoproteins. And these particles are part of the endogenous pathway of cholesterol metabolism.

The different types of lipoprotein containing particles, in addition to the chylomicrons, vary in their concentration of lipid and protein and therefore in densities. The three main lipoprotein classes are: 1) the ß-lipoproteins or low density lipoproteins (LDL); 2) the

pre-ß lipoproteins or very low density lipoproteins (VLDL); and 3) α-lipoprotein or high density lipoprotein (HDL) (Kaplan et al. 1988). The protein constituent associated with the lipids are called apoproteins (Flier ed. 1985). The purpose of the apoproteins is mainly recognition by the different receptors and enzymes that will act on the lipoprotein for their metabolism.

The very low density lipoprotein is formed in the liver by a process very similar to that which takes place in the intestine for the synthesis of chylomicrons, including the utilization of apoprotein B as the major component of this lipoprotein. However, VLDL has a larger protein than the one used in chylomicrons, called apoprotein B-100. VLDL is transported to adipose tissue and other extrahepatic tissues. As it leaves the liver, VLDL also requires the addition of apoprotein C so that the particles are recognized as a substrate for the enzyme lipoprotein lipase. As the enzyme removes triglyceride from the VLDL core, the particle is reduced in size as it increases in density. Once most of the triglyceride is metabolized by the action of lipoprotein lipase, apoprotein C is lost from the molecule (Ganong 1987).

However, VLDL has a role which transcends this triglyceride transporting function, since VLDL is the precursor of low density lipoprotein (LDL). As the triglyceride concentration decreases further under the

action of lipoprotein lipase, the final product results in the formation of LDL (Vander et al. 1985). The LDL particle serves as the major lipoprotein for the transport of cholesterol and cholesteryl esters (Rifkind and Levy 1977). In steroid biosynthesis, LDL provides the lipid necessary for the conversion of cholesterol into steroids. LDL is also the main lipoprotein for the transport of lipid back to the liver where it can be fused with more triglyceride to regenerate more VLDL particles or for the conversion of cholesterol into bile acids (Ganong 1987).

The role of LDL in the regulation of cholesterol metabolism is due mainly to the presence of the low density lipoprotein receptor which recognizes the apoprotein B-100 moiety on the LDL particle. However, the intermediate of VLDL, namely intermediate density lipoprotein (IDL), also bears apoprotein B-100 but more importantly apoprotein E, which has a higher affinity for the LDL receptor and consequently it is cleared from the circulation at a higher rate than LDL. Because the LDL particle only has apoprotein B-100 which has lower affinity for the LDL receptor than apoprotein E, the LDL particle has a longer half life ($t_{\frac{1}{2}}$) than IDL particles and the LDL achieves higher cholesterol concentrations in plasma (Mahley and Innerarity 1983).

Much of our understanding of the regulation of cholesterol metabolism has come from the studies of Michael S. Brown and Joseph L. Goldstein whose investigations using

cultured fibroblasts from both normal individuals and those suffering from familial hypercholesterolemia (type II hyperlipidemia) led to the discovery of the LDL receptor.

When Brown and Goldstein began their work in 1972 in an attempt to understand the genetic disease known as familial hypercholesterolemia, they found that the concentration of cholesterol in the blood of these patients was elevated many times above normal and heart attacks tended to occur early in life. They postulated that "this dominantly inherited disease results from a failure of the end product repression of cholesterol synthesis. The genetic disease of familial hypercholesterolemia was shown to be caused by inherited defects in the gene coding for the LDL receptor which disrupted the normal uptake of cholesterol and therefore control of cholesterol metabolism" (Brown & Goldstein 1986). The discovery of the LDL receptor earned Brown and Goldstein the Nobel Prize in Physiology and Medicine in December 9, 1985.

In an article written in 1979 and published in Nature, Brown, Anderson and Goldstein first described their understanding about the process by which the LDL receptor recognized the LDL particle and induced receptor-mediated endocytosis. "The macromolecules that bind to specific cell-surface receptors are internalized via coated pits at a much greater rate than the rate at which substances dissolved in the extracellular space enter cells by fluid

phase endocytosis". Cholesterol uptake by cells proceeds by the former fashion. If this uptake is blocked, cholesterol accumulates in the blood and can contribute to the formation of atherosclerotic plaques in the blood vessel walls (Brown et al.1979, Alberts et al. 1983).

The structure of the human LDL receptor is described by Brown and Goldstein as a large complex molecule made up of cysteine rich regions of about 292 amino acid residues that binds LDL (ligand binding domain); a region of about 400 amino acid residues that is homologous to the precursor for epidermal growth factor; a 58 amino acid region that is rich in serine and threonine and is the site of glycosilation by O-linked sugars; a stretch of 22 hydrophobic amino acid residues that spans the cell membrane; and a portion of 50 amino acid residues that projects into the cytoplasm (Ganong 1987). The LDL receptor is synthesized in the rough endoplasmic reticulum as a precursor that contains high mannose N-linked carbohydrate chains and a core sugar (N-acetyl-galactosamine) of the O-linked chains (Brown & Goldstein 1986).

The genetic defects which cause familial hypercholesterolemia arise from mutation in LDL receptor biosynthesis. These mutations do happen at different points of the process, namely:

a) the receptors are not synthesized;

b) the receptors are slowly transported from the

rough endoplasmic reticulum to the Golgi apparatus;

c) receptor may fail to bind LDL normally;

or d) the receptor may fail to cluster in coated pits and endocytose the LDL particle (Brown & Goldstein 1986).

