Jpn. J. Vet. Res. 48(2-3): 147-166(2000)

Review Article

Cardiovascular Disease : A Historic Perspective.

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(Accepted for publication : September 18, 2000)

Abstract

Cardiovascular disease (CVD) is the leading cause of death and disability in the United States and in most industrialized nations. Major breakthroughs to modern day cardiovascular/lipid research have been attributed to the findings of the Framingham Heart Study and Gofman and colleagues who made associations between lipoprotein levels (LDL, VLDL and HDL) and CVD. Unfortunately, half of all CVD patients have none of the established coronary risk factors (hypertension, hypercholesterolemia, cigarette smoking, diabetes mellitus, obesity) and new strategies for identifying patients need be considered.

Although there remains little disagreement regarding the necessity to lower elevated plasma cholesterol levels, there remains much controversy regarding appropriate dietary means to accomplish this goal. The National Cholesterol Education Program (1993) proposed a dietary reduction (Step I and Step II diets) to the percent saturated fat and cholesterol consumed by at-risk patients. Many currently question about the effectiveness of these diets and an alternative diet, replacing saturated fats by monounsaturated fats (olive oil), has attracted recent attention.

While diet modification is considered the foundation of primary treatment, other interventions are frequently required. Although early drug trials demonstrated that agents such as nicotinic acid, clofibrate, gemfibrozil, bile acid-binding resins generally slowed progression of atherosclerotic lesions, lowered plasma cholesterol levels and decreased mortality from CVD, the greatest advance to current drug therapy involved the discovery of the "statins" (HMG-CoA reductase inhibitors).

In the current work, mechanisms for vascular dysfunction resulting in myocardial ischemia were explored and potential nutritional (dietary) and pharmacologic interventions were reviewed.

Key words : cardiovascular disease, coronary artery disease, lipid, LDL, atherosclerosis

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Cardiovascular disease (CVD) is the leading cause of death and disability in the United States and in most industrialized nations, and has been so for the last few decades[7,61,83,88]. Death from CVD currently accounts for 29% of the deaths worldwide (second leading cause) and is projected to become the leading cause of death, accounting for 36% per cent of all deaths, by the year 2020[7]. In economic terms, the direct cost of CVD in the United States was estimated to be $259 billion in 1996[7] and more than 1 million patients are hospitalized for unstable angina, alone, annually[94].

Although lifestyle factors attributed to modern society have been associated with the high incidence of severe atherosclerosis and CVD, the disease is not a new or unique disease, as demonstrated by findings of atherosclerotic plaques in Egyptian mummies[68]. Myocardial infarction (MI), per se, has only been identified as a clinical entity since 1912[7]. Although investigators haverelated the findings of angina pectoris and MI to the severe hypercholesterolemia associated with familial hypercholesterolemia as early as the 1930's, elevated blood cholesterol levels were not considered to be pathogenic and findings of cholesterol accumulations in atherosclerotic plaque were considered only as incidental findings as recently as 1948[88]. In response to the *heart attack epidemic* of that era, the National Heart Institute (currently known as the National Heart, Lung, and Blood Institute) was founded in 1948 to promote strategies regarding the treatment and prevention of coronary artery disease (CAD)[7,45]. Progress was slow and even though physicians had accepted hypertension, cigarette smoking and diabetes as high risk factors for CAD, patients were not considered to be hypercholesterolemic unless their plasma levels exceeded 300 mg/dl as recent as the 1960's[88].

The major breakthroughs to modern day cardiovascular/lipid research have been attributed to the findings of the Framingham Heart Study (an on-going prospective study to determine the risks associated with CAD)[10,37] and Gofman and colleagues (who correlated lipoprotein class to CAD)[27]. For the first time, direct associations were made between low density lipoprotein (LDL), very low density lipoprotein (VLDL) and inverse associations with high density lipoprotein (HDL) and CAD -findings which were confirmed by Miller[55] in a review of three major epidemiologic studies (Framingham, Tromso and Honolulu). Subsequent studies have identified that elevations in levels of triglycerides[13], the small dense forms of LDL, and the intermediate-density lipoproteins (IDL) must also be considered as high risk factors for CAD[88].

Lipid Metabolism (Overview)

Plasma Lipids[26,29]

The major plasma lipid classes include: *triglycerides / triacylglycerols* (Tg) (primary storage form of fatty acids consisting of a glycerol backbone with three fatty acids attached), *phospholipids*-primarily *lecithin / phosphatidylcholine* [glycerol backbone with two fatty acids (polyunsaturated fatty acid on the 2 nd position) and a phosphorylcholine (attached at the 3 rd position)], *cholesterol* and *cholesterol ester.*

Lipoproteins

Lipoproteins are spherical particles consisting of a triglyceride and cholesterol ester core and a surface that consists of phospholipid, free cholesterol and apolipoproteins. Historically lipoproteins have been classified on the basis of: 1) charge by agar or paper electrophoresis into: origin (chylomicrons); pre-beta (VLDL), broad-beta (IDL), beta (LDL) and alpha (HDL); 2) density (kg/L) by ultracentrifugation into: chylomicrons (<0.95),

VLDL (0.95-1.006), IDL (1.006-1.019), LDL (1.019-1.063) and HDL (1.063-1.21) ; and 3) flotation rate (Sf) into: chylomicrons (>400), VLDL (60-400), IDL (20-60), LDL (0-20) and HDL (0-9). The apolipoproteins, found on the surface of the lipoprotein, provide structural stability to the lipoprotein, act as an interface between lipid and aqueous environments, determine particle membranes by binding to specific receptors and act as co-factors for enzymes.