One of the most important functions of the LDL receptor in living organisms was elucidated from the results of the studies performed on Watanabe Heritable-Hyperlipidemic (WHHL) rabbits. These rabbits had a mutation in which the LDL receptors, although synthesized, were slowly transported from the rough endoplasmic reticulum to the Golgi apparatus where the receptor protein gets glycosilated. When the genetic defect was present in the homozygous form, the LDL cholesterol levels were extremely high and the rabbits developed atherosclerosis very rapidly. Normally, when there is accumulation of free cholesterol in the cell the activities of two microsomal enzymes are altered: 1) 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase which is the rate-limiting step in *de novo* synthesis of cholesterol is suppressed; and 2) acyl cholesterol CoA: acetyltransferase (ACAT) which esterifies free cholesterol is activated. Accumulated cholesterol also causes the feedback inhibition of LDL receptor synthesis. In the Watanabe rabbits it was found that the low metabolic degradation of LDL particles and the overproduction of LDL

were associated with the absence of a mechanism to increase the biosynthesis of LDL receptor, thereby increasing the cholesterol concentration in the blood (Watanabe 1980). Now it is understood that the absence of the LDL receptor is considered, at least in part, responsible for hypercholesterolemia and the precipitation of the atherosclerotic process.

Experimental data have indicated that modifications on the lipid constituents of the LDL particle may exert its atherogenic properties by routes other than the interaction between LDL and its specific receptors. Several reports have actually demonstrated that LDL induces cytotoxicity, injury, or inhibition of proliferation of cultured endothelial cells. Such toxicity needs the presence of modified LDL which probably occurs as a result of LDL lipoprotein oxidation (Malmendier et al. 1987).

Therefore, if the LDL receptor mediates the removal of the LDL from plasma, the maneuvers that increase LDL receptor activity or number will affect the rate at which the LDL particles are metabolized. Pharmacological treatments have been developed which lower plasma LDL and cholesterol concentrations. These therapeutic regimes shall be discussed in detail in the section about hypocholesterolemic agents.

While high levels of LDL particles are associated with a high risk for atherosclerotic development, another

lipoprotein of the endogenous pathway of cholesterol metabolism, high density lipoprotein (HDL), has been suggested to have an opposite effect in reducing total plasma cholesterol levels (Miller and Miller 1975; Erickson and Carlson 1973). That is, HDL is believed to function in reverse transport or efflux of cholesterol out of the arterial wall (Boucek et al. 1984). The hypothesis that HDL may play a role in reverse cholesterol transport was first proposed by Glomset (1968). Later, Carew et al. (1976), in studying the role of HDL in cellular cholesterol efflux, demonstrated that HDL had the same capability to bind to endothelial cells in pigs as LDL. But HDL internalized cholesterol whereas LDL deposited cholesterol in the arterial wall (Carew et al. 1976).

HDL, like LDL, is also synthesized in the liver and contains several different apoproteins including apoprotein A, apoprotein C, and apoprotein E. The latter two apoproteins are released by HDL into the circulation and taken up by both chylomicrons and VLDL (Stryer 1981). The HDL particle contains 50% protein, 25% phospholipid, 15% cholesterol ester, 5% free cholesterol and 5% triglyceride. HDL has two types of apoprotein A, apoprotein AI and apoprotein AII in a 4:1 ratio. While apoprotein AI is important for the activation of LCAT, apoprotein AII is responsible for the proper activation of the enzyme lipoprotein lipase (Woolf 1982).

"Free cholesterol from extrahepatic tissues is transferred to HDL. It is esterified by LCAT, enabling HDL to take up more free cholesterol. Esterified cholesterol formed in HDL is then incorporated into the LDL lipid core . The LDL, carrying a load of cholesteryl ester, reaches the liver where these cholesteryl esters are hydrolyzed. Free cholesterol so formed enters the pool of free cholesterol in the liver that is available for removal by the bile, conversion into bile acids or reincorporation into plasma lipoproteins" (Woolf 1982).

HDL is also involved in the removal of cholesterol from various organs and tissues, and indirectly transporting cholesterol ester to the liver for further metabolism (Ganong 1987). If the effectiveness of this reverse cholesterol transport system is directly proportional to the concentration of HDL in the plasma, the negative correlation between HDL levels and the risk of development of atherosclerosis may indicate that reverse cholesterol transport is important in removing cholesterol out of the endothelium (Steinberg 1987). However, there are still no totally conclusive demonstrations *in vivo* of reverse cholesterol transport (Steinberg 1987). Since 1973 there has been data to indicate that the concentration of HDL is negatively correlated (Miller 1980) with the incidence of coronary heart disease (CHD), and that is why it has been commonly referred to as "good cholesterol". In

experiments performed by Miller and Miller (1975) it was shown that the body cholesterol pools increase with decreasing HDL concentration. And when coronary angiography was performed in 104 men between the ages 35 and 65 years, it was discovered that the men with high coronary scores (i.e., more atherosclerotic lesions) tended to have lower plasma HDL concentrations than those of similar age with low coronary scores (Miller et al. 1981). Some investigators suggest that a low concentration of HDL may increase the susceptibility to atherosclerosis but not that low concentrations of HDL accelerate the rate of the atherogenic process (Tan et al. 1980; Miller et al. 1981). Thus, whether a high HDL level protects against coronary heart disease or whether low levels of HDL may predispose an individual to coronary heart disease is still uncertain (Woolf 1982).

Foam Cells and Cholesterol

The mechanisms by which lipid accumulates in the arterial intima as a result of endothelial injury are very complex. The biochemical steps of atherogenesis have not yet been completely elucidated.There are several theories about the mechanisms by which the body is able to cope with the problem of intimal lipid accumulation and the ways the vascular system resolves such lesions.

The "response to injury" hypothesis of atherosclerosis is now widely held as most consonant with the large body of accumulated data. This theory, as presented by Robbins and Kumar (1987), states that; "(1) atherosclerosis is initiated as a response to various forms of injury to the arterial endothelium; and (2) endothelial injury leads to: (a) attachment of monocytes and platelets to the intimal surface; (b) proliferation of smooth muscle cells in the arterial intima; (c) synthesis by these cells of large amounts of connective tissue matrix, including collagen, elastic fibers, glycosaminoglycans (GAGS), and proteoglycans; and (d) depositions of intracellular and extracellular lipid that eventually results in the formation of a pool of lipid and cell debris in the core of advanced lesions" (Robbins and Kumar 1987).

According to this hypothesis, two main cell types contribute to the "response to injury" on the arterial

wall, namely the monocytes and smooth muscle cells. Both give rise to a single and histopathological form known as the foam cell: a characteristic subendothelial accumulation of lipid that conglomerate within cells as droplets giving these a foamy appearance (Robbins and Kumar 1987). Furthermore, abundant epidemiological, experimental and clinical data adjudge a primary atherogenic role of lipid accumulation and foam cell formation to LDL-cholesterol, the major component of the lipid content in plaques (Smith 1974; Smith and Slater 1972; Steinberg 1987). LDL and certain plasma proteins accumulate in the internal layers of the atherogenic wall. And it has been demonstrated that the amount of detectable LDL in the intima-media of human arteries correlates very well with the circulating levels of plasma LDL (Bourne 1985). Foam cells arise as a result of such lipid accumulation in the internal layers of the arterial wall.

Foam cells are derived from two cellular sources: the arterial smooth muscle cells and the monocyte derived macrophages (Malmendier et al. 1987). First, macrophages in the atherosclerotic lesion are thought to be derived from monocytes. "The factors responsible for the attraction of monocytes to the arterial wall are poorly understood. Chemotactic factors have been described as well as changes in the endothelial cells themselves induced by agents such as viruses, that enhance monocyte sticking. Monocytes are

observed adhered to the endothelium over an atherosclerotic plaque" (Bates & Gangloff 1987). Woolf (1882) reports that Adams and colleagues have shown that macrophages are "indeed present in the lesions of both human and rabbit atherosclerosis, since these cells can be identified histochemically by the use of either catalase or cytochrome-oxidase techniques".

A general consensus that some foam cells are derived from mononuclear phagocytes was reached in the years 1960-1962 on the basis of electron microscopic examination of experimental and human atherosclerotic lesions (Woolf 1982). "These mononuclear phagocytes may originate either from the circulating blood or wandering tissue histiocytes, but they phagocytize fat *in situ*, i.e., in the intima. However, this is not the initial step in the evolution of the lesion as it represents a sequence to an earlier change involving the native cells (endothelial cells)" (Geer & Haust 1972). It is at the fatty streak stage that the macrophages appear to be the predominant element in the foam cell population. These macrophages are not only the most common source of foam cells, but also they can easily ingest extracellular cholesterol from the arterial wall and metabolize it providing the means for excess cholesterol removal (Brown, Ho and Goldstein 1980). It is still not clear how macrophages can internalize the cholesterol in the intima and how these cells are recycled so that new

macrophages can arrive at the site of excess cholesterol accumulation and remove it effectively. But data from observations using scanning and transmission electron microscopy suggests that macrophages can actually penetrate the arterial wall through the tight junctions of the endothelium enabling these to removed the lipid trapped within the arterial wall. And, as suggested before, some investigators believe that atherosclerotic lesions generate chemoattractants that recruit circulating monocytes to the intima by monocytes (Stary 1987).

Macrophages are not the only type of lipid-laden cell in the atherosclerotic plaque. Smooth muscle cell-derived foam cells have also been described (Gown 1986). The first reports of lipid phagocytic activity by smooth muscle cells was made by Jores in 1903 when he noticed a "mixed type of hyperplasia of the musculo-elastic layer with fatty degeneration and superimposed proliferation of connective tissue" (Woolf 1982). But it was not until 1960 thát fat-containing smooth muscle cells were unequivocally identified in the fatty streaks of experimental and human atherosclerosis with the help of the electron microscope.

In the early atherosclerotic lesions (fatty streaks) lipid accumulates in macrophages and later in smooth muscle cells. Hence, foam cells result from the accumulation of large amounts of fat in both types of cells (Geer & Haust 1972). Through histological analysis, fat accumulation in

smooth muscle cells appears as reticulated cytoplasmic inclusion containing strands of dense material (Woolf 1982).

Unlike macrophages which can be made to accumulate large amounts of cholesteryl ester *in vitro* as demonstrated by the detection of lipoprotein-antibody complexes, smooth muscle cells do not have receptors for modified lipoproteins and the synthesis of LDL receptors of the latter are efficiently down regulated with small increases in cellular cholesterol. Smooth muscle cells internalize the lipid by mechanisms that are still not known (Klimov 1988).

Even though extensive experimentation has not been performed to outline the actual mechanisms of foam cell formation, very recent studies are bringing new evidence which may in part clear some uncertainties about foam cell cytogenesis. It was observed by Brown and Goldstein (1983) that cultured macrophages, in the presence of native LDL, were unable to convert into foam cells despite the high LDL concentration in the culture plates. This phenomenon gave rise to the postulation that "the circulating LDL particles must first undergo some kind of post secretory modification which then enables the macrophages to develop rapidly into a foam cell". They found that chemical acetylation converted LDL to a form which macrophages could endocytose more rapidly than native LDL particles. Moreover, it was

also found that smooth muscle cells in culture were also able to implement a similar modification of LDL particles into an oxidized or acetylated form (Hendriksen 1983).

LDL particles in a medium with cells found in arterial wall underwent peroxidation of polyunsaturated fatty acids in the LDL lipid. The reaction for LDL modification takes place in the presence of a transition metal ion like copper or iron at low concentrations. The reaction is inhibited by the addition of a metal chelator like ethylenediamine tetraacetic acid (EDTA). The free fatty acid lecithin, present in LDL, is converted to LDL lysolecithin by the action of oxidative lipid modification. The enzymatic reaction for the conversion of LDL lecithin into LDL lysolecithin is carried out by the enzyme phospholipase A_2. Although the precise mechanisms are not known, once some of the free fatty acid lecithin gets converted to lysolecithin, the reaction proceeds at a much faster rate, especially in the presence of low concentrations of metal ions (Steinberg et al. 1989).