Apolipoproteins

The apolipoproteins and the corresponding lipoproteins and their functions are sumarized in the Table 1.

Table 1. Lipoproteins and their functions

apo*	lipoprotein	synthesis	function
A I	HDL	small intestine, liver	activation of LCAT
			ligand for HDL binding
A II	HDL	small intestine, liver	activation of hepatic lipase
A IV	HDL	small intestine, liver	role in activation of LCAT
B48	chylomicron	small intestine	assembly and secretion of chylomicron
B100	VLDL, IDL	liver	assembly and aecretion of VLDL
	LDL		ligand for removal of LDL by LDL receptor
C I	all (trace LDL)	liver	inhibit removal of chylomicron and VLDL remnants by liver
C II	all (trace LDL)	liver	inhibit removal of chylomicron and VLDL remnants by liver
C III	all (trace LDL)	liver	inhibit removal of chylomicron and VLDL remnants by liver
			inhibitor of LPL
E	chylomicron VLDL, IDL, LDL	liver (neurons, macrophages)	uptake of lipoprotein by LDL and LDL receptor-related protein (LRP)

* The apolipoproteins are named in an alphabetical order.

Metabolism

Lipoprotein lipase (LPL) mediates the hydrolysis of VLDL and chylomicron triglycerides to free fatty acids (burned for energy or stored as triglycerides in adipose tissue) and glycerol. Most circulating LPL is associated with LDL (and macrophages).

Hepatic triglyceride lipase (HTGL) is synthesized in the liver and can remove triglycerides from VLDL remnants (IDL) (conversion of VLDL to LDL), clear chylomicron remnants and convert HDL_2 to HDL_3 by hydrolyzing the triglyceride and phospholipid in HDL.

Lecithin : cholesterol acyltransferase (LCAT) mediates the reaction on the surface of HDL where a fatty acid (linoleate) attached to lecithin is transferred to free cholesterol to form cholesterol ester which, in turn, is transferred to VLDL (and LDL) (Apo AI is a required cofactor).

Cholesterol ester transfer protein (CETP) mediates the exchange of cholesterol esters from HDL with triglyceride from chylomicrons or VLDL (LDL cholesterol esters can also be exchanged with triglyceride from chylomicrons or VLDL to produce the small, dense LDL).

Lipid Transport

Intestinal mucosal triglyceride and cholesterol are incorporated into the core of the nascent chylomicron (surface coat consists of phospholipid, free cholesterol, apo B48, apo AI, apo AII and apo AIV), secreted into the lacteals and transported via the thoracic duct. Once in the plasma, apo C proteins are transferred from HDL (see Table 1 regarding apolipoprotein function) ; the attachment of the apo E enables the remnant to attach to hepatic LDL and/or LRP for processing [Function : dietary triglyceride (as fatty acids) delivered to muscle cells or adipose cells and cholesterol to liver for processing (including bile formation)].

In the liver, triglycerides and cholesterol (synthesized in liver or delivered by chylomicron remnants) combine with the apo B 100 and phospholipids to form VLDL, which is secreted into plasma, and apo CI, apo CII and apo CIII are added (Very large triglyceride-rich VLDL are formed with triglyceride abundance and small VLDL with triglyceride "depletion"). VLDL interacts with LPL and, as triglycerides are hydrolyzed, become smaller (and more dense) and converted to IDL which can enter the liver or give rise to LDL. The LDL receptor is present on the surface of most cells and is a major factor in determining plasma LDL cholesterol levels.

Reverse cholesterol transfer (removal of cholesterol from peripheral cells in liver) is thought to be the primary mechanism by which HDL protects against atherosclerosis. Apo AI may be the essential structural apoprotein for HDL and apo AI/phospholipid complexes may fuse with other complexes containing apo AI and apo IV to form the various HDL subtypes. These small, cholesterol poor HDL particles are referred to as HDL_3. Free cholesterol is transferred to the HDL_3, acted upon by LCAT to form cholesterol esters (which move to the core and increase capacity to accept more free cholesterol) to enlarge and become HDL_2 and cholesterol esters transferred to apo B lipoproteins (chylomicrons in fed state and VLDL in fasted state) (mediated by CETP) (triglycerides transferred to HDL which can be substrate for lipolysis by LPL or HTGL) to revert to HDL_3 or HDL_2 particle removed from plasma.

High Risk Factors

Subsequent to Kannel et al's initial report[37], the concept of risk factors for the risk of development of CAD were established ; hypercholesterolemia and hypertension were identified as major risk factors for developing

CAD (1). Current goals to reduce plasma cholesterol levels to < 160 mg/dl are based largely on data regarding the low incidence of CVD-related deaths in Japan during the 1960's-70's in the presence of other high risk factors (e. g. almost universal incidence of cigarette smoking and hypertension)[73,88]. Elevation in triglyceride levels, especially in the presence of glucose intolerance (type II diabetes), elevation in levels of LDL or reduction to levels of HDL, were added to the list of high risk factors as a result of work reported by Criqui et al[13].