The cytotoxicity of oxidized LDL could conceivably, as once suggested by Malmendier et al. (1987), induce functional changes in the subendothelial space and thus accelerate the formation of the fatty steak and the subsequent accumulation of LDL cholesterol in the arterial intima (Steinberg et al. 1989). Furthermore, oxidized LDL is also a potent chemoattractant for circulating human

monocytes, an effect elicited by the lysolecithin generated during the conversion of LDL to its oxidized form (Quinn et al. 1987).

Atherosclerotic Regression and Diet

Throughout this paper it has been suggested that a high cholesterol level promotes atherosclerosis. The question we must now address is what are the effects of lowering the plasma cholesterol levels on coronary heart disease. In a consensus conference held in December 1984 by the National Institute of Health authorities on cholesterol and its relationship with cardiovascular disease arrived at the conclusion that reduction of blood cholesterol reduces the rate of coronary heart disease (Consensus Conference 1985). Clinical trials and studies have provided evidence that the incidence of coronary heart disease can be ameliorated by reducing cholesterol intake and supplementing the diet with fiber and the administration of certain cholesterol lowering drugs or hypolipidemic agents (Consensus Conference 1985). Some of these diets and drugs that help reduce cholesterol levels is supported by a growing body of evidence which indicates that the process of atherosclerosis is almost completely preventable and that it is substantially reversible (Wissler and Vesselinovitch 1977), both in primates and humans (Malinow 1981).

Atherosclerotic regression, as defined by Malinow (1980), indicates anatomical changes observed after a drastic reduction in marked hypercholesterolemia induced by

diet. "At the microscopic level, regression includes:

(1) restored integrity of the endothelium lining the plaques;

(2) arrest of intimal cell proliferation;

and (3) a decrease in the number of cells, in the amount of intracellular and interstitial lipid, and in the extent of necrotic and calcified foci in the plaques".

Atherosclerotic regression occurs in a stepwise fashion. First, capillary sprouts derived from endothelial cells penetrate the atherosclerotic plaque clearing debris from the lesion and oxygenating the area to be repaired (Adams 1984). Second, blood-borne monocyte/macrophages transmigrate into the arterial wall and phagocytize the debris (Gaton & Wolman 1984). Finally, the structure of the arterial endothelium is repaired by the action of fibroblasts which synthesize collagen restoring the arterial wall to normal thickness resulting in a fibrous scar on the vessel wall which has a low content in ground substance and cells (Linder et. al 1984).

In order to achieve atherosclerotic regression, accumulated cholesterol must be removed or at least lowered in concentration in the lesion as well as in blood. Thus the aim of inducing regression by initiating a diet or other lipid lowering regimens, is to decrease the size of the atherosclerotic plaque which may be occluding blood

flow (Wissler and Vesselinovitch 1977). "When plasma cholesterol is lowered to 150mg/dl, lipids are mobilized from the lesions and regression gradually occurs. This loss of lipid reduces the volume of the lesion appreciably, which should increase luminal area an blood flow and ameliorate the clinical effects of the disease. After prolonged periods of low plasma cholesterol, cholesterol esters and foam cells disappear and [cholesterol monohydrate crystals] gradually dissolve, leading to true regression" (Small 1988).

In a human prospective study performed by Dr. Buschwald from the University of Minnesota School of Medicine, regression of coronary lesions based on sequential arteriograms occurred after a partial ileal bypass (i.e., removal of a section of the ileum (Wissler and Vesselinovitch 1977). This surgical procedure appears to elicit regression by reducing the intestinal surface available for absorption of bile acids which diminishes the absorption of cholesterol and increases hepatic expression of LDL receptors (Barndt et al. 1977). A year later, Blankenhorn presented a summary of human angiographic studies in which a considerable number of study groups reported cases of atherosclerotic regression. It was Blankenhorn's impression that most of these studies were performed on the femoral artery because it offered the advantage of being more accessible for observation than

coronary arteries. Some of the studies provided by Blankenhorn (1977) have indicated 3 cases of atherosclerotic regression out of 31 patients and in others 9 cases of atherosclerotic regression out of 25 patients. The fact that these observations were assessed by only two serial femoral angiographies in each case, the short length of these studies and the variable severity of atherosclerotic lesions may have posed some limitations to the interpretation and results of these studies. A case report of a 46 year old white male who suffered from exertional angina and mild hyperlipidemia showed a spontaneous regression based on a series of arteriograms of the left anterior coronary artery upon modification of his diet to a low cholesterol intake which reduced his blood cholesterol level (Roth and Kostuk 1980). Although it is just a single case atherosclerotic regression, this is a representation of the possibility of atherosclerotic regression in humans.

Atherosclerosis is due mainly to an elevated concentration of cholesterol. Much of this cholesterol arises as a result of a diet high in saturated fat. The first step in treating high levels of cholesterol is by modifying the diet. Phillipson and colleagues (1985) they discovered that dietary fish oil, which is rich in polyunsaturated fatty acids, had profound hypolipidemic effects on each of the 20 patients diagnosed with

hypertriglyceridemia. The total plasma cholesterol and triglyceride fell in every patient without exception. Polyunsaturated fatty acids like linoleic acid (C 18:21 w-6) and oleic acid (C 18:1 w-9) have hypocholesterolemic properties. When compared with saturated fatty acids, both types of unsaturated fatty acids caused similar reduction in the LDL cholesterol level. In contrast, linoleic acid lowered the HDL cholesterol while oleic acid did not. This perhaps could account for the apparent extra hypocholesterolemic effect of linoleic acid over oleic acid. The capability of polyunsaturated fatty acids to lower serum cholesterol appears to have a beneficial effect in the incidence of coronary heart disease. Diets high in polyunsaturated fat seem to influence the composition of arterial tissues and atheromas by reducing the availability of cholesterol (Dayton et al. 1965) However there was no concrete evidence in this study on whether or not polyunsaturated had the capability of promoting atherosclerotic regression. Both fatty acids act in the liver replacing the saturated fat pool for lipoprotein metabolism, although they have no known effect on the LDL receptor (Grundy 1986).

The most convincing evidence of regression of advanced atherosclerosis in non-human primates was performed by Armstrong and Megan (1972). They reported that prominent atheromatous lesions in the coronary arteries decreased in

their cholesterol content when monkeys were fed a high fat diet and then placed in a diet high in polyunsaturated fat for 40 months. Also there was a significant decrease in collagen and elastin content in the atherosclerotic arteries (Armstrong and Megan 1972). Atherosclerotic regression in these monkeys was suggested by the fact that the lumen of the coronary arteries increased in size and because the reduction in the thickness of the intima was accompanied by a decrease in the lipid content of the arterial wall. These studies have also been repeated and supported by other investigators (Malinow 1980; Clarkson at al. 1979; Wagner, St. Clair and Clarkson 1980).

Dietary fiber has also important hypocholesterolemic effects and may reduce risk for coronary heart disease (Anderson 1987; Anderson & Tietyen-Clark 1986). These endogenous components of plant materials in the diet that are resistant to digestion by enzymes produced by man can be classified into water-insoluble fiber (e.g. wheat bran and alfalfa) which decrease transit time and fecal weight; and water-soluble fiber (e.g. oat bran) which have shown to decrease the glycemic response of foods and lower cholesterol concentration (Anderson 1987). A diet low in cholesterol and high in dietary fiber may achieve serum cholesterol reductions exceeding 20% by the implementation of a diet high in fiber which may lower the risk for coronary heart disease and may even reverse the

atherosclerotic process (Anderson & Tietyen-Clark 1986). Barndt et al. (1985) demonstrated that after 25 patients were treated with a combination of a high fiber diet (which resulted in weight loss) and hypocholesterolemic drugs, femoral angiography revealed regression of atherosclerotic lesion. The regression of the atherosclerotic lesion in the femoral artery was accompanied by a significant reduction in plasma cholesterol levels. However, the regression observed in this study was only observed on atherosclerotic plaques that were small and not very much developed. Lesions that have not developed into complicated plaques regress more rapidly because their cholesterol turnover is faster. Studies performed by Katz et al. (1982) on cholesterol turnover suggest that cholesterol ester droplets located proximal to the arterial lumen can leave the arterial wall more rapidly if the atherosclerotic lesions have not developed into extremely necrotic lesions. Thus, if cholesterol levels are lowered and in turn the rate of cholesterol efflux is enhanced, then small atherosclerotic lesions (fatty streaks and intermediate plaques) can regress rapidly, and complicated lesions may also regress but at a much slower rate.

When water-soluble fibers from oat bran and bean products were incorporated into a diet low in fat and cholesterol for hypercholesterolemic men, serum cholesterol concentrations decreased 26% below initial values at 24

weeks and decreased 23% below initial values at 99 weeks (the latter 3% increase resulted from an increased HDL concentration). Previous studies in humans and rats have shown that oat bran intake lowers serum LDL cholesterol concentrations and raises the HDL concentrations (Kirby et al. 1981). Other human and animal studies suggest that these hypocholesterolemic effects of oat bran are related to the water soluble gum, ß glucan (Anderson et al. 1984). The mechanisms respónsible for the hypocholesterolemic effects of oat bran and other water-soluble fibers are still under intensive investigation. However it is thought that oat bran may act as a bile-acid binding agent which accelerates the removal of LDL cholesterol from the peripheral circulation (Anderson 1987). Although there is no evidence that oat bran actually induces atherosclerotic regression, its mechanism of action is suggested to be similar to that of the synthetic bile acid binding resins and therefore may prove useful in the future in the treatment of atherosclerosis.

In studies performed by Malinow and colleagues (1978), a decrease in serum cholesterol levels, normalization in the distribution of plasma lipoproteins, and reduction in the extent of aortic and coronary atherosclerosis was found when monkeys (*macaca fascicularis*) were fed a semipurified diet containing alfalfa. Even though these were fed a usual American diet, that study suggests that alfalfa counteracts

the atherogenic effect of dietary cholesterol. A diet consisting of 50% monkey chow and 50% alfalfa reduced plasma cholesterol from 287 ± 23mg/dl to 200mg/dl. In the group of monkeys receiving alfalfa there was evidence of "healing" from aortic and coronary atherosclerotic lesions caused by an atherogenic diet. The foam cells population was either very low or completely absent in the coronary arteries. The intimal lumen widened although the intima was thicker than normal. And the accumulation of fat-laden cells in the adventitia had largely disappeared. Malinow (1980) suggests that "the mechanism by which alfalfa may counteract atherogenicity of dietary cholesterol might be due to the insoluble complexes formed by alfalfa saponins with cholesterol which tend to precipitate micellar cholesterol suspension restricting their diffusion and impairing their absorption" (Malinow et al. 1978; Malinow 1980). A similar study performed by Srinivasan et al. (1980) in monkeys (*macaca fascicularis*) also showed that alfalfa produced a decrease in aortic tissue lipids.

Atherosclerotic Regression and Drug Treatment

Decisions to use drug therapy for hyperlipidemia must be based on the specific physiologic defect and its potential for causing atherosclerosis. Dietary measures, like the ones described in the previous section, are always initiated first and may obviate the need for drugs (Katzung 1987). The use of these drugs in the treatment of atherosclerosis is based on the effect that these exert on plasma lipoproteins. Some hypolipidemic drugs could act as agents of regression by either lowering the availability of exogenous cholesterol, decreasing the synthesis of endogenous cholesterol, or by altering lipid catabolism and hence the total lipid profile (Yong and Koda-Kimble ed. 1988).

One of these hypocholesterolemic agents, cholestyramine, was originally used to control pruritus in patients with elevated concentrations of bile acid due to cholestasis (Goodman and Gilman 1985). The bile acid binding resins are one of the drugs of used for the treatment of patients with primary hypercholesterolemia, particularly those with heterozygous familial hypercholesterolemia (Illingworth 1987). Colestipol (Colestid) and cholestyramine (Questran) are useful only in hyperlipoproteinemias involving LDL elevations. Tennent (1959) reported that a significant reduction in plasma

cholesterol occurred in dogs fed cholestyramine and subsequent studies have demonstrated that cholestyramine is a very effective drug for reducing plasma cholesterol and LDL in man (Rifkind and Kevy 1977).