More recently, Welch and Loscalzo[90], in a review article, identified elevated plasma homocysteine levels as an independent risk factor for development of atherosclerosis and atherothrombosis. The authors described homocysteine as a sulfur-containing amino acid formed by one of two pathways (remethylation and transsulfuration) during the metabolism of methionine and requiring folate, vitamins B_{12} and B_6 as essential cofactors. Accordingly, Selhub et al[76] suggested that deficiencies of one or more of these B vitamins could account for up to two thirds of all cases of homocysteinemia. Although Ross[66] and Welch and Loscalzo[90] noted that elevated homocysteine levels were toxic to the arterial wall and resulted in arterial endothelial dysfunction, obesity, elevated LDL or homocysteine levels and decreased HDL levels[7,61,73,88,85]. Other potential risk factors have been cited recently and include: estrogen deficiency, fibrinogen, factor VII, plasminogen-activator inhibitor type I, tissue plasminogen activator, d-dimer, lipoprotein (a) and C-reactive protein[7]. Other contributing/co-existing factors such as exercise (to lower LDL levels)[86] and inflammation resulting from infections such as herpes virus or *Chlamydia pneumoniae*[66,64] may modulate risk factors.

Pathology

Description of Early Lesions

Early (type 1) endothelial (atherosclerotic) lesions are best described as those involving an increase in the number of macrophages filled with lipid droplets/cholesterol ester in the endothelial intima (foam cells). The type 2 lesion is characterized by layers of foam cells within the intima with droplets of extracellular lipid (fatty streak). Type 3 lesions are characterized by type 2 changes with visible pools of extracellular lipid. These lesions may progress to advanced lesions which involve direct disorganization of the intima and deformity of the artery and may predispose or result in overt clinical manifestations, including ischemic episodes[16,84].

that a possible mechanism could be the removal of cholesterol by HDL (reverse cholesterol transport) for transport and subsequent elimination by the liver.

Vascular Dysfunction

Levine et al[46] described normal endothelial (arterial wall) function as "regulation of vascular tone, inhibition of platelet activity, maintenance of the balance between thrombosis and fibrinolysis, and regulation of the re- availability and lipoprotein oxidation was shifted towards a self-reinforcing cycle of NO relative unavailability and accelerated lipoprotein oxidation, thus favoring the promotion of atherosclerotic lesions. Regardless of the mechanism resulting in decreased EDRF/nitric oxide activity, the resultant impairment of normal endothelial vasodilation could well account for the apparent ischemic effects not otherwise attributed to visible stenosis[46].

cholesterol deposition in monocyte/macrophages or smooth muscle cells in vitro, the authors concluded that circulating LDL did not cause lipid accumulation in the endothelium, but in fact, resulted from transport by the monocyte/macrophages to the endothelium. Henriksen et al[32] subsequently demonstrated that LDL, incubated overnight with monocyte/macrophages, resulted in physically and chemically modified (oxidized) LDL that was rapidly taken up by the macrophages. Oxidation of LDL has been subsequently demonstrated in vivo[89].

Recent reports suggest that prevention of thrombosis or rupture of the atherosclerotic plaque may be one of the most critical steps in the prevention of acute MI[48]. Accordingly, one of the mainstay treatments for the prevention of MI has been the attempt to prevent thrombus formation by the use of aspirin (platelet cyclooxygenase inhibitor), heparin or warfarin (anticoagulants)[1].

Arterial Stenosis and Vascular Dysfunction

Arterial Stenosis

Although many drug (e. g. nicotinic acid, clofibrate, gemfibrozil, bile acid sequestrants), diet and surgical procedure (partial ileal bypass) clinical trials had demonstrated a slowing of the progression of atherosclerotic lesions, actual regression of atherosclerotic lesions have only rarely been reported[88]. Further, angiographic evidence regarding the severity of stenosis seldom correlate with the physiologic and clinical effects (e. g. basal and reserve coronary blood flow, reactive hyperemia) and the average change (if any) in the severity of the stenosis resulting from plasma cholesterol reductions are not of a magnitude that could explain the improvements to cardiovascular function and survival[46]. Further, Levine and colleagues[46] noted that there is no apparent relationship between severity of lesions and the probability of future cardiac episodes; most lesions that resulted in cardiac failure are, in fact, not stenotic; and that cholesterol reduction may in fact have benefits, not previously described, that could reduce ischemic events. In spite of the lack of improvement to atherosclerotic lesions, declines in measurable parameters such as MI or sudden death are frequently reported, even in short term (< 2 yr) interventions involving reductions to plasma lipid levels[88]. Since regression of existing stenotic lesions do not appear to result in these cardiovascular improvements, alternate theories that could better explain the benefits of lipid-lowering needed to be considered. Davies and Thomas[14] demonstrated that a majority of the MI observed involved plaque (especially plaque with large, necrotic, lipid cores) rupture with subsequent thrombosis. Falk[19] and Fuster et al[24] proposed the concept of lesion activation in which a quiescent atherosclerotic plaque undergoes changes that make it susceptible to and ends in rupture (resulting in MI). Richardson et al[70] provided evidence for this notion by reporting MI post mortem findings in which 83% of patients had demonstrable plaque rupture. This observation was confirmed by Sherman et al[77] who demonstrated the presence of thrombi and/or plaques in arteries of patients with unstable (at rest) angina. Richardson et al[70] further demonstrated findings of fissured plaques containing pools of extracellular lipid in plaques from 87% of patients who had died from coronary thrombosis while Friedman and van der Bovenkamp[23] observed that fissures occurred most frequently at the junction of plaque and normal intima and at sites containing lipid-laden macrophages. It was, therefore, proposed that the reduction to the cholesterol pool by lipid-lowering drugs resulted in plaque thinning and a reduction of plaque rupture[46]. Several authors[24,28,82] suggested

that a possible mechanism could be the removal of cholesterol by HDL (reverse cholesterol transport) for transport and subsequent elimination by the liver.

Vascular Dysfunction

Levine et al[46] described normal endothelial (arterial wall) function as "regulation of vascular tone, inhibition of platelet activity, maintenance of the balance between thrombosis and fibrinolysis, and regulation of the recruitment of inflammatory cells into the vascular wall" and endothelial dysfunction to be a general abnormality in one or all of these factors. The principal mediator of normal vascular tone was identified as endothelium derived relaxing factor (EDRF) and this substance was suggested as being responsible for the prevention of platelet and leukocyte adhesion to the endothelial wall[75]. Although acetylcholine (an EDRF agonist) induced vasodilation in normal coronary arteries, arteries with early or advanced atherosclerotic plaques exhibited paradoxical vasoconstriction when infused (angiographically) with acetylcholine[51], thus suggesting a loss of ERDF action. Okumura et al[59] subsequently demonstrated the impaired vasomotor function in infarct-related arteries when compared to similarly stenosed arteries.

Chin et al (11) noted that oxidized LDL or HDL_3 were highly potent blockers of EDRF/nitric oxide (NO) while Liao et al[47] demonstrated decreases in NO synthase mRNA expression and reduction in NO synthase activity (NO synthesis and release) when human aortic endothelial cells were exposed to oxidized (but not native) LDL. EDRF was, in fact, identified as being NO and, in arteries subjected to atherosclerotic lesions, lipoprotein oxidation was observed to suppress its activity (in spite of a normal rate of production)[35].

Jessup[35] further noted that, in the atherogenic state, the normal balance between NO availability and lipoprotein oxidation was shifted towards a self-reinforcing cycle of NO relative unavailability and accelerated lipoprotein oxidation, thus favoring the promotion of atherosclerotic lesions. Regardless of the mechanism resulting in decreased EDRF/nitric oxide activity, the resultant impairment of normal endothelial vasodilation could well account for the apparent ischemic effects not otherwise attributed to visible stenosis[46].

Others have reported similar paradoxical vasoconstriction as a result of mental stress[95], cold pressor tests[56] and exercise[25]. Such responses could, undoubtedly, result in abnormal vessel dilator responses resulting in myocardial ischemia[46]. In summary, the endothelial dysfunction associated with hypercholesterolemia and atherosclerosis could result in the development of acute coronary ischemia and MI, thus prompting the theory that correction of abnormal circulating cholesterol levels could improve coronary endothelial function[46].

Oxidation of Low Density Lipoprotein

The most likely mechanism for LDL modification is oxidation, an essential component of many cellular functions, but one which could result in free-radical mediated damage. When phagocytes (e. g. macrophages) are stimulated or activated, an increase in oxygen metabolism results and superoxide anions may be produced. Although the reactivity of superoxide ions in themselves may be limited, in the presence of transition metal ions, partially reduced forms of superoxide (hydrogen peroxide, the hydroxy radical and the hydroperoxyl radical) generated in the arterial subendothelial space could damage the lipid and apoprotein B moieties of LDL[16,60,93]. Lipoxygenases, peroxynitrite, and/or myeloperoxidase could also be involved[30].

Jialal and Devaraj[36] and Diaz et al[15] presented a potential model of oxidatively modi-

fied LDL (OX-LDL) that could be involved in foam cell development. In that model, LDL initially accumulates in the sub-endothelial space and is mildly/minimally modified (MM -LDL). MM-LDL, in turn, induces local vascular cells to release monocyte chemotaxic protein 1 (MCP-1), granulocyte and macrophage colony stimulating factors (M-SCF) and monocyte adhesion molecules. Monocytes would be attracted to and would bind to the endothelium, migrate into the subendothelial space and differentiate into macrophages. Once monocytes cross the endothelial layer, they could become trapped in the sub-endothelial space (in part because oxidized LDL inhibits egress from the arterial wall) and M-CSF could promote monocyte differentiation to tissue macrophages. The accumulation of more monocytes and macrophages, in turn, would stimulate further oxidation of the LDL. MM-LDL would be modified to a more oxidized form with further generation of chemically reactive products of lipid peroxidation and aldehydic products (such as malondialdehyde and 4-hydroxynonenal) which induce covalent modification of LDL[30]. The monocyte-derived macrophages would in turn take up the OX-LDL by attachment to the scavenger receptor. Ross[66] suggests that OX-LDL may in fact exacerbate the inflammatory response by stimulating (up-regulating) the replication of the monocyte-derived macrophages resulting in increased numbers of new monocytes entering the lesion. This processing could result in significant cholesterol accumulation and possibly foam cell development. Unlike the uptake of unoxidized LDL by the LDL receptor (apolipoprotein B and E) on macrophages, the uptake of the OX-LDL by the scavenger-receptor (a second macrophage receptor) pathway is not under negative feedback regulation, thus resulting in dramatic uptake of the OX-LDL cholesterol by the macrophages[83].