Cholestyramine and colestipol are basic anion exchange resins, quaternary ammonium chlorides able to exchange chloride for the cholate ion of the bile acid (Meyers et al. 1980), hence often referred to as ion-exchange resins. They bind bile acids in the intestinal lumen and prevent their absorption (Katzung 1987). Once the chloride ion is exchanged for the bile acid, the complex so formed is unabsorbed as it is eliminated in the feces (Goodman and Gilman 1985). Colestipol hydrochloride is a tetraethylene-pentamine and epichlorhydrin copolymer(Kuo et al. 1979). If the bile acids are bound by a resin like cholestyramine or colestipol, their daily excretion is greatly increased (Meyers et al. 1980; Illingworth 1987). Studies in normal and hypercholesterolemic subjects demonstrated that bile acid binding resins markedly increase the fecal excretion of bile acids (Rifkind and Levy 1977).The hydroxylase enzyme that is responsible for the synthesis of bile acid from cholesterol is normally inhibited as bile acid feeds back in its synthetic pathway. However, if there is little or no bile acid returning through the enterohepatic circulation, then the inhibition of the hydroxylase enzyme is removed and it increases the synthesis of more bile

acids from cholesterol (Goodman and Gilman 1985). In response, the liver increases the biosynthesis of LDL receptors which consequently leads to even further decrease in the concentration of LDL cholesterol (Goodman and Gilman 1985).

The bile acid binding resins have no beneficial effect on patients who suffer from the homozygous form of familial hypercholesterolemia because the genes that code for the LDL receptor are not functional in these individuals. However, heterozygous have a partial mutation in the genes that code for the LDL receptor so that treatment with resins may prove useful (Katzung 1987).

The National Heart, Lung and Blood Institute (NHLBI) Type II Coronary Intervention Study with hyperlipoproteinemic subjects showed that the group that was treated with cholestyramine achieved a 26% reduction in the LDL-cholesterol while the group that was placed in a placebo achieved only a 5% reduction (both averaged over the 5 year period of the follow-up), indicating a statistically significant ($p < 0.001$) effect of drug treatment on LDL cholesterol (Brensike et al. 1984). Reports of an increase in HDL concentration have also been described in which an increase in 16 to 21% in HDL cholesterol levels with cholestyramine resulted in a lowering of the total/HDL cholesterol ratio from 8.0 at baseline to less than 4.7 (Hoeg et al. 1987). Studies with

Watanabe rabbits have shown that cholestyramine treatment early in life increases resistance to dietary cholesterol-induced hypercholesterolemia and atherogenesis in adult life (Subbiah et al. 1987).

Although the NHLBI Type II Intervention study was unable to provide conclusive evidence of regression of atherosclerotic lesions, it suggests that the lowering of cholesterol elicited by the implementation of cholestyramine plus diet lowers the rate of progression of observed coronary atherosclerotic lesions assessed by arteril angiography (Brensike et al. 1984). The study could have been limited by the small sample of patients, and the lack of consistently strong, statistically significant angiographic observations which may have given a wrong indication about the beneficial effects from the use of cholestyramine.

Studies in rhesus monkeys conducted by DePalma et al.(1979) revealed that regressive changes in the aortic, carotid and femoral arteries were produced when cholestyramine was added to an atherogenic diet. Regression of lesions was associated with lowering of serum cholesterol levels. Surgical and angiographic examinations were used in order to evaluate regression of experimental atherosclerosis (DePalma et al. 1979). When the treated animals had cholesterol levels lower than 250mg/dl for a period of 11 months, biopsy and angiographic evaluation

revealed evidence of plaque regression. At serum cholesterol levels above 300mg/dl, treated animals showed evidence of plaque regression at one site and progression at other sites. DePalma (1979) suggests that cholesterol levels above 300mg/dl may be a threshold above which regression can no longer be achieved. Other investigators have similar results with cholestyramine as an effective drug to lower cholesterol levels and to exert a regressive effect on the arterial wall of nonhuman primates (Wissler and Vesselinovitch 1977).

Srinivasan and colleagues (1980) in experiments with *macaca fascicularis* showed that cholestyramine treatment produced a remarkable depletion of both free and esterified LDL cholesterol from aortic tissue and regression of atherosclerotic plaques after 18 months of treatment. Furthermore, Malinow (1980) reported that a group of rhesus macaques receiving cholestyramine had shown regression even with cholesterolemia around 300mg/dl. Scanning electron micrograph of the aortas of these monkeys showed that, in contrast to the intimal surfaces of the aortic plaques at the end of the induction period, the intimal surfaces of the aortic plaques at the end of the study were covered by a continuous endothelium. "Transmission electron microscopy showed tight junctions and basement membrane reduplication, probably signs of repair" (Malinow 1980; Weber et al. 1977).

The resins are also used in combination with other drugs to achieve further hypocholesterolemic effect. One of these drugs is nicotinic acid, more commonly known as niacin. Nicotinic acid has been known for considerable time to reduce plasma cholesterol concentrations (Altschul et al. 1955; Carlson 1977). Nicotinic acid is a drug used for the treatment of patients with primary hypercholesterolemia (Illingworth 1987). The hypolipidemiclipid properties of nicotinic acid is separate from its role as a vitamin or coenzyme (Goodman and Gilman 1985).

Nicotinic acid is a water-soluble vitamin. Nicotinic acid is incorporated into the body and through a series of amide additions and nucleotide synthesis reactions is incorporated into nicotinamide adenine dinucleotide (NAD). "It is excreted unmodified in the urine and as nicotinamide, N-methyl-2-pyridone-3-carboxyamide, N-methyl-2-pyridone-5-carboxyamide, and other less abundant metabolites" (Katzung 1987). The normal human nutritional requirements for nicotinic acid is less that 30mg, while the doses required to lower serum cholesterol are often 100 times as much (about 3000mg/day) (Dukes ed. 1988).