Duell[16] noted that these oxidized LDL particles possess many properties that may subsequently promote the progression of atherogenesis and include: cytotoxicity; immunogenicity; stimulation of adhesion of leukocytes to the endothelium, chemotaxis for circulating monocytes, IL-1 release, cytokine expression [e. g. monocyte chemoattractant protein-1 (MCP-1), macrophage colony stimulating factors (M-CSF's), tissue factor], smooth muscle proliferation, foam cell formation from macrophages; inhibition of motility of arterial wall macrophages, endothelin-1 secretion; and decreased endothelial relaxation. Monocyte-derived macrophages and neutrophils are able to initiate lipid peroxidation while the oxidative role of smooth muscle and endothelial cells may solely be to generate hydrogen peroxide or other oxidants[60].

As previously discussed, Welch and Loscalzo[90] identified elevated plasma homocysteine levels as an independent risk factor for development of atherosclerosis and atherothrombosis. The authors noted that homocysteine accentuates the endothelial dysfunction when it is auto-oxidized in plasma (forming homocystine, mixed disulfides, and homocysteine thiolactone) resulting in the production of superoxide and hydrogen peroxide, thus supporting further oxidation of LDL. Ross[66] and Welch and Loscalzo[90] noted that homocysteine was toxic to the arterial wall with decreased availability of nitric oxide to endothelium resulting in arterial endothelial dysfunction.

Of interest, in a review of several clinical trials, Nenster and Drevon[57] noted that LDL particles rich in the n-6 polyunsaturated fats were more susceptible to oxidative modification than saturated or monounsaturated fats (n-3 polyunsaturated fats were observed to have variable oxidative susceptibility).

Role of High Density Lipoproteins in Protection of Coronary Heart Disease (CHD)

HDL is considered to protect against CHD by a process known as reverse cholesterol transport, a process by which free cholesterol in peripheral tissues is incorporated into plasma HDL, esterified [by lecithin : cholesterol acyltransferase (LCAT)], transported to the liver and excreted as bile[12]. The reverse transport of cholesterol involves the incorporation of free cholesterol from peripheral cells into a nascent HDL molecule with esterification by LCAT, transfer to the particles core to become the HDL_3 ; which becomes further enlarged (by esterification of acquired free cholesterol by LCAT) to become the larger HDL_2 molecule. Much of the resultant cholesterol ester is transferred (for removal from plasma) to VLDL and LDL by the cholesterol ester transfer protein (CETP)[26].

More recent reports[22] of *in vitro* and *in vivo* trials suggest additional protective actions of HDL. These reports suggest that HDL, because it is more susceptible to oxidation than LDL, may act as an oxidative *sacrificial target* to reduce the potential oxidation of LDL. Further, HDL may have specific antioxidant capabilities as a result of: the antioxidant potential of apoAI, the blocking of LDL oxidation by the LCAT (associated with HDL) and the binding of free metal ions by HDL-associated transferrin and ceruloplasmin[22]. Additionally, since HDL carries the bulk of paraoxinase[22,4] (which cleaves oxidized fattly acids fron the phospholipids of oxidized LDL), it may indirectly block the inflammatory response of oxidized LDL in the arterial endothelial cells. Francis[22] also notes that HDL can block the oxidized LDL's inhibition of endothelial cell NO synthesis and may inhibit the retention of oxidized LDL by the matrix components of the arterial wall.

Inflammation

Although cardiovascular death currently accounts for 29% of the worlds mortality, half of all the patients have none of the established coronary risk factors (hypertension, hypercholesterolemia, cigarette smoking, diabetes mellitus, obesity)[1] and new strategies for identifying these patients need be considered. Ridker et al[64] and Ross[66] described atherosclerosis as an inflammatory disease. In addition to the classical risk factors for CHD, Ross[66] characterizes the disease, at all stages, as one of a progressive inflammatory cycle mediated by monocyte-derived macrophages and specific subtypes of T lymphocytes. In a prospective three year study of 28, 263 postmenopausal women, Ridker et al[64] assessed the risk of cardiovascular events by evaluating markers of inflammation. Of the 12 plasma measures studied, four markers of inflammation (hs-CRP, serum amyloid A, interleukin-6, and sICAM- 1) were found to be significant predictors of risk of future cardiovascular events ; one marker, hs-CRP, clearly distinguished women at high risk from those at low risk, even in the subgroup of women with LDL cholesterol levels below 130 mg/dl [the target considered safe in the current guidelines of the National Cholesterol Education Program[18]].

Therapeutic Interventions

Role of Diet in Atherosclerosis

Although a dramatic decline of CAD occurred during World War II when animal fats were limited[88], Keys[38,39] and Keys et al[40] were among the first to demonstrate the correlation of dietary intake of fat (in seven countries) to CAD. Later, others[31,53,92] clearly demonstrated that intake of diets rich in saturated fat elevated plasma cholesterol while diets rich in unsaturated fat lowered cholesterol levels. Data from the second National Health and Nutrition Examination Survey (NHANES

II) demonstrated that the average American diet provided 385 mg cholesterol per day and 37% total energy from fat (7% PUFA, 17% MUFA, 13% SFA). It is speculated that the diets rich in saturated fatty acids [palmitic (C16 : 0), myristic (C14 : 0) and lauric (C12 :), but not stearic (C18 : 0)], which are found commonly in animal and dairy product based fats, increase serum cholesterol levels by increasing 3 -hydroxy- 3 - methylglutaryl coenzyme A (HMG-CoA) reductase levels in the liver to produce more cholesterol[17,43,54]. Since the increased hepatic synthesis of cholesterol directly suppresses LDL receptor levels, there is a decreased capacity of the liver to remove cholesterol from the blood (for degradation to bile)[9]. Although there remains little disagreement regarding the necessity to lower elevated plasma LDL-cholesterol levels to reduce the risk of CAD, there remains much controversy regarding the appropriate dietary means to accomplish this goal[33].

In 1993, the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults[18] was released and proposed a dietary reduction in the percent saturated fat and cholesterol consumed by an at-risk patient. The Step 1 diet consisted of a diet in which the total calories consumed were made up of a maximum of 30% fat (10% PUFA, 10% MUFA, 10% SFA) (i. e. maximum of 10% of the calories from saturated fat) and less than 300 mg cholesterol per day. The Step 2 diet consisted of the same 30% total calories as fat but with a maximum of 7% saturated fat (10% PUFA, 13% MUFA, 7% SFA) and a maximum of 200 mg cholesterol per day.

Historically, monounsaturated fatty acids[20,31] and stearate (C18 : 0)[20,54] were considered neutral with respect to effects on lipid profiles. Mensink and Katan[54], in a meta-analysis of 27 trials, concluded that: 1) all fatty acids elevated HDL cholesterol when substituted for carbohydrates but the effects were diminished with increased unsaturation of fatty acid, 2) fat replacement by carbohydrate lowered serum cholesterol (regardless of saturation), and 3) replacement of saturated fatty acids by unsaturated fatty acids raised the HDL to LDL ratio (replacement by carbohydrates had no effect). Hegsted et al[31] undertook an extensive review of existing data (both metabolic and field studies) and similarly concluded that both dietary saturated fatty acids and cholesterol increased serum cholesterol, dietary polyunsaturated fatty acids decreased serum cholesterol and monounsaturated fatty acids had no independent effect on serum cholesterol. Howell et al[33] concluded that step 1 and 2 recommendations resulted in 4.5 and 7.7% (respectively) reductions in LDL cholesterol. Schaefer et al[74] observed that although a low fat, weight maintenance diet was effective in reducing total cholesterol and LDL levels in their subjects, there was a decrease in HDL and increase in triglyceride levels. The authors noted that this was consistent with the findings of others that high carbohydrate (increased consumption of fruit and vegetables) diets increase triglyceride levels. The authors further noted that the low-fat *ad libitum* phase of their study was characterized by a further decline in total and LDL cholesterol, a decline in triglycerides but no decline in HDL. This was consistent with Barnard's[6] findings that a 3 week residential program consisting of a high-complex-carbohydrate, high fibre, low-fat, low cholesterol diet combined with daily aerobic exercise (walking) was associated with a 23% reduction in LDL (males > females), a 16% reduction in HDL (females> males) and a 33% reduction in triglycerides. Kris-Etherton et al[42] noted that

when saturated fat energy is replaced by carbohydrate (a low-fat, high-carbohydrate diet), the diets do have beneficial effects on total cholesterol and LDL cholesterol but plasma triacylglycerol concentrations increase and HDL-cholesterol concentrations decline, resulting in potentially increased CVD risk.

It is for this reason that many currently question whether a Step I or Step II diet is an appropriate diet for reducing CVD risk. Accordingly, an alternative diet that has attracted recent attention is one in which saturated fat energy is replaced by one containing high levels of monounsaturated fatty acid (MUFA) (primarily diets utilizing olive oil, although canola oil peanut, soybean, rice bran and cottonseed oils may also be utilized) -a diet which may result in a higher total fat intake (i. e., > 30% of energy) than a Step I or Step II diet. It is possible that MUFAs may also reduce CVD risk as a result of their antioxidant, antithrombotic, and antihypertensive properties[20]. Kris-Etherton et al[42] confirmed that such diets did, in fact, lower total and LDL cholesterol but did not lower HDL or raise triacylglycerol levels. In doing so, the authors speculated that since most serum triacylglycerol was transported by VLDL, hepatic production and clearance of VLDL and circulating triacylglycerol may be altered as a result of the amount and type of fat in the diet. In interpreting Kris-Etherton et al's data[42], Feldman[20] noted that olive oil had the greatest benefit, peanut oil and peanut products had an intermediate benefit, and the Step II diet had the least effect (reductions in CVD risk of 18%, 15% and 12%, respectively). The authors went on to note that although the oils utilized in the study (olive and peanut) were similar in their fatty acid composition (77% oleic acid), they differed in other constituents (tocopherols, tocotrienols, antioxidants, plant sterols, and other phytochemicals) which could instill added benefits. As an example, the author cited the antiatherogenic effects associated with refined rice bran oil (which has a MUFA comparable to study oils) which have been attributed to the plant sterol ferulate complex, oryzanol. Similarly, Anderson et al[1] demonstrated that the consumption of soy protein, rather than animal protein, significantly decreased serum concentrations of total cholesterol, LDL cholesterol, and triglycerides. They further commented that others have reported that most soy protein products contain soy estrogens (isoflavones or phytoestrogens) which have weak estrogenic effects that may, in themselves, decrease serum cholesterol and LDL cholesterol concentrations.

Because there remains much controversy regarding the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults[18] recommendations, a third Adult Treatment Panel is currently revising recommendations for proposed release in 2001[45].