Nicotinic acid has been demonstrated to be effective in lowering plasma cholesterol, LDL, triglycerides, and VLDL in various types of hyperlipoproteinemias. Nicotinic acid is readily absorbed from the gastrointestinal tract. However, the effect of nicotinic acid on VLDL and

triglyceride levels is more noticeable than the reduction of the cholesterol levels (Goodman and Gilman 1985).

The primary mechanism of action of nicotinic acid involves the inhibition of VLDL secretion which in turn decreases the production of LDL and IDL. The precise mechanism by which nicotinic acid reduces plasma concentrations of VLDL and LDL is unknown (Katzung 1987). But it is likely related to several of the drug's diverse actions. Fat is stored in adipose tissue as triglycerides but is released from fat cells for transport as free fatty acids (Meyers et al. 1980). The basic mechanism of action of nicotinic acid probably lies in its ability to block lipolysis by inhibiting the intracellular lipase system of adipose tissue (Woolf 1982); which in turn reduces the levels of free fatty acids in plasma. Nicotinic acid may also decrease the hepatic esterification of triglycerides and decrease the activity of lipoprotein lipase as a possible direct effect on hepatic production of apoprotein B-100 (Illingworth 1987; Goodman and Gilman 1985). Due to the ability of nicotinic acid to effectively reduce VLDL levels, the combined therapy of bile acid sequestrants with nicotinic acid has been demonstrated to have a synergistic effect.

Nicotinic acid did not appear to have any effect on mortality from coronary heart disease in rabbits (Parwaresch et al. 1978). Its effects on cholesterol levels

and the arterial intima have been reported in experimental studies (Woolf 1982). Parwaresch et al. (1978) showed that in rabbits after a period of atherogenic induction, nicotinic acid had a clear hypocholesterolemic and anti-atherogenic effect as measured by plasma cholesterol concentrations and planimetric evaluation of the atherosclerotic lesions in the aorta. Nicotinic acid was able to increase HDL:LDL ratio (Parwaresch et al.1978).

In 1981, Illingworth et al. reported in The Lancet about the treatment of familial hypercholesterolemia (FH) with the use of nicotinic acid in conjunction with one of the bile acid binding resins, colestipol. Sequential treatment of thirteen patients with fat restricted diet, diet plus colestipol, and diet and colestipol and nicotinic acid revealed cholesterol levels of 415 ± 69 mg/dl, 327 ± 54 mg/dl, and 246 ± 49 mg/dl, respectively. These results indicate the usefulness of combined drug therapy with a bile acid sequestrant and nicotinic acid to reduce plasma cholesterol levels in patient with heterozygous FH. Although the long term use of nicotinic decreased the progression of atherosclerotic lesions, this study in particular did not include any concrete evidence of atherosclerotic regression in FH patients (Illingworth et al. 1981).

The Cholesterol-Lowering Atherosclerotic Study (CLAS) (Blakenhorn et al. 1987) which included study of nicotinic

acid suggested that blood cholesterol lowering has directly beneficial effects in human atherosclerotic lesions after venous bypass graft surgery. In this double blind study coronary angiography was performed by percutaneous femoral technique before the randomization of patients into a placebo and a nicotinic acid-colestipol group. Treatment produced significant reduction in progression (but not regression) of atherosclerosis in native coronary arteries and venous grafts. The CLAS is the first angiographic study providing clear evidence of a treatment effect on human atherosclerotic lesions. This study demonstrates that reducing LDL concentration to very low levels with the use of colestipol and nicotinic acid results in a significant benefit to both native coronary arteries and venous bypass grafts (Blankenhorn et al. 1987).

Probucol (Lorelco) is considered a drug of second choice in treatment of primary hypercholesterolemia (Gotto 1987). The hypocholesterolemic effect of probucol was determined in the early 70's in laboratory animals and man (Rifkind and Levy 1977). The chemical structure of the drug sets it aside from the other hypocholesterolemic agents, although it is considered a congener of clofibrate (a fibric acid).

The mechanism of action of probucol in humans is not clear. In rabbits, it appears to promote removal of LDL cholesterol through a pathway not mediated by the LDL

receptor (Steinberg 1986). Probucol increased the fractional removal rate of LDL, an action linked to increased bile acid excretion (Nestel & Billington 1980). Probucol has been demonstrated to be incorporated into LDL particles inhibiting the oxidative modification. The "antioxidant" property of probucolprevents the LDL from being taken up by macrophages which can only internalize oxidative modified LDL through its acetyl LDL receptor (Carew et al. 1987). THus even though the LDL concentration in blood is still high, the LDL-cholesterol cannot de incorporated into the atherosclerotic lesion by the macrophage scavenger pathway. The lowering action of probucol on HDL levels is profound, and it is opposed to the common principles of treatment of hypercholesterolemia in which other drugs like cholestyramine and niacin which tended to increase serum HDL cholesterol concentration (Berg et al. 1988). The drug clearly suppresses apoprotein AI synthesis which is necessary for HDL formation.

Although probucol decreases HDL as well as LDL levels by 10 to 30%, respectively, regression of xanthomas and improvement of ischemic EKG changes (increased Q-T interval), and coronary morphology measured by arterial angiography (diminished atherosclerotic progression) is induced by probucol administration in spite of HDL decreases (Berg et al. 1988). That is, probucol may have the ability to prevent the atherogenic process of

atherosclerotic plaque formation, but it still remains unclear whether treatment with probucol effectively produces atherosclerotic regression. This study was performed in only 16 patients between the ages of 20 and 40 years of age which is a very small group sample. However, studies performed by Wissler and Vesselinovitch (1983) suggest that the combined therapy of probucol with cholestyramine may actually cause regression in the aortas of rhesus monkeys. However, its efficacy and safety still remain to be tested by a long term trial (Oliver 1983). Thus, for safety reasons, treatment of a hypercholesterolemic patient with probucol is not recommended unless other means like diet, bile acid binding resins,niacin or lovastatin have failed to cause any change in LDL cholesterol levels.