The Mediterranean Diet

Willett et al[91] and Kushi et al[44] described the Mediterranean diet (based on food patterns typical of Crete, Greece, and southern Italy in the early 1960s) as one associated with adult life expectancy among the highest in the world and rates of coronary heart disease, certain cancers, and other diet-related chronic diseases among the lowest. The diet is characterized by abundant plant foods (fruit, vegetables, breads, other forms of cereals, potatoes, beans, nuts, and seeds), fresh fruit as the typical daily dessert, olive oil as the principal source of fat [total fat ranging from<25% to > 35% of energy; low in saturated fat (7-8 % of energy)], dairy products (principally cheese and yogurt), low to moderate fish and poultry consumption, low red meat consumption, zero to four eggs consumed weekly and

low to moderate wine consumption. Although, Kushi et al[44] noted that the moderate alcohol consumption, typical of the area, was associated with a protective effect against CHD (probably due to increased HDL levels), they suggested that the olive oil consumption (compared with saturated and partially hydrogenated fats) typical of this diet was responsible for reduced LDL and increased HDL (compared with carbohydrates). Others[5,62,81] have reported similar findings relating favorable lipid profile (low risk factors for CHD) to the diet, especially as it relates to the consumption of olive oil.

Essential Fatty Acids

Human beings evolved consuming a diet that contained approximately equal amounts of n-3 and n-6 essential fatty acids, yet, for the last decade or so, there has been an enormous increase in the consumption of n-6 fatty acids due to the increased intake of vegetable oils from corn, sunflower seeds, safflower seeds, cottonseed, and soybeans. As a result, in modern Western societies, the ratio of n-6 to n-3 fatty acids in diets range from 20-30 : 1 instead of the traditional range of 1-2 : 1[80]. The authors note the findings of others that such diets, richer in n-6 fatty acids, tend to shift the physiologic state to one that is prothrombotic and proaggregatory and are characterized by increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time. The authors note, however, that diets rich in n-3 fatty acids [fish and fish oils which are rich in eicosapentaenoic acid (EPA ; 20 : 5) and docosahexaenoic acid (DHA ; 22 : 6)] have physiological effects that tend to be anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and possess vasodilatory properties which have been shown to be beneficial in the secondary prevention of CHD, hypertension, type II diabetes, renal disease, rheumatoid arthritis and inflammatory bowel disease (i. e. ulcerative colitis, Crohn's disease). The authors do note, however, that consumption of α-linolenic acid (found in green leafy vegetables, flaxseed, rapeseed, and walnuts) may be converted (desaturated and elongated) to EPA and DHA. More recently, Nestel[58] reported that fish oils affect VLDL metabolism by reducing VLDL triacylglycerol secretion, generally increasing VLDL apoliprotein B secretion, reducing triacylglycerol transport (resulting in smaller VLDLs, which are largely converted to LDLs) and increasing VLDL clearance ; reduce cholesterol absorption, hepatic synthesis and cholesterol secretion within VLDLs in humans ; depress cholesterol synthesis and reduce cholesterol absorption ; increase HDL_2 at the expense of HDL_3 (very high intake of fish oil may lower HDL concentrations) ; reduce triacylglycerol formation (due to reduced fatty acid availability) and plasma fatty acids (increased hepatic uptake and reduced fatty acid synthesis due to suppression of key enzymes) ; and increase oxidation of fatty acids. In summary, the current consensus is that eating fish is beneficial at surprisingly modest intakes but that the benefit probably depends on the fatty acid profile of the fish consumed.

Trans (Hydrogenated) Fatty Acids

Because of lower costs and purported health benefits, the use of butter (high saturated fatty acids) has gradually been replaced by the margarines. In order to produce solid fats (shortening and margarine), more desirable for human consumption, partial hydrogenation of fats is necessary[3]. The resultant fat is solid at room temperature but is rich in trans fatty acids (carbon atoms adjacent to their double bonds are on opposite sides) as compared to naturally occurring unsaturated fatty acids which contain double bonds as cis isomers (adjacent carbons on the same side of

the double bond) but a liquid at room temperature. Although initial reports indicated that the LDL cholesterol levels were not as markedly elevated for fats high in trans fatty acids, compared to fats rich in saturated fats, later works demonstrated that trans fatty acids also lower HDL cholesterol levels[3]. In fact, trans fatty acids actually increase LDL cholesterol to levels similar to those produced by saturated fatty acids and also decrease HDL cholesterol levels, resulting in a LDL : HDL cholesterol that is actually double that of saturated fatty acids. Hu et al[34] demonstrated that risk of CAD was high with dietary intakes high in saturated and trans unsaturated fats and low with dietary intakes high in monounsaturated and polyunsaturated fatty acids. In the logical extension of this work, Lichtenstein et al[49] reported that the use of soybean oil (lowest in trans fatty acids) or semi-liquid margarine resulted in optimal total and LDL cholesterol levels and ratios of total cholesterol to HDL cholesterol; whereas stick margarine or butter resulted in the worst, thus confirming previous reports suggesting that diets rich in saturated fatty acids or trans fatty acids have a detrimental effect on serum lipid profiles. Further, the authors noted an HDL cholesterol-lowering effect of trans fatty acids (especially stick margarine) comparable to the HDL cholesterol-raising effect of saturated fatty acids. As the health risks of CAD associated with the consumption of trans fatty acids have become more broadly accepted[34,49], consumption of the harder (stick) margarines have declined and softer margarines have become popular[3]. In summary, both metabolic and epidemiologic studies have strongly suggested a strong adverse effect with respect to CHD associated with the replacement of fats rich in saturated fats (e. g. butter) with those rich in trans fatty acids (stick margarine).

Role of Dietary Antioxidants in Atherosclerosis

Dietary intake of the lipid soluble antioxidants (e. g. tocopherol and probucol) results in incorporation into the LDL and an associated increase in resistance to oxidative modification[2,15,83]. Accordingly, it has been estimated that, on average, six molecules of tocopherol are present in each LDL particle[16]. Although the National Research Council's recommended daily allowance for vitamin E is 30 IU[83], optimal vitamin E levels for CAD antioxidant effects range from 100-400 IU per day[2,15,83]. Carotene (despite being incorporated into the LDL particle) and ascorbic acid (vitamin C) (not incorporated into the LDL particle) may provide an increase in resistance to oxidative modification[2,15]. Although antioxidant supplementation has been associated with a decline in CAD without necessarily reducing plaque size, it's action may, instead, be directly upon increased resistence to subendothelial oxidation of LDL, stabilization of the plaque (prevention from rupture), correction of the endothelial dysfunction (including platelet adhesion, paradoxical vasoconstriction and vasospasm) and/or reduction in vascular cell cytotoxicity[2,15].

Drug Therapy and Atherosclerosis

Lowering of Serum Cholesterol

The target goal[18] for serum LDL is < 160 mg/dl for patients with no risk factors, < 130 mg/dl for patients with 2 or more risk factors and < 100 mg/dl for those with demonstrable cardiac disease; while serum triglyceride levels > 200 mg/dl are considered high and > 400 mg/dl are considered very high. While diet modification is considered the foundation of primary treatment, other interventions are frequently required. Early drug trials evaluated such agents as nicotinic acid (inhibits mobilization of free fatty acids from peripheral tissues, thus reducing hepatic synthesis

of triglycerides and secretion of VLDL), clofibrate (resembles short chain fatty acids and increase oxidation of fatty acids in muscle and the liver), gemfibrozil (a fibrate similar to clofibrate), bile acid-binding resins (cholestyramine and cholestipol) (bind intestinal bile acids, thus interrupting the enterohepatic circulation of bile acids, increasing hepatic conversion of cholesterol to bile acids ; unfortunately, this results in increased hepatic synthesis of cholesterol, which increase the secretion of VLDL into the circulation and raise triglyceride levels) generally demonstrated : a slowing of progression of atherosclerotic lesion (with no regression), a lowering of plasma cholesterol levels and a decreased mortality from CAD[41,50,52,88]. [Partial ileal bypass procedures demonstrated similar results[88]].

Unquestionably, the greatest advance to current drug therapy involved the discovery of the "statins" (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors)[88]. This class of drug resulted in the inhibition of cholesterol synthesis with a subsequent increase in hepatic expression of the LDL receptor, resulting in increased clearance of circulating LDL and a 20-60% reduction in plasma LDL[71,72,78]. Of interest, not only was a decrease in CAD observed but also a reduction in stroke (cerebral vascular accident) (which historically had only weak associations with plasma cholesterol levels)[63].

Other therapies currently noteworthy include dietary supplementation with soluble fiber (e. g. psyllum husk, oat bran, guar gum, pectin) which has been demonstrated to lower serum LDL levels by 5-10% and margarine with sitostanol which can inhibit the gastrointestinal absorption of cholesterol[41].

Other

Although the primary goal for treatment of the lipid disorders typically involve strategies for the reduction of serum cholesterol levels, other plasma lipids require attention. Nicotinic acid has cholesterol lowering effects, however its use, especially in combination with other lipid lowering drugs, reduces serum triglyceride and remnant lipoprotein concentrations, raises HDL concentrations and improves the LDL-subclass profile[41]. Modest alcohol consumption[21,79] has been demonstrated to breakdown ethanol in the liver to acetate which is released into plasma (which reportedly has inhibited lipolysis in peripheral tissues by 53% and whole-body lipid oxidation by 73%). The principal change in serum lipids related to alcohol consumption, however, is the reported increase in HDL [both light (HDL_2) and heavy (HDL_3) subfractions]. Since the light particles have been most consistently associated with cardiac protection, it is unlikely that the effect of alcohol consumption on HDL species provides a simple and complete explanation for the relation between alcohol consumption and coronary artery disease. Rumpler et al[69] demonstrated a greater HDL elevation in the HDL_2 subfraction of women with moderate alcohol consumption.

Summary

Cardiovascular disease (CVD) is the leading cause of death and disability in the United States and in most industrialized nations[7,61,83,88]. The major breakthroughs to modern day cardiovascular/lipid research have been attributed to the findings of the Framingham Heart Study[10,37] and Gofman and colleagues[27] who, for the first time, made direct associations between low density lipoprotein (LDL), very low density lipoprotein (VLDL) and inverse associations with high density lipoprotein (HDL) and CAD. Unfortunately, half of all CVD patients have none of the established coronary risk factors (hyper-

tension, hypercholesterolemia, cigarette smoking, diabetes mellitus, obesity)[7] and new strategies for identifying these patients need be considered. More recently, CAD risk factors have been expanded to include[7,61,64,73,85,86,88] : elevations in triglyceride levels, cigarette smoking, hypertension, diabetes, obesity, elevations in homocysteine levels, estrogen deficiency, fibrinogen, factor VII, plasminogen-activator inhibitor type I, tissue plasminogen activator, d-dimer, lipoprotein (a) and C-reactive protein.

In the current work, mechanisms for vascular dysfunction resulting in myocardial ischemia were explored and potential nutritional (dietary) and pharmacologic interventions were reviewed.

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Accepted by Editorial Committee