Dujovne et al. reported that probucol and colestipol showed a synergistic effect if used concurrently. The probucol and colestipol combination reduced mean serum LDL levels from 242 ± 51 (SE)mg/dl during the diet and placebo to 171 ± 41 mg/dl.

Clofibrate and its recent congener gemfibrozil, have been one of the most widely used drugs for the treatment of hyperlipidemia since 1963 (Woolf 1982). Although its mechanism of action at the cellular level is not well known, the principal effects of clofibrate and other fibric acid derivatives, with the exception of probucol, is

to increase the rate of metabolism of triglyceride rich proteins due to increases in the activity of the lipoprotein lipase clearance system (Kesaniemi & Grundy 1984; Katzung 1987). This mechanism of clofibrate, in turn, enhances the rate of intravascular catabolism of VLDL and IDL to LDL. It increases the synthesis of VLDL or perhaps the protein component of that lipoprotein (Meyers et al. 1980). The secretion of VLDL from the liver is unaffected in hypertriglyceridemic patients and actually appears to be increased in normotriglyceridemic subjects. This could explain the lack of significant synergism in most patients with heterozygous familial hypercholesterolemia treated with a combination of bile acid binding resin and clofibrate (Katzung 1987). Clofibrate appears to have very little effect, if at all, on the plasma LDL cholesterol; although it slightly increases HDL cholesterol levels (Meyers et al. 1980).

Many studies have been carried out to evaluate the lipid lowering effects of clofibrate. But until 1974 there was no clinical end point or data suggesting that clofibrate may have some effect on atherosclerosis . Cohn, Sakai, and Langston (1975) performed an angiographic study in 40 patients in their late forties using clofibrate, 16 of whom underwent treatment with clofibrate. The study did not show any significant effect of clofibrate on the coronary arterial wall, but rather observed a significant

difference between the placebo and clofibrate groups in the degree of coronary disease progressionas measured by arteril angiography. These results were limited by the short duration of the study, crude estimates of change in coronary pathology, small numbers of patients and the patients' extensive degrees of coronary disease (Cohn, Sakai and Langston 1975).

Experiments with swine performed by Daoud et al. (1984) showed that a diet low in cholesterol did not result in a decrease in the size of the atherosclerotic lesion, but it did prevent their progression. However, the addition of clofibrate to such a diet caused regression which involved a significant size of the lesion in abdominal aortas.

Erikson et al. (1988) recently demonstrated that the use of fenofibrate, a derivative of clofibrate, caused regression in 5 out of 31 male patients between the ages of 35 and 65 years treated with the drug. Although the femoral angiography was performed up to three times without any complications, the visual analysis of the arteriograms revealed considerable inter-observer variation (Erickson et al. 1988). This study was limited by the fact that only 31 patients were receiving the drug and that variations were found in the analysis of the computerized angiograms.

Recent pharmacologic developments for the treatment of hypercholesterolemia has led to the discovery of a new type

of hypocholesterolemic drug. These drugs are inhibitors of the enzyme 3-hydroxy-2-methylglutaryl CoA (HMG-CoA) reductase which is the rate limiting step enzyme in the conversion of HMG-CoA to cholesterol (Goodman and Gilman 1985). Lovastatin (Mevacor) is extremely effective in lowering serum cholesterol by stimulating the removal of LDL-cholesterol from plasma via the LDL receptor mediated endocytosis (Prescription insert). Lovastatin has proven to be in many studies much more effective in reducing serum cholesterol levels than gemfibrozil alone (Tikkanen et al. 1988). In a comparison study lovastatin proved to be the only treatment free of side effects when compared to cholestyramine, neomycin and niacin (Hoeg et al. 1987). However, there are no published studies thus far that have demonstrated any evidence of the effect of HMG-CoA reductase inhibitors in the treatment of atherosclerosis. However, since this drug is indeed effective in reducing LDL-cholesterol levels, it certainly has the potential of being a candidate for inducing regression of atherosclerosis.

Conclusions and Recommendations

The primary aim in the treatment of atherosclerosis has been directed towards diminishing the content of cholesterol so as to prevent further lipid deposition in atherosclerotic plaques. With the use of animal models and arterial angiographies, evidence suggests that reduction of serum cholesterol and the anti-atherogenic effect of some hypocholesterolemic drugs promotes the regression of atherosclerotic lesions.

Studies which evaluate the effects of a modified diet low in cholesterol have demonstrated a beneficial effect in arresting the progression of atherosclerotic lesions. Animal models have demonstrated that the implementation of diets high in fiber and polyunsaturated fat modifies the lipid composition of plasma and reduces lipid accumulation in the arterial wall (Blankenhorn 1986; Armstrong and Megan 1972). Decreased deposition of cholesterol in the arterial wall results in the enhancement of cholesterol efflux from existing arterial lesions and the size of the atherosclerotic plaques is reduced. However, the mechanisms by which polyunsaturated fat and water soluble and insoluble fiber are capable of affecting lipid deposition is still unknown. Hypocholesterolemic drugs like cholestyramine and clofibrate have not only been effective in reducing serum cholesterol, but also have been shown to

have a positive effect in the progression and regression of atherosclerotic lesions as well. New discoveries in the pharmacologic field have led to the development of other drugs like mevinolin (Tobert 1987; Grundy and Vega 1985) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Nimer et al. 1988) which lower serum cholesterol but have not yet been proven to cause regression of atherosclerotic lesions.

There is need for further investigation with patients and animal models to prove that atherosclerotic regression can occur in humans. Correlation between observations of atherosclerotic regression in treated animals and clinical trials need to be enhanced. Studies with longer use of hypocholesterolemic drugs need to be performed in order to prove their capability for producing atherosclerotic regression.

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